P001 3D cell printing technology for precise fabrication of tissue analogues

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Tissue engineering and regenerative medicine is a field that has evolved for over two decades and is progressively gaining more interest. The goal of tissue engineering is to regenerate the form and function of damaged tissues and organs to their original state by fabricating living replacements for parts of the body. Mimicking the forms and functions of native tissues and organs are major challenges. For this, sophisticated three-dimensional(3D) cell printing is a necessary technology for fabrication of tissue analogues.

In the process of 3D cell printing based on micro-extrusion, precise stacking along the z-direction and printing of complex tissue form have been preferentially required. 3D cell printing system equipped with heating modules for precise stacking, which developed by ourselves, showed excellent efficacy for precise stacking, there was no cell damage due to this heating system while cell printing. As well as precise stacking technology, we have printing process for fabrication of complex tissue analogues (such as dermis and epidermis complex, and alveolar bone and periodontal ligament complex) using multiple printing modules (Micro-extrusion and ink-jet printing). Micro-extrusion and ink-jet printing modules enable to print complex tissue analogues composed of tissues with various scales. Complex tissue printing technology may be useful for fabrication tissue analogues similar to native tissues because it allows us to study tissue integration between eachtissue.

P002 Characterization of Poly(Lactic Acid) scaffold synthesized by 3D printing technology

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The goal of tissue engineering is to create functional tissues and organs for regenerative therapies, and ultimately organ transplantation/replacement [1]. Recently with 3D printing or additive manufacturing with controlled microarchitectures has gained traction in a wide variety of fields, including in tissue engineering because it represents an interesting alternative for the synthesis of new scaffolds due to rapid manufacturing, high precision, inexpensive and easy-to-use [2]. Thus; the objective of the present investigation was to manufacture and characterize a Poly(Lactic Acid) Scaffold (PLA) made by 3D printing technology. Methodology: A 3D scaffold was designed and constructed by additive manufacturing of PLA polymer in the form of layers. The 3D scaffolds manufactured were characterized by scanning electron microscopy (SEM), X-ray, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and porosity analysis by profilometry. Using cultured osteoblastic cell lines, cell-scaffold interaction was assessed by confocal microscopy, cell adhesion assays, and cell viability by CCK-8. Our results of the scaffold characterization showed the presence of PLA without alterations, and a homogeneous surface between the scaffolds, with greater porosity in the lateral surface than the superior deposition. The cellular interaction of the osteoblasts analyzed by confocal microscopy showed membrane projections in the manner of filopodia or pseudopodia with a polygonal morphology characteristic of osteoblastic cells. The 3D scaffold promotes an increase in cell adhesion between 50 and 120% at 4 and 24 h when compared to nanofibrilar scaffolds (35 and 95%). Cell proliferation indicates that there is an obvious increase in viability in the 3D scaffold when compared to nanofibers scaffold between 1 to 7 days of cell culture. In conclusion, the 3D impression of PLA scaffolds allows to obtain a reproducible and stable structures, both with physically and thermodynamically and also having a good cellular biocompatibility properties. Acknowledgment: Authors want to thank the grant to the project by DGAPA-UNAM program: PAPIIT IN210815 and CONACyT for the support to the fellow to the student.

P005 Freeze-drying as a biofabrication method for achieving a controlled microarchitecture within large complex natural biomaterial scaffolds

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Where regeneration of a tissue with a complex shape is required, fabrication of a suitable scaffold geometry and microarchitecture presents a challenge. 3D printing does not offer the resolution needed when used with natural materials [1] and electrospinning can affect the bioactivity of natural materials while a homogenous porous microarchitecture is unachievable. Freeze-drying (FD) (or lyophilization) is used to create bioactive porous scaffolds and is typically only used to generate planar 3D geom etries. Therefore, the overall goal of this study was to use FD to develop a biofabrication process to create a collagen-glycosaminoglycan (CG) scaffold with a homogenous porous microarchitecture in a large, complex geometry. A semilunar heart valve (HV) shape was chosen to demonstrate this technique as it presented challenges- a large width to height ratio and also, complex leaflet components. The specific aims were to: (1) identify the optimal FD parameters to reproducibly produce a CG-HV shaped scaffold by investigating the effect of different mould materials and different freezing temperatures on the resultant scaffold geometry and microarchitecture and (2) to establish the optimal collagen concentration for a HV shaped geometry, in terms of pore size and mechanical properties.

Two HV shaped moulds were fabricated (one aluminium and one polyoxymethylene (POM)). A CG suspension was created [2] and pipetted into these moulds prior to FD. To map the temperature profile within the moulds during FD, thermocouples were placed at multiple positions in the CG suspension. The microarchitecture of the resultant CG-HV scaffolds was quantified by taking samples from the top, leaflet and bottom positions of each scaffold. Based on the initial results, an optimised *final mould* was manufactured. To establish the optimal collagen concentration for a HV shaped geometry, different concentrations were assessed in terms of scaffold pore size and mechanical properties.

Using the aluminium mould, HV shaped scaffolds were consistently fabricated which contained a homogenous porous microarchitecture. In contrast, the scaffolds produced within the POM mould showed evidence of uncontrolled FD. The *final mould*, in tandem with identified process parameters, facilitated repeatable, controlled fabrication of a collagen-based biomaterial into a HV geometry. The temperature profile observed during FD, the geometry of the scaffold and the resultant pore architecture, provided validation of this biofabrication process. Using a collagen concentration of 0.75% at a freezing rate of 1°C/min and at a final FD temperature of -10°C within the *final mould*, a CG-HV shaped scaffold with an average pore size of 130 µm, and suitable mechanical properties was produced. This scaffold was suitable for use as a template for HV regeneration. This study demonstrates the suitability of FD for achieving a controlled microarchitecture within large complex natural biomaterial scaffolds. This technique allows the advantages of FD to be exploited for use in applications where a porous collagen-based scaffold in a complex shape is required for tissue regeneration.

P006 Fabrication of thermoresponsive 3D dual hydrogel systems via 3D bioplotting

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Despite the great developments in biofabrication in recent years, there are still major criticalities to overcome regarding the required physical and biological properties of bioinks.

The main bottleneck is that the fabrication of biomimetic structures requires distinct boundaries for the physical properties of the hydrogels. Thus, recently, an increasing attention is paid by researchers when formulating a precursor hydrogel solution and a constant research is carried out to synthesize new polymers with improved hydrogel performances.

Thermoresponsive hydrogels which have sol-gel transition temperature close to physiological temperatures hold great importance in biofabrication as the gelation of such polymers occurs in such cytocompatible manner and does not require post processing.

Das et al. have reported a new fully-synthetic polyisocyanide (PIC) hydrogel that mimics characteristics of the natural extra-cellular matrix (ECM) [1]. Polyisocyanide (PIC) hydrogels are cutting edge reverse thermo responsive hydrogels currently used in various tissue engineering applications.

Here, we demonstrate the feasibility of 3D printing PIC hydrogels and of creating dual PIC-Gelatin Methacrylate (GelMA) hydrogel system. Furthermore, we propose the use of PIC as fugitive hydrogel through dual hydrogel system as the removal of PIC hydrogel is a simple and cytocompatible procedure. 3D printed PIC hydrogel and dual hydrogel system created with PIC-GelMA hydrogels open new avenues for fundamental co-culture studies, temporal and spatial control over cell niches.

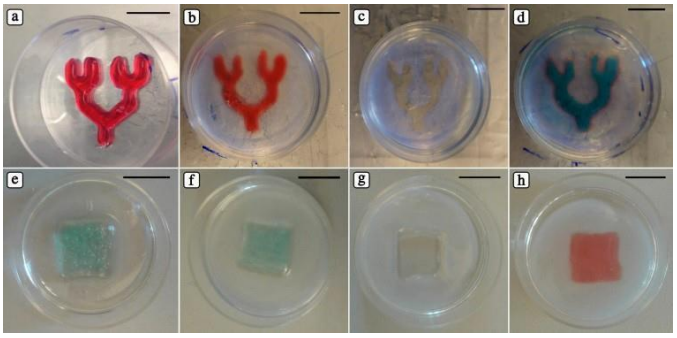

Figure 1. Optical images of 3D embedded constructs that are printed (a,e),comined with GelMA (b,f), evacuated (c,g), and perfused with a water-soluble food coloring(d,h) (scale bars : 10 mm)

P007 3D biofabrication of cell-laden gelatin methacrylate hydrogels

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Methacrylate functionalized gelatin (GelMA) is an attractive derivative of gelatin because in addition to gelatin's favourable biocompatible characteristics, GelMA can form a stable gel at physiological temperatures. Furthermore, GelMA hydrogels closely resemble some characteristics of native extracellular matrix (ECM) which induce cell proliferation and migration in GelMA-based scaffolds. Hereby, GelMA hydrogels has already been used in wide range of tissue engineering applications.

In the presence of a photoinitiator, when GelMA is exposed to UV irradiation, it crosslinks and forms hydrogels with tunable mechanical properties. Thus, it can also be used as bioink to generate cell-laden constructs with controlled architectures[1].

In this study, we demonstrated 3D printed macroporous gelatin methacrylamide constructs could be designed with interconnected pore network using 5% (w/v) GelMA concentration. Hereby, the influence of the printing temperature, the printing pressure and the printing speed on printability was analysed. Furthermore, the fabrication of cell-laden scaffolds was studied. The cytocompatibility was tested by encapsulation of the 3T3 fibroblasts and we have observed above 90 % cell viability in cell-laden scaffolds.

In conclusion, we have obtained mechanically stable cell-laden GelMA scaffolds with high cell viability.

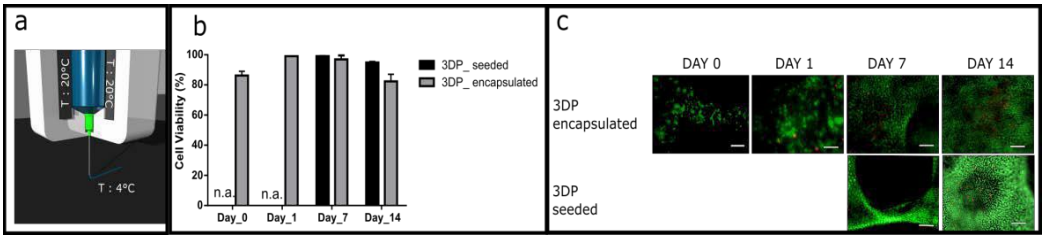

Figure 1. Printing conditions of 5% GelMA hydrogels (a), cell viability for the cell-laden and cell-seeded 3D printed GelMA hydrogels (b), live-dead staining for for the cell-laden and cell-seeded 3D printed GelMA hydrogels ; scale bar: 200µm (c)

P008 Time-dependent 3D printing of chemically crosslinked gelatin hydrogels

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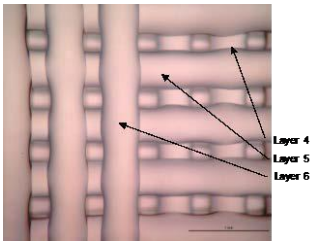
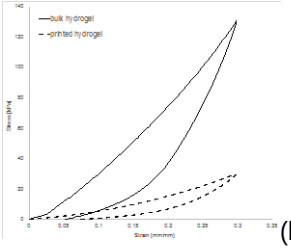
Hydrogels 3D printing has recently gained tremendous interest in tissue engineering thanks to the unique possibility of printing patient-customized scaffolds with controlled porosity, pore size and pore distribution. The successful 3D printing of a hydrogel is strictly related to the chosen printing strategy since it affects the accuracy of the printed material and the printed scaffold chemical, physical and mechanical properties.

In this study, we propose an innovative time-dependent 3D printing strategy of a chemically crosslinked gelatin hydrogel, printed by tuning the 3D printing process with the crosslinking kinetic of the hydrogel, without the need of post-curing or external treatments.

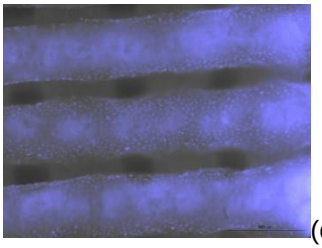
The hydrogel was prepared by a patented [1] reaction based on a Michael type addition, by mixing type A gelatin from porcine skin with *N,N'*-methylenebis(acrylamide) (MBA) as crosslinker. The 3D printing was performed by loading the gelatin/MBA reaction solution in the printer pneumatic dispensing cartridge, kept at 35 °C, and by printing on a glass substrate, kept at 4 °C to allow the shape retain of the printed scaffold. After the printing, to simultaneously promote the deposited filaments shape maintenance at low temperature (i.e., $T < T_{gelation}$) while allowing the crosslinking reaction to complete, scaffolds were sealed in petri dishes and kept at 20 °C for 48 h.



(a)



(b)



(c)

The rheological properties of the hydrogel during the crosslinking reaction allow to determine that the hydrogel is printable until 60 min after the gelatin/MBA mixing (printability time window at G'' > G', constant viscosity value). Printing parameters were optimized by using a 25 G needle and by setting 200 μm as layer thickness; 2 x 2 cm scaffolds were printed by 0 – 90° layer-by-layer deposition; filaments with 500 μm diameter were obtained and structures with more than 8 layers were successfully printed, with a final scaffold thickness > 1 mm (Fig.a). Printed hydrogels swollen in distilled water at 37 °C showed to be stable for more than 2 weeks, confirming the effective crosslinking. Mechanical compressive properties (Fig.b) were lower for the printed scaffolds compared to compact hydrogels, due to the porous structure and higher water content, suitable for soft tissue regeneration. Preliminary *in vitro* tests were performed on lyophilized printed scaffolds by dynamically seeding them in a L929 cell suspension, achieving a uniform distribution of cells (Fig.c). These preliminary results exhibit the success and efficiency of the printed strategy adopted, showing good printing shape fidelity and cell distribution.

P009 Biofabrication of a breast cancer model

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Despite significant advances in preventing, diagnosing, and treating breast cancer, this disease is still a major cause of death in women worldwide. It is nowadays widely recognized that the success rate of potential drugs entering clinical stages of development remains too low, particularly in the field of oncology(1). Success rates during drug development process greatly depend on pre-clinical research, which currently involves two-dimensional (2D) in vitro models on tissue-culture plastic and three-dimensional (3D) in vivo animal models. However, 2D in vitro models lack complexity, and in vivo animal models often fall short when translating to the human situation. To account for the unmet clinical demand and advance drug development research, more complex and clinically relevant in vitro models are required. By taking advantage of the power of additive biomanufacturing, the first steps towards establishing a robust and reproducible 3D heterogeneous in vitro model for breast cancer are taken. By means of stereolithography, two-component molds are manufactured; enabling the fabrication of constructs resembling the geometry of the ducts in the mammary gland. The resulting construct is comprised of a tube of breast cancer epithelial cells (i.e. MCF-7 cell line) surrounded by breast cancer associated fibroblasts (i.e. Hs606T cell line), the latter incorporated into a photocrosslinkable gelatin methacryloyl (gelMA)-based hydrogel. Pilot experiments regarding construct fabrication and cell viability have shown that the approach holds great promise for the future. Our model is envisioned to enhance the understanding of breast tumor biology and to improve drug development research. In addition, given the malleability in terms of matrix stiffness, cellular composition, and addition of external stimuli, this approach has potential for high-throughput screening purposes and personalized medicine applications at later stages of development.

P010 Biofabrication of a perfusable liver model

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Current in vitro systems do not allow an accurate prediction of drug-induced liver injury (DILI). This is one of the main reasons why DILI still occurs in clinical phases of drug development or even post-marketing. We developed a biofabricated 3D liver construct that can be cultured in a custom designed bioreactor system for the prediction of DILI. To achieve this, biofabrication techniques were combined with liver cells and hydrogel in a 3D bioprinter. As a source of hepatocytes, adult stem cells of the liver (organoids) were used for the constructs. Bioreactors were custom designed and produced by stereolithography technology. Liver cells were printed in 5% w/v gelMA hydrogel directly into the bioreactor and subsequently cross-linked by UV-A irradiation. The simultaneous deposition of sacrificial support material pluronic F127 allowed the formation of a porous structure. After printing, liver constructs were followed over time with cell viability assays including Alamar Blue. Cellular damage after treatment with toxic compounds was determined with an ATP assay. Cell aggregates remained viable for at least eight days after 3D printing. Aggregate formation of liver organoid cells and multipotent stromal cells (LMSCs) prior to printing in gelMA increased the albumin expression. However, gene expression profiling of the aggregates with only organoids showed a higher expression of hepatic markers compared to the combined aggregates with organoids and LMSCs. No increased hepatic function of organoid and LMSC containing aggregates was observed with cytochrome P450 assays. Acute toxicity of the liver constructs was determined with a four-fold increase in ATP levels after Triton X-100 treatment at 2-3 days post printing. Placement of the porous liver construct in the custom designed bioreactor allowed the perfusion of the liver construct. In conclusion, these results indicate that perfusable liver constructs in a bioreactor systems have the potential to predictDILI.

P011 Combining self-assembly of hierarchical synthetic extracellular matrices with microscale control of bioprinting

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Introduction Bioprinting is a rapidly expanding field within tissue engineering. Inks for bio-microfabrication need to meet printing requirements such as low viscosity, rapid gelation and structural integrity. These conditions are currently not optimal for cell culture, consequently self-assembling biomaterials have recently gained more attention, as these can be functionalized and tuned at the nanoscale to better mimic the extracellular matrix (ECM). Here we demonstrate that the co-assembly of peptide amphiphiles (PAs) with ECM proteins can be controlled and directed by 3D bioprinting to generate complex, hierarchical, and bioactive hydrogels.

Methods A simple acoustic based print head was used to generate droplets on demand (DoD) (Nozzle diameter ≤500µm). Here we demonstrate the possibility to print hydrogels using the co-assembling properties of PA molecules with keratin. PA and keratin were dissolved in cell friendly buffer at concentrations 5-20mg/mL and 10-50mg/mL respectively. A high-speed camera was used to visualise the hydrogel formation. Scanning electron microscopy (SEM) was used to characterise the gel structure. Cell viability was assessed using live/dead assay.

Results When a droplet of PA was jetted into a protein solution, a toroidal shaped gel was formed within ms of the droplet hitting the interface of the bulk solution. We showed that nanoscale control of the fibrous network within the gels can be achieved by modifying the PA structure or the solution conditions. Microscale control and precision of the system was shown by assembling structures, controlling gel sizes and by altering the final gelation structure by changing the fluid properties. Moreover, the system showed flexibility as the droplet and bulk solution are easily reversed, as well as versatility, as a variety of natural proteins were used for the co-assembly. High cell viability (>80%) was observed after printing using the acoustic print head design. Cell encapsulation was demonstrated with good cell survival, highlighting its potential for *in vitro* cellculture.

Conclusion By combining self-assembling materials with 3D DoD printing, we create a biofabrication method with both nano and microscale control. In addition, molecular co-assembly enables the possibility to use interfacial forces to direct self-assembly.

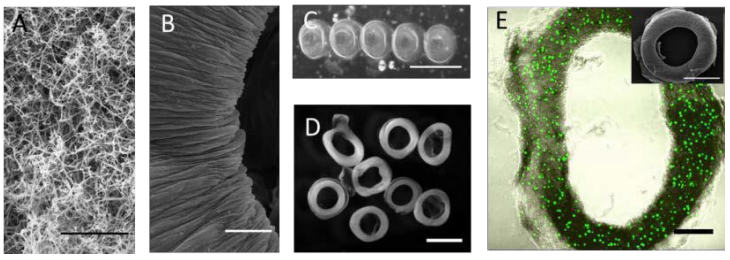

Figure 1 | (**a**) SEM of the PA/Keratin fibrous network (5 µm) (**b**) SEM of the gel surface topography (100 µm) (**c**) Gel alignment (1mm) (**d**) Toroidal shaped gels (1mm) (**e**) Live/dead assay of encapsulated NIH-3T3 cells (1mm)

P013 Rheology as a tool for validation of silk fibroin blend for 3D bio-printing

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Regenerative medicine has come a long way in last years, providing a lot of knowledge on scaffold materials, required physicochemical properties, cell behaviour in contact with scaffolds, etc. We realized that there is a necessity for patient specific tissue engineering scaffolds, since there can be a different solution for each specific case. A very common problem is an osteochondral lesion, where both chondral and bone tissue are damaged. Depending on the specific case, such lesions can have different size, ratio between chondral and bone tissue, etc. Therefore, a custom patient specific scaffold is preferred.

3D printing provides a promising solution. By acquiring CT scans from the osteochondral lesion one can reconstruct the damaged area, make a suitable scaffold to fit the lesion using 3D printing and plan all further steps prior the operation. This means we can save precious time during operation, making the whole experience easier for the patient and the medicaldoctors.

Silk fibroin has been proven to be a very suitable biomaterial for scaffolds as it shows great biocompatibility as well as good mechanical properties, needed for such osteochondral defects. However, fibroin by itself cannot be directly used as a bioink in its natural state. Direct 3D printing usually requires printing cross-linkable polymers or addition of thermo/photo-sensible synthetic polymers. We found a suitable thermo-sensible polysaccharide polymer gellan gum to be a good combination with silk fibroin, allowing the latter one to be directly printed.

Using rheological measurements, we could asses the ideal composition of the gellan gum and silk fibroin blend in order to balance the printing temperature while not overheating silk fibroin. By addition of Ca^{2+} ions we have also improved initial stiffness of the 3D printed scaffold. Rheology does not only provide temperature of gelation, but also behaviour of the solution/suspension while it is being printed, the rate of gelation and the expected precision of the printed scaffold. We have done some preliminary prints using a custom extruder (in the figure on the right) mounted on an off the shelf 3D printer.

Silk fibroin blend shows promise for future tissue engineering applications, as it allows easy use in 3D printing and all the benefits of a silk fibroin scaffold.

P014 Production of engineered-islets and their transplantation to cure type 1 Diabetes

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Introduction

The donor shortage is a rate-limiting factor for the transplantation of islets in type 1 diabetes. If it is possible to make artificial islets having efficient insulin secretion activity, we may be able to reduce the amount of islets for transplantation. To explore this possibility, we made various types of artificial islets using alpha cells, beta cells and hydrogel beads, and evaluated their insulin secretion activity both in vitro and in vivo.

Materials and Methods

Mouse derived pancreatic alpha (alpha TC1.6) and beta (MIN6m9) cell lines were used in this study. Alginate hydrogel beads, approximately 20 µm in diameter, were produced by an inkjet system. In order to fabricate artificial islets, we injected 1 µl of normal culture medium suspending cells and alginate hydrogel beads into 3% methylcellulose medium. Injected cells were immediately gathered and additional culture in a CO_2 incubator allowed cells to organize islet-like spherical tissues. The islets in basic condition were formed with 2000 beta cells. In some conditions, alpha cells and/or alginate hydrogel beads were mixed before injection to make hetero or hybrid islets. Two days later from injection, artificial islets were recovered from methylcellulose medium, washed well with glucose free medium for 1 hour, and stimulated by high-glucose to detect secreted insulin. In addition, islets were transplanted into the kidney capsule of streptozocin-treated C57Bl/6 mice, and blood glucose concentration was measured.

Results

Compared with basic islets comprising 2000 beta cells, islets containing 8000 beta cells showed higher insulin secretion rate. Enhancement of insulin secretion activity was also detected when we mix alpha cells in a ratio of alpha cells to beta cells of 1:8. Mixing of hydrogel beads was effective to make microchannels inside of the artificial islets and to enhance insulin secretion from the core part of the islets. Transplantation of these islets into the streptozocin-treated mice revealed that engineered-islets were better than basic islets to reduce blood glucoselevel.

Conclusion

These findings indicate that engineering of islets is a considerable option to increase therapeutic effect on islet transplantation.

P015 Effects of quercetin-functionalized collagen/hydroxyapatite composites for enhancing osteogenic differentiation of bone marrow-derived mesenchymal stem cells

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Chonbuk national university, Jeonju, Republic of Korea

INTRODUCTION: Quercetin(Qtn) is flavonoid materials and is known for increase alp activity . Qtn can induce osteoblast. Collagen possess of human about 30%, main ingredient of ECM. Collagen is mainly used for cartilage, bone and skin regeneration owing to its well-known biocompatibly, biodegradably. And they are known for low antigenicity, hemostatic effect, and cell adhesion. Previously, duck's feet-derived collagen(DC) showed reduced inflammation reaction [1]. In this study, 25μM Qtn sponges had possibility of applications usage in bone tissue engineering.

METHODS: Control, 25μM Qtn, 50μM Qtn, 100μM Qtn sponges made by lyophilized methods. All samples characteristics were studied by FT-IR, compressive strength, porosity and SEM(Fig. 1). Also, osteogenic differentiation of rabbit bone marrow mesenchymal stem cells (rBMSCs) cultured in scaffold was evaluated by ALP assay, MTT assay, RT-PCR, SEM.

RESULTS: We found decrease in porosity with increasing Qtn content in the scaffolds. In contrast, compressive strength was increased. Notably, cell proliferation and osteogenic differentiation are highest in 25μM Qtn.(Fig. 2).

DISCUSSION & CONCLUSIONS: This study showed the highest cell proliferation and osteogenesis in 25μM AA/DC/HAp sponge.

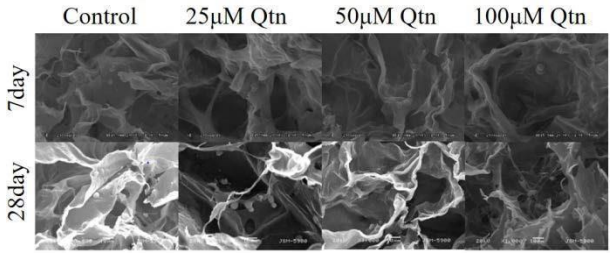

Fig. 1: SEM images of Control, 25μM Qtn, 50μM Qtn, 100μM Qtn sponges as a function of duck's feet- derived collagen sponge coated with hydroxyapatite.

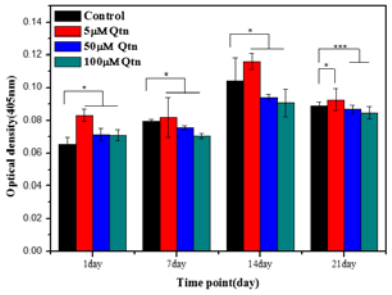

Fig. 2: ALP assay 1, 7, 14 and 21day of Control, 25μM Qtn, 50μM Qtn, 100μM Qtn sponges as a functional of duck's feet-derived collagen sponge coated with hydroxyapatite.

P017 Approach for standardized evaluation of printability and structural deformation of extruded bioink filaments

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Introduction: Bioinks that exhibit high shape fidelity and printability are necessary for bioprinting tissue-mimicking structures. In bioprinting, bioink filaments can collapse or undergo deformations after extrusion, compromising the quality of the print. In particular, shape stability is undermined primarily by two forces: i) gravity, causing unwanted sagging or compression, and ii) surface tension, which drives the material to adopt the most favourable shape to minimize surface energy. Sufficiently high yield stresses exert a protective effect against these effects, and are desirable for bioprinting, as long as the parameters determining yield stress (i.e. polymer concentration and crosslinking density) do not negatively affect cell function However, to date evaluation of printability is rather subjective and performed qualitatively on visual observation of the print, rather than on physical parameters of the material. Thus, the aim of this study was to describe the performance of candidate bioinks as a function of yield stress, through a set of tests allowing for quantitative analysis. **Methods:** A library of printable hydrogel compositions having different yield stresses was generated using blends of poloxamer 407 and poly(ethylene glycol), and was characterized by rheology. To assess filament deflection due to gravity, the inks were plotted on top of pillars with increasing spacing [1], and the angle of deflection from the printing plane was measured at each gap, and correlated to the yield stress. The effect of surface tension on fusion of adjacent filaments was evaluated by printing a meandering pattern with increased strand-to-strand distance. **Results and Discussion:** Filament deflection is inversely correlated to the yield stress of the extruded hydrogel (Figure 1). Yield stress also directly correlated with higher resolution in terms of minimum printable strand-to-strand distance, sharper meanders, and porosity.

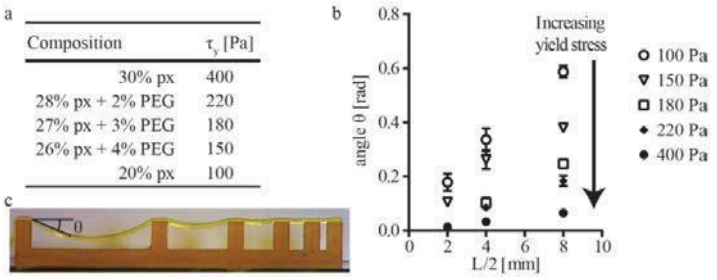

Figure 1: Yield stress dependent deflection of suspended printable bioinks

Conclusions: The proposed tests constitute a straightforward toolkit to provide quantifiable analyses of bioink printability. Finally, the experimental data will be used to validate a theoretical model that predicts the overhang and adjacent filament fusion as a function of rheological parameters (yield stress), and can guide the identification of the lower boundaries for bioink formulations.

P018 Inhibition of necrosis in multicellular spheroids by improving oxygen deficient conditions of culture medium and spheroid cores

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[Introduction] Spheroids are able to be used for drug screening. However, it is difficult to evaluate drug efficacy or toxicity because central necrosis of spheroids is induced by lacking of oxygen when spheroids become larger size. We previously reported that polydimethylsiloxane (PDMS) plates, which had a bottom of PDMS sheet, enhanced oxygen supply to culture medium. [Hamon et al., Cell Transplant, 2012] It was also reported that hydrogel beads were able to fabricate microchannel network structures in spheroids when they were mixed equal volume of cells. Microchannels were possible to supply oxygen to spheroid cores [Kojima et al., Sensor Actuat B-Chem, 2014]. It was available to combine these two methods.

[Objective] We aimed to inhibit central necrosis of spheroids by suppling oxygen to spheroid cores and culture medium.

[Methods] Hep G2 cells or beads were adjusted to 1×10^7 cells/ml or 1×10^7 beads/ml in normal medium respectively. Spheroids without microchannel were formed by injecting 1µl of cell suspension to a 3% methyl cellulose (MC) medium (without microchannel). To fabricate microchannel networks, equal volume of cells and beads were injected 1 µl to MC medium (with microchannel). Normal tissue-culture-treated polystyrene plate (TCPS) or PDMS plate was used in this study. Spheroids were cultured under four conditions: (1) TCPS + without microchannel, (2) TCPS + with microchannel, (3) PDMS + without microchannel, (4) PDMS + with microchannel. After 4, 7 and 10 days cultured, spheroids were isolated from the MC medium to investigate DNA amount of spheroids. Paraffin sections of spheroids were visualized with hematoxylin-eosin (HE) staining.

[Results] The diameter of spheroids was about 600 µm in conditions (1) to (4). We examined cell proliferation of spheroids by measuring DNA amount. In Conditions (3) and (4), DNA amount was about two times higher than (1). Condition (2) was almost same as (1) in amount of DNA. To examine the central necrosis of spheroids, spheroids were visualized by HE staining. Necrosis of spheroid cores was detected in conditions (1) and (3) after 4 days culture. The spheroid cores underwent necrosis within 7 days culture in condition (2). Necrosis of spheroids was inhibited until day 10 in condition (4).

[Conclusions] These results indicated that it was effective to supply oxygen to both culture medium and spheroid cores to inhibit central necrosis of spheroids and prolong the spheroid culture.

P019 3D bioprinting of mechanically robust cell-laden core-shell strands

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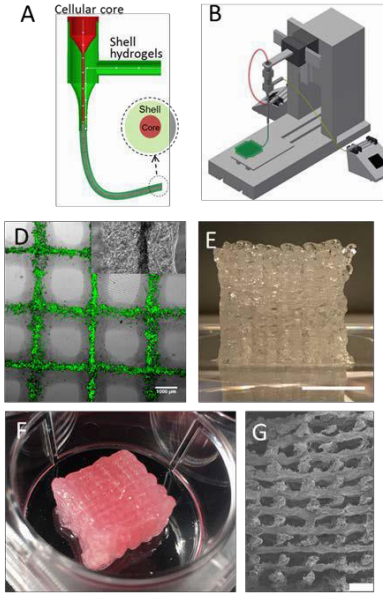
3D bioprinting can be used to pattern cells and arrange them into higher-order structures. The materials co-printed with the cells must have both biological and mechanical properties to support cell attachment and growth and also must be strong and robust enough to maintain shape fidelity.

In this work, we have developed a novel coaxial printing system that was able to simultaneously extrude a cell-laden extracellular matrix (ECM)-like core and mechanically robust shell into a single printed strand. The printer was assembled from a coaxial needle (Fig. 1A) and a commercial 3D bioprinter (Fig. 1B). Using a coaxial needle mounted onto a commercial 3D printer, we were able to produce coaxial fibres with distinct core and shell regions (Fig. 1C, D).

The shell material used was a hybrid of alginate and poly(ethylene) glycol and due to its viscous properties, soft and fluid-like materials such as Matrigel and Collagen could be incorporated into the core, whilst maintaining the ability to fabricate tall 3D structures (Fig. 1E). Tall multilayer lattices could be printed and maintained interconnected pores after culture (Fig. 1G).



Using this method, we have separately printed three cell types and demonstrated high cell viability during culture. Protein release from the core has also been studied and was found to correlate with the swelling rate of the shell material. This process of bioprinting using core-shell strands with optimal biochemical and biomechanical properties represents a new strategy for fabricating functional human tissues and organs.

Figure 1 (left): Bioprinting using cell-laden core-shell strands. A) Illustration of the coaxial needle. B) Schematic of the printer set-up. C) A single core-shell strand. D) A printed lattice with GFP-labelled 3T3 fibroblasts in the core. E) A multilayer lattice immediately after printing. F) The multilayer lattice following incubation in culture media. G) SEM showing the cross-section of the multilayer structure.

P021 Influence of bio-ink properties on viability of printed cells during drop-on-demand bioprinting

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Bioprinting is an emerging research field that has attracted huge attention; the drop-on-demand (DOD) bioprinting approach enables subtle control over the deposition pattern and material volume at pre-defined positions in a contactless printing manner. The capability to control cell deposition in a non-contact printing approach is valuable for high-throughput cell screening, fundamental biological research and fabrication of complex *in-vitro* tissue models. Although the droplet formation mechanism has been studied in great details, it is still poorly understood how the properties of bio-inks influence the viability of printed cells.

In this study, a microvalve-based bioprinting system is utilized for investigating the influence of bio-ink properties on cell viability during the DOD bioprinting process. Here, we employed polyvinylpyrrolidone (PVP) as a polymer model (0-3% w/v) to tune the physical properties (eg. viscosity, surface tension and density) of the bio-inks to evaluate its influence on the printability of bio-inks and viability of printed cells. These three key parameters of the bio-inks influence the printability can be represented by the Reynolds number (N_{Re}: the ratio of inertial to viscous forces) and the Weber number (N_{We}: a balance between the inertial and capillary forces)

$$N_{Re} = \frac{\rho v r}{\eta} \quad (1) \qquad N_{We} = \frac{\rho v^2 r}{\gamma} \quad (2) \qquad Z = \frac{N_{Re}}{(N_{We})^{1/2}} = \frac{(\rho \gamma r)^{1/2}}{\eta} \quad (3)$$

where v, ρ, η and γ are the average travel velocity, density, viscosity and surface tension of the bio-inks respectively, and r is a characteristic dimension (radius of the printing orifice). Another dimensionless number is the inverse (Z) of Ohnesorge number (Oh), which is defined as the ratio between the Reynolds number and a square root of the Weber number, and is independent of bio-ink velocity.

The printable range of Z values for the PVP-based bio-inks was determined to be within $5.75 \leq Z \leq 64.36$ (0-2.5% w/v). It was observed that the short-term viability of printed cells generally increases with decreasing Z values and a change in cell concentration (0.5 to 2.0 mil cells/ml) has no significant effect on the short-term cell. The remaining viable cells were shown to proliferate well without any impairment in the bio-inks with low Z values (≤ 9.30). To sum up, the Z value not only influences printability of the bio-inks, but also the viability of printed cells. Within this printable range of Z values, a decreasing Z value resulted in higher cell viability. Furthermore, cells can be printed without any significant impairment using bio-inks within a specific Z value threshold (≤ 9.30). This work provides critical information for formulation of new printable bio-inks that facilitates deposition of highly viable cells.

P022 3D printed microgroove patterns to spatiotemporally organize periodontal ligaments

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Introduction: The periodontal ligament (PDL) is the tooth-supportive structures to remodel, preserve, or maintain periodontia with anchorages between mineralized tissues. For functions under occlusal/masticatory loadings, controls of specific PDL orientations in micron-scaled interfaces are currently challenging in periodontal tissue engineering. We investigated the 3-D PDL architectures, which can spatiotemporally organize PDL in 3-D printing technology.

Materials & Methods: The programmed slicing with three different angulations is the key step to create oriented microgroove patterns on 3-D scaffold surfaces. The spatial scaffold for PDL regeneration was designed using CAD and wax molds were manufactured with the 3-D wax printer. The poly-ε-caprolactone was casted into 3-D printed molds and surfaces were analyzed by micro-CT, SEM, and confocal microscope. After *in vitro* human PDL cell cultures for 7 days and 21 days, cell orientations were statistically analyzed using fluorescence staining of cell nuclei. *In-vivo* subcutaneous experiments are performed to evaluate PDL cell/tissue alignments and tissue formations.

Results: Microgrooves with 0°, 45°, and 90° angulations on the scaffold were identified using micro-CT, confocal microscope for topography, and SEM and the individual topography had high reproducibility and predictability of manufacturing by 3-D printing technology. In in-vitro cell cultures, the 25um microgroove-patterned scaffolds provided more predictable cell alignments and cell orientations on three angulated microgroove patterns were significantly angular-controllable with statistical difference.

Discussion and Conclusions: The additive manufacturing can create various microgroove patterns on scaffold surfaces with consistence. 25μm-distant microgroove patterns can organize fibrous tissue constructs with specific cell orientations in periodontal tissue engineering. In particular, the 3-D printing technique will facilitate PDL regeneration in PDL interfaces with the controls of oblique or perpendicular orientations to tooth-root surface.

P023 A cell-responsive, photocrosslinkable bioink for extrusion bioprinting of 3D hydrogel constructs

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Bioprinting is an attractive technology to create complex 3D constructs through the precise layer-by-layer placement of hydrogels and live cells in predesigned locations. The design of hydrogel bioinks for extrusion-based bioprinting is a challenging task since their formulation must satisfy several requirements, such as viscous fluid behaviour, printability and, at the same time, exhibit suitable biophysical and biological properties. In this context, the objective of this study was to design a polysaccharide-based bioink fulfilling those requirements and simultaneously being capable of instructing cell behaviour.

Pectin, a structural polysaccharide extracted from the primary cell wall of plants, was selected as the sole material component of the bioink for its biocompatibility, anionic nature and lack of cell-adhesive sites in native composition. To render pectin photocrosslinkable, it was chemically modified by the introduction of alkene double bonds into the backbone. These bonds were subsequently reacted with a cell-adhesive peptide containing the amino acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD), yielding cell-adhesive and photocrosslinkable pectin macromers. A detailed characterization regarding the photocrosslinking of pectin hydrogels indicated the ability to tailor the mechanical properties of hydrogels in a broad range (79.6 ± 7.7 Pa – 2.6 ± 0.3 kPa) by simply changing the degree of chemical modification and polymer concentration, independently on the cell-adhesive ligand density. Rheological properties of the bioink were tailored to exhibit shear thinning properties and adequate viscosity for extrusion bioprinting. Human dermal fibroblasts were homogeneously mixed within the bioink with printable consistency, and printed into 3D constructs with high levels of shape fidelity. After 14 days of *in vitro* culture, printed hydrogel constructs supported high levels of cell viability, as qualitatively determined by the live/dead assay. Furthermore, cells assumed a typical spread, spindle-shaped morphology within the hydrogel, and promoted *de novo* deposition of extracellular matrix proteins. These results demonstrate the ability of this novel bioink formulation for the direct bioprinting of cell-responsive hydrogels.

Acknowledgments: This work was financed by European Regional Development Fund (ERDF) through the COMPETE2020 Operational Programme for Competitiveness and Internationalization (POC I), Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, and Portuguese funds through Portuguese Foundation for Science and Technology (FCT) in the framework of the project Ref. PTDC/BBBEC T/2145/2014. Ruben F. Pereira is grateful to FCT for the doctoral grant SFRH/BD/91151/2012.

P024 Robocast zirconia toughened alumina scaffolds: processing and human primary osteoblast response

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Nano-structured zirconia-toughened alumina (ZTA) is a tough, strong and low-wear bio-inert ceramic widely used for hip and knee prosthesis. However, integration of standard ZTA with bone is weak. Rapid prototyping techniques such as robocasting have the advantage of producing highly reproducible scaffolds with fully interconnected porous networks, which can facilitate integration with bone. Therefore, the goal was to assess ZTA robocast scaffold potential by (i) optimizing the ZTA ink, (ii) characterizing the robocast structure and (iii) investigating human primary osteoblasts (hOb) behaviour on ZTA *in vitro.*

A stable alumina - zirconia (w:w=84:16) suspension (73% w/v) was obtained in a 1.5% v/v Darvan C-N dispersant and 2% w/v carboxymethyl methylcellulose solution in deionised water. 3D-ZTA scaffolds were robocast (330 µm nozzle, 660 µm center to center rod distance, sample size: 1.5 cm diameter and 7 layers) in an acidic bath (pH 1.5). Samples were dried in air at room temperature for 24 hrs and then sintered at 1350°C. Structural features were analysed by scanning electron microscopy (SEM), mercury intrusion porosimetry and computed tomography (CT). Human primary osteoblast (hOb) proliferation, gene expression and mineralization were assessed at 10, 20 and 30 days of culture in osteogenic medium. 2D-ZTA discs (fabricated with the same ceramic ink) and Thermanox® (TMX) were used as controls.

Struts with 196±15.45 µm thickness were found by SEM analysis (Fig. 1a) and interconnected pores with 163±99.40 µm size were observed by mercury porosimetry and confirmed by CT. Cell proliferation was comparable on 2D- and 3D-ZTA, but lower cell density values were registered on the 3D-ZTA scaffolds at all time points. In terms of mineralization potential, higher ALP/DNA ratios were found on 2D-ZTA compared to TMX and 3D-ZTA, with a peak at day 20 (Fig. 1b). Osteogenic markers mRNA levels were upregulated from day 10 on already. Both cell density and mineralization on 3D-ZTA could be improved by optimising cell retention inside the 3D structure, for instance by culturing the scaffolds in perfusion bioreactors, further modifying the ZTA surface with acid etching and/or optimizing the scaffold design.

In conclusion, 3D-ZTA robocast scaffolds with interconnected pores of >100 µm were successfully produced. Further work is needed to optimize cell retention inside the structure.

Acknowledgement: European Commission funding under the 7th Framework Programme (Marie Curie Initial Training Networks; grant number: 289958, Bioceramics for bone repair).

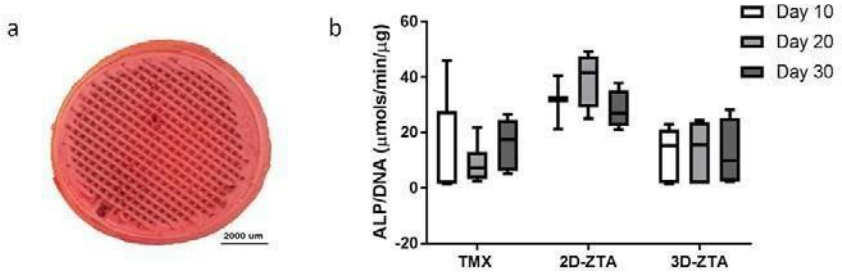

Fig.1: a) Overview of the robocasted 3D-ZTA; b) ALP to DNA ratio (n=3 independent donors).

P025 3D bioencapsulated structures using multiple size hydrogel spheres as building blocks by perfusion based layer-by-layer technique

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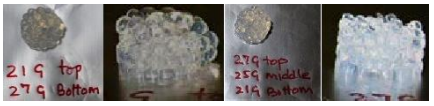
The rise in organ failure presents a serious risk to a public healthcare system which gets compounded by the shortage of donors for an organ transplant. Currently, 3D printing, using a robotic mechanism that requires special setups, that enable fabricating artificial organs such as skin, liver, kidney. But still there is a need to expand this research area by introducing more cell friendly environment, introducing spatial organization and utmost the economically viable options.

Recently in proof of concept study, we have demonstrated a novel, simple, desktop and cell friendly process to produce 3D bioencapsulated constructs using hydrogel beads and fibers as building blocks using the perfusion-based layer-by-layer technique without using binders/crosslinking agents. These constructs can be chelated that switch the initial solid microenvironment to liquified form. These self-supporting modified constructs favor diffusion thereby increasing cell viability. This work aims to biofabricate 3D hierarchical constructs using multiple sized cell encapsulated hydrogel spheres as building blocks, thereby showcasing the spatial aspect of this technique.

This process termed as perfusion-based layer-by-layer technique is based on a drop-wise method that allows, in real time, to coat and bind building blocks by nanometric multilayer membranes into freeform shapes. The production of building blocks is made by simple ionotropic cell encapsulation process through 27, 25 and 21 G needles using 1.5% alginate as a hydrogel and 1% calcium chloride as a crosslinking agent. The obtained bioencapsulated hydrogel spheres were then configured accordingly and followed by coating 5 bilayers using perfusion based layer-by-layer using 1mg/ml water-soluble chitosan and alginate as complementary polyelectrolytes. The 3D constructs can also be used as such or further processed by liquefaction using EDTA. Cell study will be characterized by cell viability and microscopy.



A B C D

A & B: Top and side view of a 3D construct obtained by two different sizes of hydrogel spheres. C & D: Top and side view of a 3D construct obtained by three different sizes of hydrogel spheres.

This investigation will enhance the fabrication prospects of using perfusion based layer-by-layer by introducing multiple sizes of building blocks in the same construct which is still not possible using current form of 3D biofabrication. Along with this feature, we predict the liquefaction and possible increase in the contact points may lead to high intra-transfer of media and nutrients.

P026 Alginate-fibrinogen and -collagen scaffolds for skin tissue engineering

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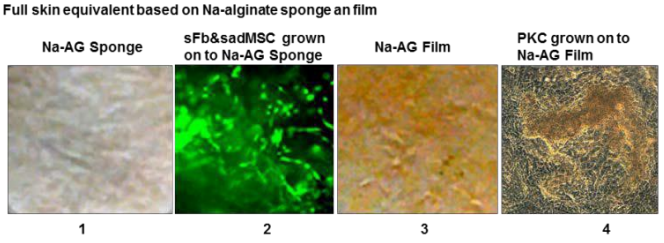
In the world clinical practice, including burn and reconstructive dermatology, there is a high demand of scaffolds suitable for generation of implantable or *in situ* forming tissue. Most of the derma-epidermal bioengineered skin constructs are based on collagen since it is a major and abundant protein in mammalian skin highly supportive for fibroblast growth also *in vitro*. Nevertheless, the use of conventional collagens such as bovine or porcine in biomedical applications is problematic due to the risk of disease transmission and expensiveness. Based on sodium alginate - anionic polysaccharide derived from brown algae we fabricated a series of thrombin modified alginate-fibrinogen sponge scaffolds for the dermal reconstruction and a number of alginate-collagen films (a basal membrane bioequivalent) to be used not only as a keratinocytes scaffolds for a full sickness epidermal construct but also as a barrier to prevent desiccation and infection during in vivo reconstruction of damaged skin. We selected Ca^{2+} and Ba^{2+} crosslinking cations for fabrication the scaffolds with requred mechanical properties. We fabricated a number of composite alginate-collagen (AG-CL) film scaffolds with addition of collagen to improve adhesiveness and composite alginate-fibrinogen (AG-FG) sponge scaffolds to improve angiogenic properties. The AG-FG sponge scaffolds possessed the suitable biocompatibility for the primary skin fibroblasts (sFb) and subcutaneous adipose tissue derived multipotent stromal cells (sadMSC) and the AG-CL film scaffolds possessed the suitable biocompatibility for the primary keratinocytes (PKC). We noticed that sodium alginate supplementation by 15-30 % of collagen resulted in two fold increase of PKC adhesion to the AG-CL compare to 100 % AG film scaffold. Our technology to produce the skin bioengineered construct based on sodium alginate is represented on figure below and can be described by the main steps: 1 – fabrication of AG-FG sponge scaffold, 2 – sFb and sadMSC culture on to AG-FG sponge scaffold; 3 – fabrication of AG-CL film scaffolds, 4 - PKC culture on to AG-CL film applied on top of AG-FG sponge with sFb and sadMSC for 7-10 days depending of hypoxia or normoxia conditions. The personal full skin construct could be obtain by using the AG-FG sponge scaffold with autologous or allogenic fibroblast, which could be prepared in advance and kept in liquid nitrogen for long term storage, and the autologous keratinocytes seeded on AG-CL film scaffold.



In summary we have found that the base sodium alginate sponge and film scaffold crosslinked by Ca^{2+} and Ba^{2+} cations have high adsorption capacity, adequate mechanical properties and biocompatibility and could represent an efficient and low cost scaffold material for the skin tissue reconstruction and could be used for other type of tissue reconstruction and bioengineering.

P027 A range of gelatin derivatization strategies lead to biofabrication versatility

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Biofabrication is a specific area closely related to the field of tissue engineering, which takes advantage of additive manufacturing (AM) techniques to generate 3D structures which mimic the natural extracellular matrix (ECM). A popular material in this respect is gelatin, as it is a cost-effective collagen derivative, which is the major constituent of the natural ECM. The material is characterized by an upper critical solution temperature (UCST), resulting in hydrogel formation at low temperatures and a gelatin solution at elevated temperature. Due to this UCST behaviour, the material will dissolve in cell culture and physiological conditions. To tackle this problem, the present work focusses on different gelatin functionalization strategies which enable covalent stabilization of 3D gelatin structures. To this end, three derivatisation strategies were applied and compared. The first modification consists of methacrylation of the primary amines present in gelatin type B using methacrylic anhydride to obtain gel-MOD. As a consequence of the presence of the introduced functionalities, the material becomes (photo-)crosslinkable using a chain growth polymerization mechanism. As a result, both deposition (e.g. Bioplotting) as well as irradiation-based AM techniques (e.g. stereolithography and two-photon-polymerization) can be applied for biofabrication. Although this derivative has a proven track record in biofabrication, it exhibits limitations in terms of post-production swelling and mechanical properties. To overcome these limitations, additional crosslinkable methacrylates were introduced by additional carboxylic acid modification of gel-MOD with 2-aminoethyl methacrylate via conventional carbodiimide coupling chemistry (EDC/NHS) to generate gel-MOD-AEMA. As a result, more densely crosslinked hydrogels can be obtained with a low swelling degree, which makes the material more suitable towards the use of high resolution AM techniques including two-photon-polymerization (2PP).

To further increase the gelatin versatility, a third derivative has been developed of which the primary amines were modified into norbornene functionalities. These functionalities have a very interesting reactivity towards thiols, as they can be applied for very fast, orthogonal thiol-ene “photo-click” reactions. When using multivalent thiol crosslinkers, crosslinked hydrogels can be obtained using significantly shorter irradiation times. Furthermore, the norbornene groups can be applied for one-step grafting of functional thiolated compounds with a high degree of spatiotemporal control.

All developed derivatives were characterized in depth for their material properties (NMR, rheology, swelling, etc) as well as their biocompatibility by monitoring the metabolic activity of L929 fibroblasts and MC3T3 osteoblasts seeded onto thin crosslinked films. The metabolic assay indicated no significant difference between the different gelatin derivatives in terms of biocompatibility. Furthermore, proof of concept biofabrication experiments were performed. Micro-scaffolds were generated using 2PP which indicated superior processing capabilities for the gel-MOD-AEMA and the gel-norbornene derivatives. In addition, macro-scaffolds were generated using indirect AM to demonstrate the versatility of the established material toolbox towards biofabrication.

P028 Combination of microfluidics and electrohydrodynamics as tools for the development of *in situ* cell-laden 3D biografts

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INTRODUCTION: With developing technologies, requirements for biomimetic tissue engineering scaffolds are constantly increasing. Not only the choice of material but also incorporation of specific cells and the concomitant cell-material interactions are crucial for the success of novel tissue engineering concepts. However, many biofabrication techniques rely on the usage of cytotoxic components, limiting the *in situ* incorporation of cells into scaffolds. In our work we report a combined approach of microfluidic encapsulation of cells into a protective carrier system to enable their delivery in a combined electrospinning – cell biospraying approach. By that, related cytotoxic effects of the solvents used for electrospinning can be overcome, since these residues can be washed out whilst the cells are still protected.

METHODS: Cell encapsulation into gelatin microgels was achieved by a droplet based microfluidic setting. C2C12 murine skeletal myoblasts were processed and potential negative effects of the process on cell viability or phenotype was evaluated via immunohistochemistry and flow cytometry. The obtained capsules were subsequently electrosprayed into a electrospun nanofibrous poly(vinylidene fluoride-co-hexafluoropropylene) membrane to fabricate cell-laden electrospun scaffolds in a layer-by-layer approach. Viability of encapsulated cells was compared to non-protected cells that were also sprayed into the polymeric membrane.

RESULTS: Using microfluidics and tailoring channel dimensions as well as flow, homogenous capsules in the size of 112 µm ±30 µm were obtained. When the encapsulated cells were electrosprayed at a voltage of up to +10 kV no loss of viability was measured compared to the seeded control group. Release of cells from the capsules occurred within 30 minutes followed by cellular adhesion to the substrate. By inducing differentiation of the C2C12 cells and staining for myosin heavy chain, a specific marker for differentiated myoblasts, it was shown that the cells retained their phenotype. The majority of the encapsulated cells survived the electrospraying process whereas all of the non-encapsulated cells died when sprayed into the polymeric membrane. Scanning electron and fluorescence microscopy revealed the incorporation and attachment of the cells within the fiber scaffold (Fig.1).

DISCUSSION & CONCLUSIONS: The results of the microfluidic cell encapsulation show great potential to combine cells with tailored polymeric structures to obtain complex 3D cell networks. In a next step the fabrication of multi-cellular tissues are envisioned.

ACKNOWLEDGEMENTS: This work is part of the Zurich Heart project of Hochschulmedizin Zürich.

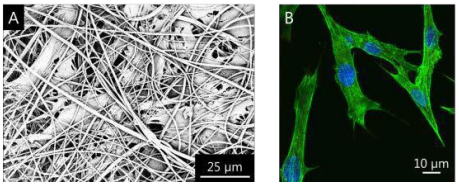


Fig.1: A) Scanning electron micrograph of C2C12 cells incorporated into the electrospun membrane. B) Cells from the same experiment fluorescently stained for Actin and DAPI showing their attachment to the material.

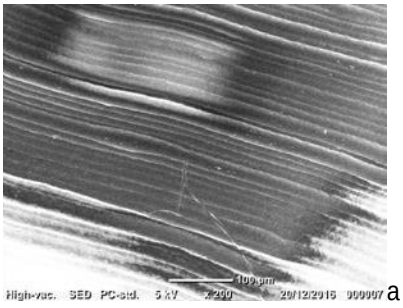
P029 Melt Electrospinning Writing for Tissue Engineering

Shavini Wijesuriya, Valeria Annibaldi, Gillian Hendy, Shane Finnegan, Ellen Byrne, Shirley O'Dea, Michael Macguire

Spraybase, Avectas, Maynooth, Ireland

Melt electrospinning has recently emerged as an alternative polymer processing technology; as it enables the fabrication of 3D scaffolds. This method is solvent free and enables the use of polymers which do not dissolve easily. This developing technology provides multiple opportunities in the Tissue Engineering field where solvent retention and toxicity are a concern. Despite the great potential observed, this technology is under-investigated. Approximately 150 publications are present in the literature on melt electrospinning. The main reason for this is the complexity of the apparatus where high voltage and high temperature need to coexist, in addition there is a lack of commercialised instruments available on the market.

Spraybase® has designed a novel melt electrospinning instrument capable of melting polymers with melting points up to 250^{o}C. Furthermore, the system enables melt electrospinning writing (MEW) through the use of an x-y stage as the collection platform. The aim of this study is to present data generated with our novel MEW instrument using several FDA approved polymers. We investigated the fibre deposition, control of subsequent layers, porosity, fibre morphology and fibre size. Preliminary results highlighted the accuracy of fibre deposition, with 100 layers effectively deposited (Fig.1a); resolution of pore size as low as 100µm and control of the fibre size, with fibres as low as 2µm melt electrospun, within a controlled (Fig.1b) and staggered format (Fig.1c).



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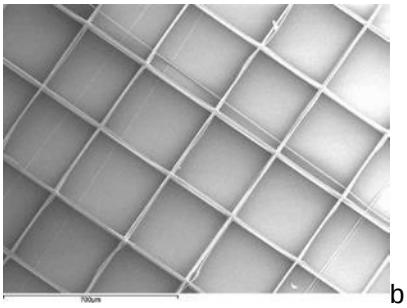
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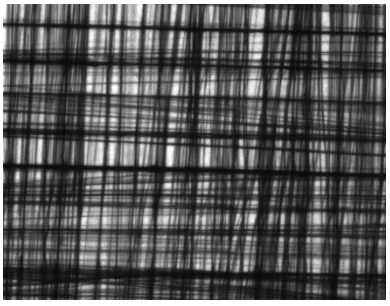
c

Figure 1: SEM images (a&b) and light microscope image (c) to show melt electrospun writing of polymer with a) controlled layer deposition, b) overall appearance of a controlled aligned scaffold and c) controlled staggered scaffold.

P030 3D-cell cultivation of human adipose-derived mesenchymal stem cells via magnetisable nanoparticles

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It is known that 3D-cell cultivation approximates the *in vivo*-situation more than the 2D-cultivation since extracellular matrix (ECM) secretion and intercellular interaction are more physiological in 3D than in 2D. To generate 3D-cell spheroids different methods are available (e.g. hanging drop, cultivation on cell repellent surface, scaffold based). However, these methods entail various drawbacks such as the use of artificial ECM as scaffold, non-defined cell numbers in the spheroids, or the loss of cellular material during the regular changes of cell culture medium.

In this study a scaffold-free model system was applied to evade the use of artificial ECM and to allow long term cultivation with regular changes of medium without the loss of cellular material. For this purpose human adipose-derived mesenchymal stem cells (adMSC) were loaded with magnetisable nanoparticles and afterwards placed in a cell repellent plate. Spheroid formation was induced by putting a magnet under the plate and thus forcing the aggregation of the nanoparticle-equipped cells. Initially, the minimal amount of applied nanoparticles which is sufficient for spheroid formation was evaluated. A nanoparticle concentration dependent difference in spheroid formation time occurred. Furthermore nanoparticle concentration affects the maintenance of a long term culture. After the adjustment of adequate nanoparticle amount, the formation of adMSC-spheroids was achieved within hours. The spheroids are characterized by a defined cell number at starting time. Initial testing was performed with cell numbers from $0.1 \times 10^5 – 1.25 \times 10^5$ cells per spheroid. The spheroid size differs in dependency on the initial cell number (300 - 800 µm). The exposure of spheroids to adipogenic and osteogenic differentiation stimuli led to changes in the spheroid size. Both differentiation conditions led to spheroids which were bigger and more stable than the non-stimulated spheroids. Microscopic analysis on the cellular distribution within the complete spheroids was difficult since confocal microscopy was only successful on the spheroids` surface due to its compactness. Also cell counting and life/dead staining turned out to be difficult since the process of enzymatic dissolution of the spheroid was hardly reproducible. Actual histological preparations of spheroid slices with regards to the depiction of cell distribution and viability are under progress. Future work comprises a comparison of cytoskeleton and the intercellular contacts between 2D and 3D culturing. Moreover the quantification of adipogenic and osteogenic differentiation capacity of the spheroids is in the works.

In summary, the characteristics of the nanoparticle/magnet-generated spheroids are dependent on several parameters such as concentration of nanoparticle, cell number, cultivation time, and differentiation conditions. 3D spheroids are a challenge with respect to some analysis methods compared to 2D culturing. Further research is necessary to elucidate the value of this nanoparticle/magnet-generated 3D cultivation.

Acknowledgement: This work was financially supported by the European Union and the Federal State Mecklenburg-Vorpommern (EFRE-project ARENA, project-No. TBI-V-1-003-VBW-001)

P031 Isolation and characterisation of pepsin soluble type II collagen from mammalian and marine tissue sources for cartilage regeneration

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Collagen is the predominant component of extracellular matrix in various connective tissues and makes up to 25% to 35% of the whole protein content in animal bodies. Type II collagen was first extracted from chicken sternal cartilage and provides supportive function in cartilaginous tissue. Since type II collagen is the major component of cartilage in the joint, this study aims to determine an optimal type II collagen source for the development of medical devices for articular cartilage regeneration. In order to make more effective use of underutilized food waste, type II collagen from mammalian tissue sources (porcine tracheal cartilage; auricular cartilage; articular cartilage) and marine tissue sources (cuckoo ray, blonde ray, thorn back ray, lesser spotted dogfish) was isolated through acid-pepsin digestion under 4°Cand characterized by various biological, biochemical and biophysical assays. The number and size of collagen chains were evaluated by SDS-PAGE. Intermolecular crosslinking density was quantified by ninhydrin assay. Thermal stability was tested by differential scanning calorimetry (DSC) and enzymatic degradation was assessed using a collagenase assay. Human chondrocytes were seeded into collagen hydrogels at a density of 50,000 cells/ml. Cell morphology, viability (LIVE/DEAD® assay), proliferation (PicoGreen®) and metabolic activity (alamarBlue®) were assessed at different time points (day 1, 3, 7, 14). Quantitative morphometric analysis was carried out using ImageJ. SDS-PAGE image of isolated type II collagen products demonstrated that high purity type II collagen can be obtained from porcine articular cartilage tissue and there is no difference in purity as a function of gender (**Fig. 1**). Therefore, pure type II collagen isolated from porcine articular cartilage can be further used as an ideal material for articular cartilage regeneration.

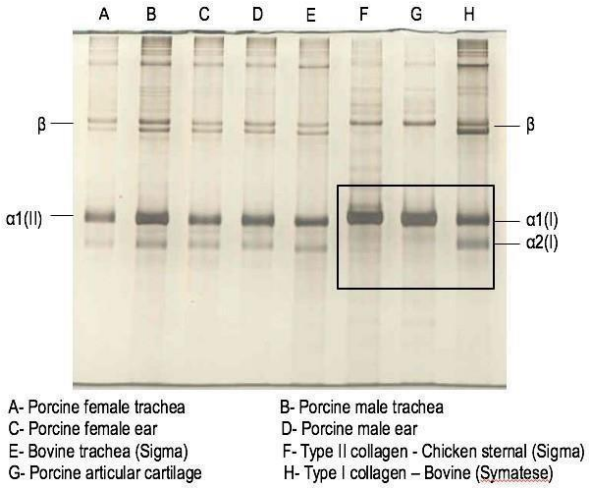

Figure 1: SDS-PAGE image of porcine isolated type II collagen products.

P033 3D inkjet printed branched vascular networks with feature size < 50 μm

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Tissue engineering has great potential to create tissue and organ constructs for clinical applications. However, fabrication of vascular networks within engineered tissues is still one of the greatest challenges in this field. 3D printing is potentially a powerful tool for fabricating controlled vascular networks with hierarchical structures within tissue scaffolds. 3D inkjet printing has particular advantages including readily available technology, multiple material delivery, high spatial resolution and cytocompatibility.

This study aims to build 3D branched vascular networks within hydrogel scaffolds using dual-head inkjet printing. Pluronic F127 is used as a sacrificial material to provide temporary support during the printing process and will be removed to generate hollow channels. The permanent matrix material is photocrosslinked gelatin methacrylate (GelMA) and thiol-terminated Polyethylene glycol (PEG-SH). PEG-SH inks are shown to be better suited for inkjet printing than conventional GelMA-based inks and improves the mechanical strength of final matrix hydrogel. The minimum channel size achieved by this method is 35 μm. By altering jetting voltage and drop spacing, channels with various sizes can be obtained as well.

This approach provide a possibility to create sophisticated 3D vasculature replicas within artificially engineered tissues.

P034 Controlled release of GET peptides for sustained and highly efficient intracellular delivery

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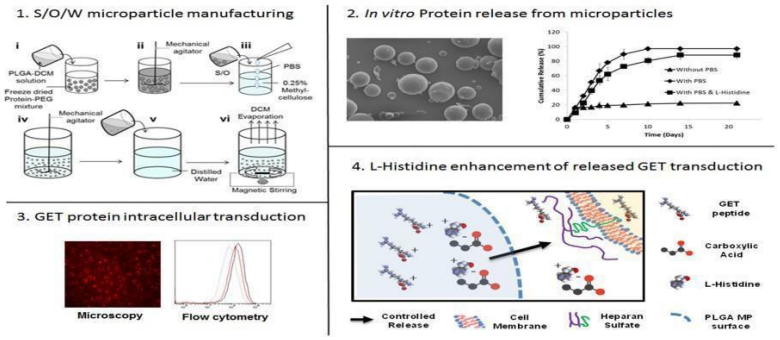
University of Nottingham, Nottingham, UK

Controlled release systems for therapeutic molecules are vital to allow the sustained local delivery of their activities which direct cell behaviour and enable novel regenerative strategies. Direct programming of cells using exogenously delivered transcription factors can by-pass growth factor signalling but there is still a requirement to deliver such activity spatio-temporally. We have developed novel fusion proteins that couple a membrane-docking peptide which binds heparan sulfate glycosaminoglycans (GAGs) with a CPP [1]. This GET (GAG-binding enhanced transduction) system can be utilised to enhance delivery of different cargos, including proteins (i.e. transcription factors and enzymes), nucleic acids, nanoparticles and drugs. Herein we demonstrate that GET system can be used in controlled release systems to mediate sustained intracellular transduction over one week. In this research, we have investigated all the manufacturing process parameters which may affect GET – protein molecule activity during encapsulation and release processes. We assessed the stability and activity of GET peptides in poly(DL-lactic acid-co-glycolic acid) (PLGA) microparticles (MPs) prepared using S/O/W double emulsion method. Efficient encapsulation (~65%) and tailored protein release profiles could be achieved, however intracellular transduction was significantly inhibited post-release. To retain GET peptide activity we optimised a strategy of co-encapsulation of L-Histidine, which may form a complex with the PLGA degradation products under acidic conditions. Simulations of the polymer microclimate showed that hydrolytic acidic PLGA degradation products directly inhibited GET peptide transduction activity, and use of L-Histidine significantly enhanced released protein delivery. We have demonstrated a successful controlled delivery of a GET – Protein molecule, retaining the high transduction activity of GET system. The ability to control the intracellular transduction of functional proteins into cells will facilitate new localised delivery methods, with minimised risk of systemic dosing that may lead to non- targeted activity, and allow approaches to direct cellular behaviour for many regenerative medicine applications.



P035 Microwave synthesis of fluorescent carbon nanoparticles for use in bio-imaging applications

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[1]university of sheffield, sheffield, UK, [2]kroto Research institute, sheffield, UK

Fluorescent carbon nanoparticles (CNPs) were produced by using a chemistry-based bottom-up approach via microwave heating to synthesis CNPs within a time scale of 5-10 minutes. A diverse amount of carbohydrates starting stock solutions were used to prepare CNPs resulting in an inexpensive one-step production method, which produced CNPs. Additionally, the CNPs were stable in aqueous solution without the need of surface passivation. This study shows that highly fluorescent carbon nanoparticles can be produced both from glucose and alginate stock solutions. These nanoparticles were characterized via a range of analysis techniques such as Transmission Electron microscopy (TEM), UV-VIS spectrometry, fluorescence, Raman and FT-IR spectroscopy, XPS and XRD. The carbon nanoparticles (CNPs) emit strongly in the green under pulsed femtosecond laser in the near-infrared region excitation, indicating that they are excellent absorbers for 2-photon-microscopyand imaging. These CNPs show good cell-imaging applications. They enter into cells without any further functionalization, and the fluorescence property of these particles can be used for fluorescence-based cell imaging applications.

P036 Enhanced loading and controlled delivery of TGF and VEGF with mesoporous calcium phosphates bone substitutes

Janina Moeller-Siegert[1], Julien Parmentier[1], Patrice Laquerriere[2], Ali Ouadi[2], Olivier Raisslé[3], Edouard Jallot[4], Jean-Marie Nedelec[3], Cathie Vix-Guterl[1], Karine Anselme[1]

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The performance of calcium phosphates (CaP) in bone regeneration can be improved if they are associated with active substances such as growth factors (GFs). Here, a large panel of high surface area CaP ceramics was prepared by using endo- and exo-templating synthesis routes giving interparticular mesopores. These materials were loaded with TGF-β1 or $VEGF_{165}$, both with pivotal roles in bone regeneration, using low GF concentrations (max 1 µg/mL), in order to reach low and physiological GF loading. The *in vitro* kinetics of adsorption and release of both GFs loaded in CaP ceramics at two different concentrations were studied in cell culture medium. The detrimental burst release that is usually encountered within the first hours of exposure to culture medium is considerably lower for the tested samples. From 0 to 50% of the initial GF loading was released after 3 to 6 days in culture medium. The high affinity of the GFs for the ceramic materials and their slow release are attributed to i) the targeted low GF loading that promotes adsorption on the stronger adsorption sites and ii) the high difference of surface charge between the ceramic and the GF, iii) their large surface area and confinement related to the presence of mesopores. The expected strong coupling between the resorption of CaP ceramics *in vivo* and the GF desorption will be crucial for bone regenerating systems with maximum release control.

P037 Multifunctional magnetic-responsive hydrogels modulate platelet lysate-derived growth factor release and guide cell fate

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[1]3Bs research group, Guimarães, Portugal, [2]INL-International Iberian Nanotechnology Laboratory, Braga, Portugal, [3]ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal, [4]i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal, [5]INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal

Photocrosslinkable natural based hydrogel matrices have received great interest for tissue engineering strategies, especially because they provide versatile systems that can be potentially used in diverse applications. Nevertheless, to engineer complex tissues and/or tissue interfaces, it is necessary to develop systems that can accommodate various functionalities. Such systems could also benefit from the possibility of being remotely controlled and/or further manipulated *ex vivo*.

This work reports the development of magnetic-field responsive photocrosslinkable hydrogels produced with methacrylated chondroitin sulphate (met-CS) and incorporating platelet lysate (PL). CS is known to interact specifically with GFs in mammalian extracellular matrix (ECM) allowing the storage of GFs, which are presented or released as needed. Magnetic nanoparticles (MNPs) coated with met-CS (met- CS MNPs) were incorporated within developed hydrogels, being linked to the hydrogel matrix and providing magnetic responsiveness.

The proposed system was shown to be injectable and crosslinked *in situ* producing stable hydrogels, therefore likely to be used in minimally invasive approaches. Moreover, the actuation of an external magnetic field (EMF) was shown to modulate the swelling and degradation profiles of the developed hydrogels, and the release of growth factors present in the PL. Additionally, the effect of EMF in hydrogels laden with either pre-osteoblasts differentiated from human adipose derived stem cells (pre-Ost) or human tendon cells (hTDCs) was assessed in single and co-culture systems. The hydrogels incorporating PL displayed an increased ability to support cell adhesion and proliferation, as well as the expression of tendon- and bone-related markers of both hTDCs and pre-Ost cells, namely osteopontin, collagen type I and tenescin. Finally, EMF impacted cell morphology and the synthesis of a tendon- and bone-like ECM, with a more pronounced effect in co-culture systems.

Overall, our data suggests that the proposed system is a suitable vehicle for cells and PL-derived GFs delivery. Furthermore, the versatility of such photocrosslinkable systems makes them extremely interesting tools for minimally invasive tissue engineering approaches, namely targeting interface tissues.

Acknowledgements: FCT project RECOGNIZE (UTAP-ICDT/CTM-BIO/0023/2014); fellowships SFRH/BD/96593/2013 (R.A.), SFRH/BPD/111729/2015 (M.R.) and IF/00685/2012 (M.E.G).

P038 PDMS-based block copolymers for soft tissue engineering applications

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Introduction. Polydimethylsiloxane (PDMS) is an attractive material for tissue engineering due to its biocompatibility and elasticity. However, it has some intrinsic drawbacks, like hydrophobicity, lack of biodegradability and poor suture support. To solve some of these problems, we have synthesised several PDMS-based block copolymers.

Materials and methods.

PDMS-based block copolymer types:

1. Hydrophilic copolymers from 2-hydroxyethyl methacrylate (HEMA) and acrylic acid, methacrylic acid or acrylamide were chemically modified with hydrophobic α,ω-diepoxy-PDMS.

2. Poly(urethane-urea) copolymers were obtained from polyethylene glycol (PEG), hexamethylene diisocyanate and α,ω-diamino-PDMS.

3. Poly(vinyl alcohol) (PVA)-based copolymers were synthesised by polyaddition reaction from PVA, glycidyl methacrylate and α,ω-diepoxy-PDMS.

4. Hydrophilic copolymers synthesized from HEMA, acrylic acid and α,ω-divinyl-PDMS were modified with glycidyl methacrylate.

Ciba Irgacure 651 was used as a photoinitiator. Films were prepared by UV curing and characterised by FTIR, SEM and EDX spectroscopy. Tensile strengths of the materials were evaluated.

To assess the biocompatibility of the materials, primary rabbit muscle-derived stem cells

were either cultured on the copolymers or they were treated with copolymer extracts and their viability and proliferative activity were measured.

Results and discussion.

PDMS-based block copolymers showed varying degrees of swelling in aqueous media, where some materials would expand up to three times upon immersion in water. The observed swelling properties might prove promising in loading scaffolds with drugs or other bioactive molecules.

Poly(urethane-urea)-based films had high Young's moduli, exceeding that of commercial PDMS.

As expected, both the chemical structure and polymerisation conditions had a strong influence on cellular metabolism. Some of the copolymers show promise for use in tissue engineering applications.

Acknowledgement. This work is supported by the Research Council of Lithuania, grant No. SEN-

P039 Application of bioactive melatonin in anticancer therapies: new therapeutic approaches from nutrition to nanomedicine

Enrico Catalano

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INTRODUCTION: Phytochemicals as dietary constituents are being explored for their cancer preventive properties. Phytochemicals are known to have modulatory effects on cell apoptosis, migration and growth via various signaling pathways. Though, phytochemicals possess great medicinal value, their applications as a therapeutic drug are limited. Problems like low oral bioavailability and poor aqueous solubility make phytochemicals like unreliable candidates for therapeutic purposes. Additionally, the rapid gastrointestinal digestion of phytochemicals is also a major barrier for their clinical translation. Hence, to overcome these disadvantages phytochemicals-based nanoformulations are being considered in recent times. Nanoformulations of phytochemicals have shown promising results in its uptake by the epithelial system as well as enhanced delivery to the target site.
METHODS: The use of nanoparticles as a promising drug delivery system has been reported in various studies due to its low in vivo toxicity, high stability and ability to be functionalized by various ligands to achieve targeted drug delivery. In addition, nanoparticles have high surface area and ordered tunable porosity rendering them high drug loading efficiency with better release kinetics. The reported bio-distribution and excretion profile of nanoparticles makes them an opportunistic nanomaterial for controlled drug delivery. Zhang et al., showed increased oral bioavailability, ~154% compared to free poorly water soluble drug, by loading them onto mesoporous silica nanoparticles. Physical adsorption and solvent evaporation are the most employed silica nanoparticles based drug loading methods.
RESULTS: As discussed earlier that the antioxidant property of phytochemicals provide it a better therapeutic potential against cancer, similar kind of nanoparticles-phytochemicals hybrid which retains their antioxidant property can be suggested as a potent anticancer agent. Additionally, nanoparticles loaded with melatonin were also synthesized for topical application as a chemopreventive agent. They are mainly employed to immobilize the therapeutic molecules to modulate their controlled release and thus enhancing their biological activities. In 2011, US Food and Drug Administration (FDA) approved iron-oxide nanoparticles for first human clinical trial aimed at targeted imaging of cancer, reflecting the clinical acceptance of silica nanoparticles in near future.
DISCUSSION & CONCLUSIONS: Phytochemicals are competent antioxidant and can serve as potent anti-cancer agents. However, its efficiency as a therapeutic drug is low due to its rapid metabolism in the body. Thus, the use of biodegradable and biocompatible carriers as delivery systems can enhance the therapeutic competence of melatonin. However, the use of such nanoparticles at a clinical level still needs more process optimization to enhance their specificity and efficacy for their efficient clinical translation.

P040 Biomimetic apatite coating directs endothelial and smooth muscle cell fate as a polymer-free carrier for drug eluting stent

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A drug eluting stent (DES) is commonly a metallic platform coated with drug-loaded non-degradable/degradable polymers. Recent concerns regarding the mid- to long-term safety of these polymer-based DES have been raised, mostly because of the late and very late in-stent restenosis, partially attributed to the presence of polymer coating. As a consequence, there is a huge demand in improving the safety and efficacy of DES with biocompatible polymer-free coating and offering controlled eluting of drugs over two months. Apatite, a porous biocompatible coating that can be biomimetically deposited onto chemical treated metallic materials, has drawn attentions as a polymer-free carrier for DES.

In the present study, Co-Cr alloy is chosen as the stent platform, which can offer ultra-thin struts due to its high mechanical strength. The surfaces of Co-Cr are activated through a two-step chemical treatment, and on which apatite are biomimetically deposited in metastable calcium phosphate solution. The cell behaviors of human artery endothelial cell (HAEC) and human artery smooth muscle cell (HASMC) are investigated. It is found that the biomimetic apatite coated surfaces significantly enhance HAEC adhesion, proliferation, and release of nitric oxide (NO). Additionally, the biomimetic apatite coated surfaces show a remarkable ability to decrease the adhesion and proliferation of HASMC. It is noteworthy that the biomimetic apatite coating on Co-Cr shows good resistance to delamination or destruction after balloon expansion. These data suggest the potential of biomimetic apatite coating as a polymer-free carrier for the application in vascularstents.

This research was supported partly by National Natural Science Foundation of China (51502265), and partly by Zhejiang Provincial Natural Science Foundation of China (LQ16E020006).

P041 Non-invasive assessment of mesenchymal stem cells proliferation on micro-structured polyurethane-based fiber scaffolds and evaluation of the fibre orientation effect on cells differentiation

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[1]INRIM,Istituto Nazionale di Ricerca Metrologica, Turin, Italy, [2]Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Turin, Italy

Micro- and nano-structured scaffolds in combination with mesenchymal stem cells are an interesting approach to mimic human tissues, such as cardiac and skin tissues, and promote tissue regeneration. A non-invasive *in vitro* analysis of the cellularised scaffold can help the prediction of the construct effectiveness *in vivo*. Non-destructive methods, such as some metabolic assays and advanced not labeled imaging approaches, are available to assess the cell-scaffold behavior with minimal culture system perturbations. Aim of this study was to assess the biocompatibility of two different polyurethane-based membranes (random and oriented) on mesenchymal stem cells (MSCs) and to determine how the fibre orientation of engineered scaffolds affects the proliferation of MSCs. MSCs were seeded onto scaffolds and cultured for 14 days under normal conditions. Cell viability was assessed by non-destructive resazurin metabolic activity assays. Cell-scaffold interactions were visualized using traditional fluorescence (FM) and scanning electron microscopy (SEM) and quantitative atomic force microscopy (AFM). As results, cells grew in response to scaffold fibre orientation and cell viability and cell proliferation analysis showed that both fibre membranes support MSC proliferation. Moreover, aligned orientation scaffold supported higher proliferation compared to random fiber orientation (Figure 1 and 2). Results on cell differentiation assessment by gene expression analysis through digital droplet-PCR will be presented. This study supports the further investigation of an electrospun aligned fibrous scaffold for tissue repair and regeneration and highlights the potential of optimizing fibre orientation for improved utility. In addition, biomaterial degradation kinetics investigation could demonstrate the constructs capability to be further attractive for regeneration approaches.

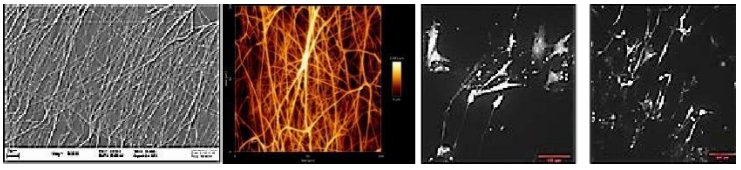

Figure 1. Imaging of aligned fibre scaffolds and of cells. From left side: SEM and AFM of scaffold and FMof MSCs (20x and 10x magnification) at day 14 of culture on scaffold.

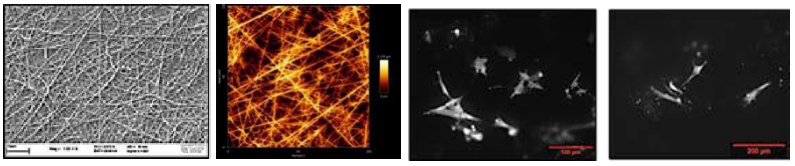

Figure 2. Imaging of random fibre scaffolds and of cells. From left side: SEM and AFM of scaffold and FM of MSCs (20x and 10x magnification) at day 14 of culture on scaffold.

P043 ELECTROSPUN SCAFFOLDS WITH POTENTIAL APPLICATION IN SKIN WOUND HEALING AND DRUG DELIVERY

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THE UNIVERSITY OF NOTTINGHAM, NOTTINGHAM, UK

INTRODUCTION

Wound healing is a complex and coordinated response where the skin repairs itself. The prolongation or failure of this process may result in a chronic wound condition [1]. Our ultimate aim is to fabricate electrospun scaffolds based on PCL or PEG_{5000}-*b*- PEG which enhance skin tissue in non-healing skin wounds. The purpose of this study was to evaluate *in vitro* cell growth, cell infiltration and the use of these scaffolds as platform of drug release, loading maggot secretion as therapeuticmolecule.

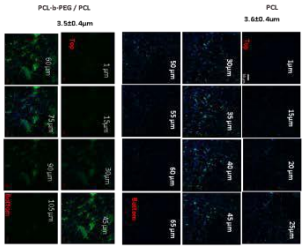
MATERIALS AND METHODS

PCL and PCL-PEG electrospun scaffolds were produced via electrospinning. To investigate biocompatibility of scaffolds, metabolic activity of fibroblasts was determined by performing the AlamarBlue™ assay over 18 days. To determine the level of cell penetration into the scaffolds, confocal microscopy with z-stacked was carried out.

Maggot secretion was loaded on PCL and PCL-PEG scaffolds during 24h, after that the membranes were washed and dried in vacuum oven. For *in vitro* drug release samples were embed in 1 mL of DMEM or PBS media and incubated at 37°C. At determinate time, supernatants were collected and replaced with fresh media. The supernatants were analysed using Bradford assay.

Statistical analysis was performed using a two way-ANOVA using Prism (GraphPad Prism7, San Diego CA).

RESULTS



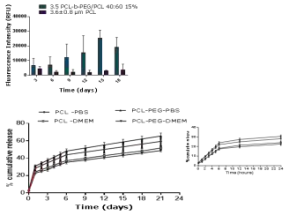


Figure I) Confocal microscope z-stack images. Magnification x20, scale bar = 50 µm. Figure II) Metabolic activity assessed using AlamarBlue™ Two way-ANOVA, multiple comparison test. N=3 (means±SD); ns for P> 0.05, * for P≤ 0.05, ** P ≤0.01, *** P ≤0.001, **** P ≤0.0001

Figure III. Cumulative drug release % of electrospun scaffolds loaded with maggot secretion. Comparison of drug release profiles between PCL and PCL-PEG scaffolds over (a) 24 days (b) 24 hours.

Fibroblast infiltrated through haft of the height until day 5 in both material. Biocompatibility analysis on Human dermal fibroblasts showed that PCL-PEG was significant more compatible than PCL alone. The release of maggot secretion on both material exhibited sustained delivery for up 24 days. In general, the release of maggot secretion was slower when DMEM media was used but not significant differences were observed between materials.

CONCLUSIONS

Both material PCL and PCL-PEG support fibroblast infiltration. And showed a sustainable release of maggot secretion. However, PCL-PEG is the material that gather all the properties including biocompatibility to be use for skin regeneration and drug release platform.

ACKNOWLEDGMENTS

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P044 Salivary Gland Radioprotection and Functional Regeneration via Nanotechnology-based Protein Therapy: A Step Closer towards Clinical Translation and Personalization?

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PROBLEM: Saliva plays a major role in maintaining oral health. This becomes more apparent when the amount and quality of saliva are reduced, often due to medications, Sjögren's syndrome and especially ionizing radiation therapy for tumors of the head and neck, during which the salivary glands are included within the radiation zone. Hyposalivation leading to symptomatic dry mouth or xerostomia, causes difficulty in mastication, swallowing and speech, changes in taste, acceleration of dental caries, oral sores, burning sensations and periodontal diseases. While temporarily alleviated via "intensive" regimens of palliative home and professional care, many Head and Neck Cancer patients are unable to maintain the diligence required to be effective. More considerably, those affected by irreversible salivary gland dysfunction (and/or using amifostine, I.V.) often choose to surrender/terminate their radiotherapy course (and cancer management/treatment) pre-maturely as they become severely malnourished and experience a significant deterioration in their Quality of Life, mainly owing to hyposalivation. **SOLUTION:** A nano-sized dual-protein release-controlled delivery system as an alternative therapy approach/strategy to protect salivary glands from ionizing radiation and potentially restore/repair the radiation-induced damage can be beneficial and desirable to suffering patients since all current radioprotectors seem to lack safety as well as efficacy whilst all commercially-available remedies and medications offer only short-term relief of symptoms. **OBJECTIVE:** Evaluate the radioprotective effect and regenerative potential of core-shell nanocapsules designed for sequential and timely protein(s) release, following a single local administration via direct injection into murine submandibular salivary glands pre-irradiation. **METHOD:** Loaded core-shell nanocapsules with the protein(s) were directly administered into the salivary glands of the experimental group 8 hours before radiation and PBS was injected into the glands, likewise, for the controls. External irradiation at dose with 15 Gy was exposed to head and neck fields of C57BL/6 mice. Salivary flow rates and salivary protein excretion/content were evaluated using an enzyme-linked immunosorbent assay (ELISA) over a 3mons. period following treatment. Histological evaluation of structures and analysis of apoptosis/proliferation were performed. Timely bio-distribution assays followed. **RESULTS:** Experimental animals demonstrated increased salivary flow rates compared to controls. Protein content was comparable to that of pre-radiation (base-line) level. Histological evaluation revealed that acinar cells showed less vacuoles and nuclear aberrance in experimental group compared to the control group and the amount of mucin stained by alcian blue was larger, in the latter. Protein therapy resulted in less apoptotic activities detected by TUNEL assay and similar proliferative indices as in the control mice. **CONCLUSIONS:** Novel, biocompatible, stable, reproducible and *customizable* core-shell nanoparticulate layer-by-layer self- assembled delivery system is formulated and presented. Our findings suggest that the local sequential release of a protein cocktail (in specific dosage and order) into murine salivary gland highly prevents radiation-induced damage via reducing apoptosis. This approach also promotes *in situ* proliferation of salivary gland cells.

P045 Formulation and Physico-Chemical Characterization of Chitosan-Hyaluronan-coated Solid Lipid Nanocapsules for the Targeted and Release-Controlled Delivery of Paclitaxel

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Providing oncology patients with an efficient and safe/tolerable therapy would lead to higher patient compliance rates and subsequently positively impact their quality of life. Paclitaxel (PAX) is a chemotherapy agent, effective in the treatment of a broad range of human malignancies, with activity demonstrated in cancers of the ovary, lungs and breast, among others. Recently, PAX demonstrated an anti-angiogenic activity via the inhibition of vascular endothelial cell proliferation, motility and cord formation, at extremely low concentrations. Despite vast developments, a more effective and stable PAX formulation with improved solubility, permeability, high anti-tumor and anti-angiogenic activity (and reduced formulation-related adverse effects) continues to be desirable and pursued. Since their introduction in 1991, solid lipid nanoparticles (SLN) have emerged as potential drug carriers for many poorly soluble-drugs. Indeed, SLN seem promising given simplicity to modulate drug release, improve anti-cancer activity and overcome MDR (multi-drug resistance). Here, the main purpose of this work is to specifically target and kill cancer cells, through the enhancement of the delivery and uptake of PAX. For this purpose, we developed a novel natural polymer-lipid hybrid formulation consisting of SLN as core and chitosan (CH)-hyaluronan (HA) as a shell, with HA as the outmost layer (to bind to CD44 receptors), to enhance selectivity toward HA receptors in MCF-7 cells (breast cancer cells).

In the present study, layer-by-layer (L-b-L) self-assembled natural polymer-coated solid lipid nanoparticles containing PAX were designed, formulated (via modified high-pressure hot homogenization) and evaluated to overcome drug resistance, reduce side effects and enhance the therapeutic effect of the loaded drug. Influence of process and formulation variables on the preparation of stable SLN were investigated, in depth. Drug release kinetics from the CH-HA coated and un-coated SLN were profiled and studied. Stability, cellular viability and uptake experiments were also conducted. Reproducible and negatively charged nanoparticles resulted. Findings reveal that CH-HA-coated SLN facilitated the targeting, cellular uptake and the time-/dose-controlled delivery and release of PAX, enhancing intrinsic chemotherapeutic activities. It was thus hypothesized that when natural polymer-coated and PAX-loaded SLN enter the cytoplasm (via endocytosis as well as thru receptor targeting), by-passing the resistance mechanism of the cell membrane can result.

Thereby, an effective concentration of the released drug can be maintained within the cancer cell to cause apoptotic death. SLN are suitable carrier candidates for nano-oncology given their localized, and potent cytotoxic potential overcoming multidrug-resistant cancer cells.

To the best of our knowledge, this is the first *proof-of-concept* investigative report depicting the potential of L-b-L self-assembled core-shell, natural polymer-SLN, as a predictable, customizable, hybrid and release-controlled PAX delivery system, against MCF-7 cellline.

P049 3D-printing materials and medical coatings - a biocompatibility evaluation

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OBJECTIVE:

Biomedical research often requires custom-made and highly specialized tools and testing devices. In regard to biocompatibility stainless steel and PMMA are among the most established materials for machining. We evaluated alternative production strategies to reduce production times, handling issues and costs while increasing flexibility. This can be achieved by using different materials for machining specialized surface coatings. A fast evolving alternative is to utilize additive manufacturing technologies. The aim of this research was to evaluate different surface coatings and 3D-printing materials in regard to their biocompatibility.

METHODS:

Cylindrical plates (ø11.3mm, A=1cm²) were used as sample geometry to fit in 24well-plates. Therefore, specialized mountings were designed to allow an easy handling of the samples in all test scenarios. To evaluate the surface coatings, aluminium samples with beneficial machining properties (ALMg4,5Mn0,7) were prepared. Six different coatings were applied. The 3D-printed samples were created solely from the evaluated material. Five materials from Stratasys Ltd. with different mechanical properties were chosen to provide an overview of available materials.

Different scenarios were defined to evaluate the influence of the materials on cell vitality, genotoxicity, proliferation and cytotoxicity. In all cases, endothelial cells (EC) were used, due to their key role in cardiovascular tissue engineering. To evaluate the direct influence of the material surface on the cells, cells were seeded on the samples and the viability was evaluated via Live-Dead assay. The cell growth on the sample surface was evaluated by SEM analysis of the samples. Additionally, cytotoxicity was assessed byWST-1 assay. To evaluate long-term effects of santicizers or released ions, samples were placed in culture media for 24h, 96h, 30d and 90d. Thereafter, EC were cultured with these conditioned cell growth media. Again, proliferation and cytotoxicity was evaluated by WST-1 assay. A total number of 1152 samples were evaluated to grant reliable results.

RESULTS:

Significant differences between different surface treatments and 3D-printing materials were detected. The biocompatibility of surface coatings highly depends on the chosen procedure. Flexible as well as dyed printing materials generally showed inferior behavior than rigid and colorless materials. Strong differences between short term (≤96h), mid-term (≤30d) and long-term (≤90d) results were identified.

CONCLUSIONS:

Surface coatings as well as printing materials with suitable properties for application in tissue engineering could be identified. The restriction to rigid and colourless materials for 3D-printing applications limits the use of this technique. However, many new materials are constantly developed and the possibility to produce rigid biocompatible geometries alone offers a multitude of new possibilities in biomedical research.

P050 Human pluripotent stem cells culture on growth factor-immobilized surface under feeder-free and xeno-free conditions

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[1]National Central University, Taoyuan City, Zhongli Dist.,, Taiwan, [2]King Saud University, Riyadh, Saudi Arabia

Human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have significant potential in regenerative medical applications due to their infinite proliferation ability and pluripotency. Therefore, large-scale culture of the hPSCs has become noteworthy in recent years. However, current culture medium (Essential 8, E8) for hPSCs is too expensive. One of the reasons is that the crucial biological cues for hPSCs cultivation, growth factors, such as FGF-2 and TGF-β1, are extremely expensive and should be kept under -80 °C ~ -20 °C. Several articles have suggested that total growth factor usages can be decreased via immobilization of growth factors on the surface compared to the usage of growth factors in the solution (culture medium). This is because the stability of growth factor immobilized surface is much higher than the growth factor in the solution owing to easier conformational deformation of growth factors in the solution. It is highly demanded to develop growth factor-immobilized dishes to decrease the usage of the culture containing growth factors.

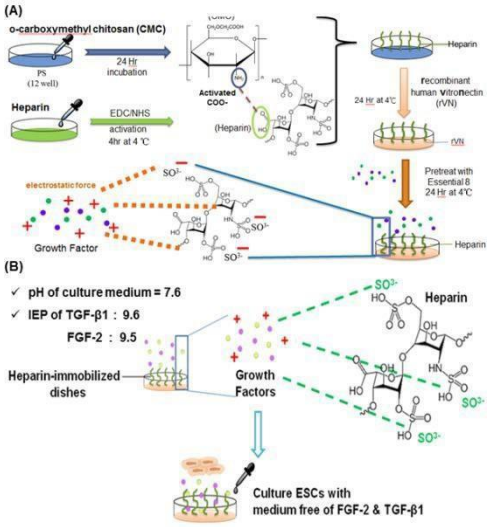
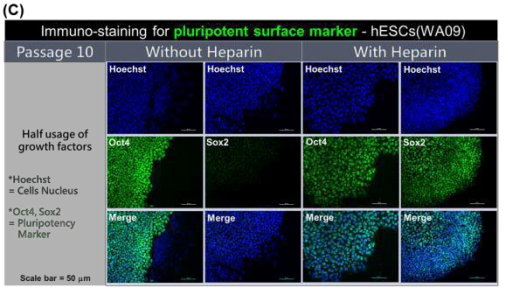


Fig. 1 (A) Scheme of preparation for heparin-immobilized dishes, (B) mechanism of growth factors binding to heparin via electrostatic force, (C) the pluripotent expression of hESCs(WA09) were analyzed after 10 passages under reduced usage of growth factors.

In this study, we reported that hESCs (WA09) cultured on optimal concentration of heparin-immobilized surface, where heparin, a well-known stabilizer for proteins, serves as binding sites for growth factors (Fig. 1A and 1B). After immobilization of the growth factors on the heparin-immobilized surface through electrostatic force, hESCs were cultured on the modified surface and the culture medium without FGF-2 and TGF-β1 (E6, Essential 6), was utilized under feeder-free and xeno-free conditions. The hESCs propagation with decreasing usage of growth factors system was successfully developed (Fig 1C) to more than 8 passages and the pluripotency examination of the hESCs was characterized by immunostaining method after long term cultivation. Further quantification of heparin and growth factors content remained on the surface after cultivation is still under investigation.



P054 A new technology for standardized separation and concentration of SVF cells during liposuction

Inge Matthiesen

Human Med AG, Schwerin, Germany

A new technology for the standardized separation and concentration of stromal vascular fraction (SVF) cells from fat tissue is being described. This new technology enables the sterile separation of SVF during liposuction in a closed system. The concentrated SVF cells can be directly sampled from the system and immediately re-injected, at the point of care. The procedure of SVF cell harvesting can be carried out without centrifugation and without enzyme digestion. However, the addition of one centrifugation step will yield a considerably higher yield of SVF cells.

The system consists of the single-use Q-graft collector including various filtration steps, and the small medical device Q-graft control. The SVF cells are separated in an essentially mechanical process. The entire process is completed in the sterile area of the operating room. The process time is less than 60 minutes.

The fat is aspirated directly into the Q-graft® collector, right on the sterile operating table.

The medical device Q-graft control regulates the heating and mixing of the fat with collagenase in the incubation chamber of the single-use collector, and the rinsing of the ultrafiltration membrane during concentration of the SVF cells.

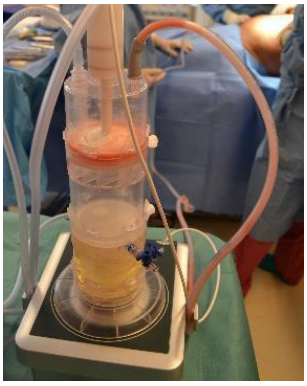


Fig. 1: Liposuction directly into the Q-graft collector in a sterile closed system

Fig. 2: Standardized SFV cell separation in various steps within the Q-graft collector

Fig. 3: Sampling of the concentrated fat-free SVF cells from the Q-graft collector

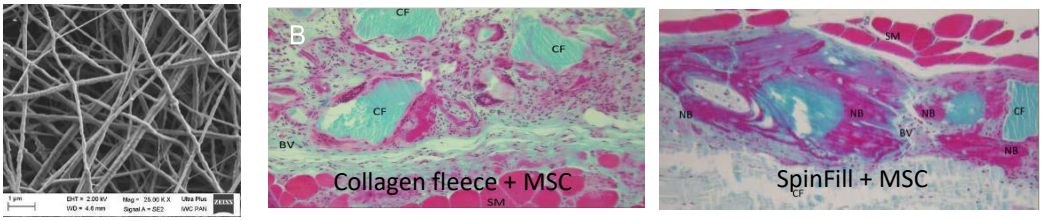
P055 Osteostimulative collagen nanofleeces to support bone regeneration

Javorina Milosevic, Claudia Heine, Sadat Hasan, Julia Tews, Timothy Ganey, Hans-Jörg Meisel
SpinPlant GmbH, Halle (Saale), Germany

SpinPlant utilizes electrospinning, a leading technique to produce biomimetic, nanoscale scaffolds for tissue regeneration, stem cell hosting or delivery in various tissue-engineering applications. Our focus is the production of bone void filler based on three-dimensional nanofleeces (SpinFill).

SpinPlant technology relies on the preservation of the collagen nativity, assuring stability of the product, which is a prerequisite for an efficient osseointegration and new bone formation. SpinFill consists of biomaterials shown to be non-toxic, biodegradable and at the same time stable and porous, promoting cell adhesion and spreading. Improved with osteoconductive supplements, these fleeces are promising candidates to provide effective bone regeneration.

The next generation of bone remodeling substitute shall provide matrix for an efficient bone ingrowth and regeneration without causing side-effects. They should primarily demonstrate high patient safety, therapeutic efficacy, unlimited availability, but also cost efficiency. In addition to the osteoconductivity, bone fillers ideally exhibit osteoinductive and osteogenic properties as well. None of the currently available therapeutic approaches combine all those features.



Figre 1. (A) 100,000x SEM image reveals a complex organization of native collagen nanofibers co-spun with nanoceramics. Osteostimulative properties of the collagen fleeces without (B) and with nanoceramics (C) are shown. CF – collagen fleece, NB – new bone, BV – blood vessel, MSC – mesenchymal stem cells.

To produce fleeces with preserved native collagen structure, a unique electrospinning process was used. By means of different techniques including SEM analysis, qPCR analysis and histology, the spun fleeces were comprehensively characterized.

SpinPlant has incorporated the above-mentioned properties in one product, ECM-mimicking, native collagen bone filler on nano-basis. It promotes cell adhesion, migration angiogenesis and new bone formation, without causing adverse effects in vivo.

P057 Silica nano-carrier as a sustained delivery system of GDF5 and TGF-β1 for intervertebral disc regenerative medicine

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Introduction: Intervertebral disc (IVD) degeneration is one of the major causes of low back pain. The sustained delivery of therapeutic factors able to promote IVD regenerative processes *in situ* is contemplated and biomaterials-based delivery systems are widely studied. Among these biomaterials, mesoporous silicas are of interest with respect to their properties and lack of cytotoxicity [1]. In this context, we propose to use mesoporous silica nanofibers (MSNFs) [2] as a drug delivery system for two nucleopulpogenic factors [3], Growth and Differentiation Factor 5 (GDF5) and Transforming Growth Factor β1 (TGF-β1).

Methods: GDF5 and TGF-β1 were associated with MSNFs at pH 7, 4°C for 48h at concentrations from 1-4 µg/mL. Release was first investigated in PBS, pH 7.2 at 37°C for 21 days. The concentration and bioactivity were measured with ELISA and by testing the Smad pathways activation in human adipose stromal cell (hASC) by western blot, respectively.

We then studied the differentiation capacity of human adipose stromal cells (hASC) when associated to a hydrogel containing the GDF5/TGF-β1-loaded MSNFs, prepared as described earlier. The differentiation was performed at 37°C, in hypoxic conditions for 7, 14, 21 and 28 days. Nucleopulpogenic commitment of hASC was assessed using immunohistology (alcian blue, type II collagen, aggrecan, OVOS2, PAX1, CD24).

Results: Release experiments showed that both GDF5 and TGF-β1 could be adsorbed and released from MSNFs for up to 28 days. Released growth factors bioactivity, evaluated by Smad phosphorylation, was evidenced until the latest release time-point for the highest GDF5/TGF-β1-loaded concentrations onto MSNFs. In addition, the immunohistological analyses revealed the expression of the main nucleopulpogenic markers.

Discussion & conclusions: Altogether, our data indicate that GDF5 and TGF-β1 can be loaded and released from MSNFs while preserving their biological activity as evidenced by the activation Smad pathways and the expression of nucleopulpogenic markers. MSNFs are promising nano-carriers for therapeutic factors delivery and are thus of particular interest for the intervertebral disc regenerative medicine.

ACKNOWLEDGEMENTS: Financial support: FARMA " ET3-683", Région Pays de la Loire "Projet LMA", ANR "REMEDIV" and FRM Projet DBS20131128442.

P058 Investigation of the use of bioinspired, star-shaped polypeptides to enhance delivery of stem cells and regenerative proteins to the ischaemic myocardium

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Currently heart transplantation represents the only curative treatment for heart failure[1]. The lack of donor hearts means that most patients rely entirely on the use of pharmacological strategies which provide only symptomatic management of their condition. Thus fulfilling the need for preventative or curative therapies has become a priority[1]. Delivery of stem cells and growth factors to the heart for tissue regeneration is impeded by rapid degradation of protein growth factors and poor retention of therapies at the target site[2]. Biomaterial hydrogels can improve ventricle wall strength but without the incorporation of therapeutics their success has been limited. This project aims to produce an innovative delivery platform for cells and regenerative proteins which may overcome some of these translational barriers. The materials being investigated are synthetic, bioinspired star-shaped polypeptides which possess advantages over traditional, natural biomaterials in that their structure can easily be modified and they have the ability to produce site specific, sustained delivery of one or multiple therapeutics. To-date their application in growth factor delivery and potential to form hydrogels for cell delivery remains largely uninvestigated. This project aims to use a negatively charged glutamic acid (PGA) based star polypeptide to bind electrostatically to the angiogenic protein vascular endothelial growth factor (VEGF) to form nanoparticles. A number of star polypeptides including PGA and star polylysine (PLL) will also be assessed for their ability to form hydrogels capable of acting as a delivery vehicle both for stem cells and nanoparticles. Dynamic light scattering, nanotracking analysis and atomic force microscopy were used to assess the size and charge of the PGA-VEGF nanoparticles and release of VEGF from these nanoparticles was investigated. Bioactivity and toxicity of the particles were determined using Matrigel®, Scratch and MTS assays. Rheological and injectability testing was conducted on a number of star polypeptide hydrogels with and without the inclusion of stem cells and nanoparticles. PGA:VEGF ratios between 30:1 and 100:1 produced particles ~200nm with a narrow size distribution and facilitated sustained release of VEGF for up to 28 days. Star PGA:VEGF treatments led to on average 33% more tubule formation than cells alone at 12 hours. Migration of human Umbilical Vein Endothelial Cells (hUVEC's) was significantly improved with PGA nanoparticle treatments and particles demonstrated little cytotoxicity on both hUVEC's and human Mesenchymal Stem Cells. Hydrogels formed via covalent crosslinking had storage moduli in the range of 1-7kPa, similar to biomaterial hydrogels currently in clinical trials. All hydrogels were shear-thinning even following incorporation of stem cells and nanoparticles and could be injected through a 1ml syringe with a 25G needle attached at forces below the maximum human pinch strength. Further modification of the star polypeptides based on the current results should allow the production of a hydrogel system which will facilitate and enhance minimally invasive cell and growth factor delivery to the ischaemic myocardium, thus promoting tissue regeneration.

P059 In situ delivery of curcumin to pancreatic islets using prolonged release-type microspheres for the prevention of apoptosis

Shiva Pathak, Shobha Regmi, Dong Ho Ha, Tung Thanh Pham, Tiep Tien Nguyen, Chul Soon Yong, Jong Oh Kim, Jee-Heon Jeong

Yeungnam University, Gyeongsan, Republic of Korea

Pancreatic islets transplantation is a promising method to insulin-dependent diabetes mellitus. Hypoxic conditions at the transplantation site induce necrosis and apoptosis of islets during the early stages after transplantation. In this study, we aimed to protect pancreatic islet cells against apoptosis by establishing a method for *in situ* delivery of curcumin to the pancreatic islets. Self-assembled heterospheroids of pancreatic islet cells and curcumin-loaded polymeric microspheres were prepared by hanging drop technique. Release of curcumin in the microenvironment of pancreatic islets promoted survival of the islets during long-term culture. In hypoxic conditions, which mimic the *in vivo* conditions after transplantation, viability of the islets was significantly improved, as indicated by a decreased expression of pro-apoptotic protein and an increased expression of anti-apoptotic protein. Additionally, oxidative stress-induced cell death was suppressed. Thus, unlike co-transplantation of pancreatic islets and free microspheres, which provided a wide distribution of microspheres throughout the transplanted area, the heterospheroid transplantation resulted in co-localization of pancreatic islet cells and microspheres, thereby exerting beneficial effects on thecells.

P060 Functional improvement on erectile and ejaculatory in a spinal cord injury after mesenchymal stem cell implantations

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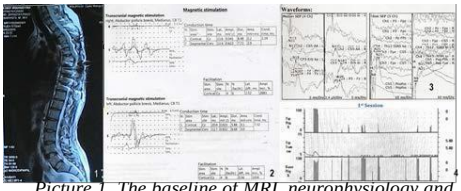
INTRODUCTION: Decompression of involved cord and stabilization of adjacent vertebrae has been the mainstay of spinal cord injury treatment. However, this strategy alone does not address the neurologic sequel that lead to poorer patient's quality of life, one of which is erectile and ejaculatory dysfunction. Mesenchymal stem cells has been known to have regenerative ability in a wide variety of tissues, included nerve tissue. Clinical studies on human have also been conducted in small basis, and so far has shown equivocal results. This paper will report the evaluation of erectile and ejaculatory function of patient with spinal cord injury, and before and after the implantation of mesenchymal stem cell.



Picture 1. The baseline of MRI, neurophysiology and Rigiscan result

Evaluation	Baseline	1 month follow up	3 month follow up
Sensory function (SSEP)	Latency of Tibialis nerve P38 disapear	N/A	Latency of tibialis nerve P38 longer
Motor function (MEP)	Central interneuron activity in right side is lower than left side	N/A	Central interneuron activity in right and left side is moderate
Ejaculation function	No ejaculation	No ejaculation	Normal ejaculation

METHODS: Thirty seven-years-old male with paraplegia as well as erectile and ejaculatory dysfunction for six years due to spinal cord injury (SCI), and since then has undergone two surgery without any improvement on his clinical symptoms. He got stem cell treatment with RSCM protocol. Every cycle contained three consecutive injections with interval of 2 weeks. The first injection administered $10x10^6$ cells via intravenous route, $16x10^6$ cells via intrathecal route, and $16x10^6$ cells via intralesional route. The second and third injections administered $16x10^6$ cells by intralesional route. The evaluations of sensory and motoric function was done by clinical and physical examination using ASIA Scoring System, Somatosensory Evoked Potential examination (SSEP), Motoric Evoked Potential examination (MEP). The patient was also evaluated his erectile and ejaculation function by Rigiscan examination.

RESULT: At baseline, the patient had no sensory or motor function at the level of Th7 and below. The patient was also examined for SSEP, which found a total functional lesion of dorsal horn of spinal cord at both sides of C7-Th12 vertebrae. At one month follow-up, the patient reported an improvement on his erectile and ejaculatory function; he can erect better and ejaculate normally. There were also sensory and motor improvement: Normal sensory function was found at dermatome of the level of Th7-8 and hypoesthesia was found at dermatome of the level of Th9 and below. The patient also reported more rigid erection and normal ejaculation. The patient will be scheduled for rigiscan at 6-month followup.

DISCUSSION AND CONCLUSION: It has been known that sexual function in different stages after SCI and the types of erections depend mainly on the completeness of the injury and the level of neurological damage. For complete SCI, both erectile and ejaculatory problems was mainly associated with neurologic injury that causes autonomic control dysfunction as well as inadequate brain stimulus toward effector side.[1] Furthermore, most of the SCI men demonstrate defects concerning the entrance of semen into the posterior urethra and the expulsion of the semen through the penile urethra and the urethral orifice, rendered the patient susceptible to retrograde ejaculation.[2]

An array of new and promising strategies is being developed to improve function after SCI. At present, two main therapeutic strategies, cell-based and gene-based therapies are being investigated. In this present case, the patient was treated with a regime of mesenchymal stem cell implantations, and uniquely resulted in immediate improvement on both erectile and ejaculatory manifestations. Normally, erection was mediated via sacral pathways in the sacral spinal cord and involve the parasympathetic nervous system.[3] In our knowledge, there was still no published article describing the recovery of sexual function of SCI patients. We hypothesized that the prompt recovery of erectile and ejaculatory functions in our patients was due to central healing of spinal cord that involves sacral parasympathetic pathway.

P061 Study on photo-patterning gelatin scaffold to mesenchymal stem cell growth

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INTRODUCTION: Gelatin is considered as a type of polymer used to fabricate bio degradable scaffold for tissue engineering. However, gelatin is developed from the toxic glutaraldehyde solution. Nowadays, a photo-induced crosslinking method has been developed in scaffold fabrication to create a more pleasant environment for cell growth. Rose bengal (Aldrich), a new type of non-toxic crosslink agent, has reported to form gelatin into gel. For further application to human body, toxicity and biocompatibility test are needed to evaluate this biomaterial.

METHODS: Gelatin-based scaffold with various consentrations starting from 1%, 2 %, 3%, 4%, and 5% were inserted into an adipose derived mesenchymal stem cells (MSCs) - filled wells and observed until the 6th day

RESULT: There were no difference on MSCs morphology between those who cultured with or without scaffold. Based on microscopic observation on the 2nd and 6th day, MSCs proliferation on the well's surface under scaffold was faster than those either in control well or in the same well outside scaffold area. Scaffold with 1%, 3%, and 4% gelatin content didn't show proliferation inhibition (proliferation inhibition less than 50%)

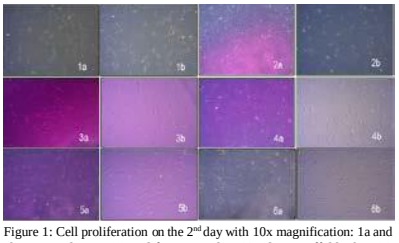

Figure 1: Cell proliferation on the 2nd day with 10x magnification: 1a and 1b as control; 2a MSC proliferation under 1% gelatin scaffold; 2b MSC proliferation outside the area of 1% gelatin scaffold; 3a MSC proliferation under 2% gelatin scaffold; 3b MSC proliferation outside the area of 2% gelatin scaffold; 4a MSC proliferation under 3% gelatin scaffold; 4b MSC proliferation outside the area of 3% gelatin scaffold; 5a MSC proliferation under 4% gelatin scaffold; 5b MSC proliferation outside the area of 4% gelatin scaffold; 6a MSC proliferation under 5% gelatin scaffold; 6b MSC proliferation outside the area of 5% gelatin scaffold.

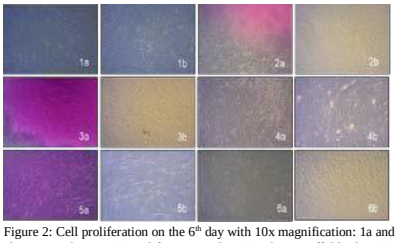

Figure 2: Cell proliferation on the 6th day with 10x magnification: 1a and 1b as control; 2a MSC proliferation under 1% gelatin scaffold; 2b MSC proliferation outside the area of 1% gelatin scaffold; 3a MSC proliferation under 2% gelatin scaffold; 3b MSC proliferation outside the area of 2% gelatin scaffold; 4a MSC proliferation under 3% gelatin scaffold; 4b MSC proliferation outside the area of 3% gelatin scaffold; 5a MSC proliferation under 4% gelatin scaffold; 5b MSC proliferation outside the area of 4% gelatin scaffold; 6a MSC proliferation under 5% gelatin scaffold; 6b MSC proliferation outside the area of 5% gelatin scaffold.

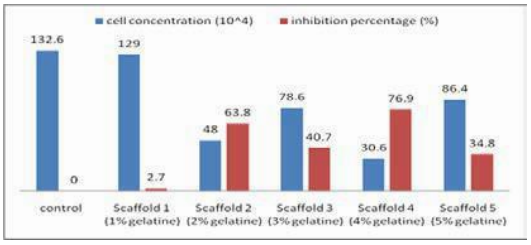

Figure 3. Gelatin concentration influence to MSCs' growth on the 6th day observation

DISCUSSION AND CONCLUSION: Various gelatin concentration on each scaffold showed effects on MSCs proliferation. Scaffold number 5 with 1% gelatin concentration showed the least inhibitory effect to MSCs proliferation.

P062 Controlling GDF6 delivery via PLGA microspheres to direct adipose-derived stem cell differentiation for intervertebral disc regeneration

Tom Hodgkinson[1], Lisa White[2], Kevin Shakesheff[2], Judith Hoyland[1], Stephen Richardson[1]

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During intervertebral disc (IVD) degeneration, the leading cause of back pain, the cells of the nucleus pulposus (NP) display changes in gene expression and extracellular matrix (ECM) secretion, leading to matrix degradation and loss of tissue integrity. Stem cell therapy has been suggested as a potential regenerative strategy to treat IVD degeneration and GDF6 has been shown to differentiate adipose-derived stem cells (ASCs) into an NP-like phenotype. However, for clinical translation, a delivery system is required to ensure controlled and sustained GDF6 release. This study aimed to investigate the encapsulation of GDF6 inside novel PLGA-PEG-PLGA microparticles (MPs) to control delivery and assess the effect of the released GDF6 on NP-like differentiation of human ASCs.

GDF6 was loaded into PLGA-PEG-PLGA microspheres at 1 mg ml^{-1} GDF6 and controlled release over 14 days determined using BCA assay and GDF6 ELISA. The effect of MP loading density on collagen gel formation was assessed through scanning electron microscopy (SEM) and histological staining. ASCs (N=3) were cultured in collagen hydrogels for 14 days with GDF6 delivered exogenously or via microspheres. ASC differentiation was assessed by qPCR for NP markers (Keratin-8, -18, -19, FOXF1, brachyury, CAXII) and sGAG production was assessed through histological staining and quantitative content analysis (DMMB).

GDF6 release from microspheres was controlled over 14 days *in vitro* and was demonstrated to be equivalent to concentrations added exogenously. SEM and histological analysis confirmed that MPs were distributed throughout gels and that gel formation was not disrupted by loading at relevant concentrations. GDF6 released from microspheres induced upregulation of all NP marker genes in ASCs in a comparable manner to exogenous GDF6. Furthermore, histological staining and quantitative DMMB assays revealed GDF6 released from microspheres up-regulated the production of sGAG by cells in constructs in comparison to unstimulated controls.

In 3D collagen gel cultures, GDF6 release from microspheres elicited equivalent ASC differentiation and NP-like matrix formation compared to that following exogenous delivery of GDF6 to media, indicating activity was not affected by microsphere encapsulation.

This study demonstrates the effective encapsulation and controlled delivery of GDF6, which was able to maintain its activity and induce ASC differentiation into an NP-like phenotype and production of an NP-like ECM. Delivery of GDF6 microspheres in combination with ASCs is a promising strategy for IVD regeneration and treatment of back pain.

P063 Micromolding method to prepare mesenchymal stem cells-containing alginate particles for intra-articular injection in osteoarthritis

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Osteoarthritis (OA) is a degenerative and inflammatory joint disease that affects cartilage, subchondral bone and joint tissues. Mesenchymal Stem Cells (MSCs) ability to secrete anti-inflammatory and immuno-modulatory factors represents an attractive tool in the treatment of osteoarthitis. Considering the risk of cell leakage and the massive cell death upon intra-articular injection, MSCs encapsulation therefore could prevent cell death, avoid cell effusion outside the articular space, and supply a suitable micro-environment supporting MSCs biological activity. Previously, we have demonstrated that alginate particles support MSCs viability and bioactivity. Nevertheless these particles obtained through a dropwise method had an average size of 1.5 ± 0.2m mm and were thus too large to be injected into joints. Here, we propose to develop a method of cell encapsulation in alginate compatible with intra-articular injection through a 26G needle.

Alginate particles were generated through micromolding method. We first manufactured photoresist molds on silicon wafers by photolithography. These molds had protruding 100 µm high patterns with square and circle shapes of 100, 150 and 200 µm of side or diameter. Polydimethylsiloxane (PDMS) molds were then generated by pouring PDMS containing 10% of curing agent onto the photoresist molds and curing it for 20 min at 70°C. Finally, the PDMS molds were peeled from the wafers and stored 1 night at 60°C. Before their use, the molds were hydrophilized by plasma treatment. A sterile 2% alginate solution was then deposited on the molds and alginate particles were crosslinked with CaCl2. After manufacture, microparticle size and shape were assessed using phase-contrast microscopy and digital imaging. To encapsulate cells, human adipose-derived mesenchymal stem cells (ASC) were suspended at 1 and 3 millions of cells per mL of a sterile 2% alginate solution and deposited on PDMS molds containing 3200 circle wells with a diameter of 200 µm. Number of encapsulated cells were then determined by a PicoGreen assay.

We obtained alginate microparticles reproducing the micromold shapes. However, both cylindrical and cubic particles exhibited a reduced size as compared to the original molds. For instance, cylindrical particles molded into 100, 150 and 200 µm cycle molds had an average size of 73 ± 0.8, 103 ± 0.7 and 136 ± 0.7 µm, respectively (n=3). Similarly, for cubic particles, we measured a size of 72 ± 0.7, 107 ± 0.7 and 137 ± 0.8 µm (n=3) instead of 100, 150 and 200 µm. The presence of cells into the alginate solution had no impact on the size or shape of the particles. Preliminary results indicate that the number of encapsulated cells by particle depends on the initial cell concentration in the alginate solution.

To conclude, we succeeded in generating alginate particles that could be injected through a 26 G needle. In addition, micromolding-derived particles of alginate allowed the encapsulation of viable MSCs. Our work will now focus on demonstrating the ability of encapsulated MSC to secrete immunomodulatory and anti-inflammatory factors. In a future step, we will consider intra-articular injection in an animal model of osteoarthritis.

P064 Development and characterization of polymer-based nano-complexes for cartilage regeneration

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The repair of damaged articular cartilage, which rarely heals spontaneously and develops osteoarthritic complications, is a common clinical issue[1] and none of the available therapies can restore the hyaline cartilage surface beyond just fibrous repair. Over the last few years, polymer-based nanoparticles (NPs) have been in the limelight as a system for the encapsulation and delivery of drugs or active biomolecules, for the treatment of chronic wounds. Water-soluble, biodegradable, polyelectrolyte NPs offer numerous advantages over the conventional delivery forms, particularly so regarding the possibility to tune surface properties, high versatility to control the release profiles of loaded molecules and to interact with membrane receptors of cells, improved efficacy and patient compliance, and reduced toxicity. Polysaccharide-based NPs have, hitherto, been applied in the field of tissue engineering to enhance tissue regeneration by sustained and localized release of appropriate biological molecules, incorporated within the biodegradable nanostructure[2]. Starting from this point, this project is mainly focused on the development and characterization of polymer-based nano-complexes, as a first step in the fulfillment of an injectable composite material for cartilage regeneration. Among different polymers, hyaluronan (HA), Chitosan and its derivatives seem to be very promising for their structural and well established biological role. The two differently charged polymers were used for the preparation of a binary mixture solution by mixing different amounts of a HA solution and a Chitosan/derivatives solution, respectively, hence obtaining different HA weight fractions. The different formulations were prepared in the presence of different concentrations of NaCl to study the influence of ionic strength on the formation of complexes; the variation of pH and the used of HA with higher molecular weight were also investigated to find the best condition for NPs preparation. First, the Transmittance (*T*) of the different solutions was measured; then the same solutions were investigated by Dynamic Light Scattering (DLS) for the evaluation of Z-average size, zeta-potential, polydispersity index. After that, the best formulation was selected to evaluate the stability of complexes, first in water, and then changing the ionic strength and the pH up to physiological conditions. Overall, these nano-complexes appear as promising biomaterials with interesting biological and physical properties for cartilage regeneration.

P065 Disulfide containing microparticles for the delivery of cells and proteins

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Introduction: Microparticles have gained increasing popularity in the field of tissue engineering for the delivery of cells and proteins. Their potential formulations are bountiful, with the possibility of being coated with or encapsulating biologically active molecules, attached or embedded within scaffolds or injected directly to injured sites. Furthermore, they are tunable regarding size and uniformity, resulting in tight control over the bioavailability of the therapeutic molecule for delivery. However, one limitation is the lack of functional groups present on biologically "friendly" and approved polymers for the formulation of microparticles, which hinders the conjugation of biologically active molecules. The aim of this study was to design and characterise a novel polymer for the synthesis of uniform microparticles incorporating a disulfide bond that can be used for the bioconjugation of molecules of interest via thiol/Maleimide chemistry. We further investigated the cytotoxicity of these microparticles using GFP equine tenocytes.

Materials and Methods: Disulfide containing polycaprolactone was produced using ring opening polymerization of ε-caprolactone initiated with 2-Hydroxyethyl disulfide, employing Tin(II) as a catalyst. The resulting polymer product was fully characterised by GPC and NMR and then used for the production of Microparticles produced using membrane emulsification.

To test cytotoxicity cells were cultured with fabricated microparticles in supplemented DMEM. Moreover, a live/dead assay employing trypan blue and fluorescent microscopy was used to visualise live cells.

Results: Membrane emulsification allowed for the rapid and facile production of microparticles. Microparticles were found to have a good degree of uniformity and an average particle size between 20-25µm. The cytotoxicity data showed that there was no reduction in cell viability after culture with disulfide microparticles after 24 hrs incubation. Fluorescent microscopy showed the presence of viable cells at all concentrations of microparticles.

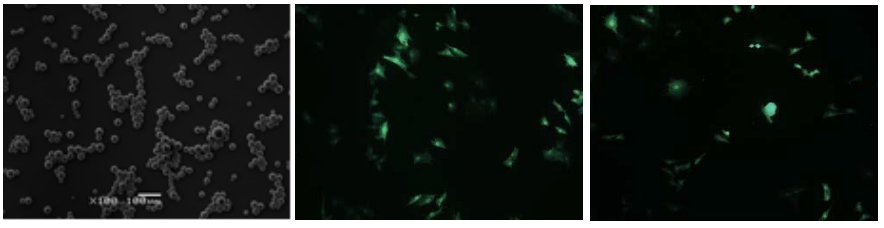

Figure 1: SEM images of disulfide containing microparticles produced using O/W membrane emulsification (left), Fluorescent microscopy of GFP equine tenocytes 24 hours after incubation with microparticles at concentrations ranging from 1mg/ml (middle) to 30mg/ml (right)

Discussion and Conclusions: Here we show the successful production of microparticles with controlled size and uniformity with the potential for conjugation and immobilisation of small molecules on the surface using disulfides present in the polymer and thiol Maleimide chemistry. We demonstrate that there is limited toxicity of these particles to equine tenocytes.

Acknowledgements: The EPSRC funded this research.

Disclosures: The authors have nothing to disclose.

P066 Development of self-assembling peptide hydrogels to release triamcinolone acetonide for the management of oesophageal strictures

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Introduction

Surgical treatment of Barrett's oesophagus is efficacious at removing pre-cancerous tissue. However, there is a 50-70% chance of oesophageal strictures (post-operatively), which is an unintended side effect resulting in severe discomfort in patients. Stricture formation is due to rapid, uncontrolled inflammatory responses and fibrotic tissue formation [1]. Whilst local delivery of steroids *via* injection reduces stricture rate, side effects include risk of ulcer formation, delayed wound healing due to the mode of delivery and adverse systemic effects [2].

In this study we have investigated an alternative mode of releasing the clinically relevant steroid, triamcinolone acetonide (TA), at therapeutic doses from a panel of different self-assembling peptide hydrogels with tailored properties. This family of peptide hydrogels are fully synthetic and have already been shown to be muco-adhesive, sprayable, injectable [3] and non-inflammatory [4].

A panel of peptide hydrogels with a diversity of properties were investigated to determine the differential release profiles of TA. We also explored the effect of simulated Barrett's conditions (media containing bile salts and acidic pH) on the rheological properties of hydrogels and the release profiles of TA.

Methods

TA (50mg/mL) was physically mixed into pre-prepared hydrogels. Release of TA from hydrogels was measured every 24 hours using UV spectroscopy (251nm wavelength). Hydrogels were exposed to either normal media or media containing the bile salt, Taurochenodeoxycholate, and acidic conditions for 10 minutes every am and pm. Rheological properties of hydrogels were measured using oscillatory rheometry.

Results

Differential release profiles were observed from the different self-assembled hydrogels investigated. Sustained release was observed from all hydrogels; but rate of initial release was different between them. Addition of TA was shown to have no effect on the rheological properties of the gels. Drug release profiles and rheological properties were minimally affected by exposure to conditions mimicking Barrett's oesophagus.

Impact

In vitro release of TA from self-assembling peptide hydrogels has been shown. By controlling the hydrogel design and therefore the physical properties of the materials the release profile of the drug could be controlled. *In vitro* investigations into the efficacy of modulating inflammatory responses are on-going. These hydrogels have been shown to hold significant promise as a sprayable and/or injectable, topical release vehicle.

P068 Autologous tissue expansion in a 3d-construct in vitro and in vivo during one single surgical procedure

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Introduction:

Cultured autologous cells can be transplanted as a method to restore tissue, but is associated with several drawbacks such as high costs and requires advanced culturing facilities and personnel. By using our own body as a bioreactor, *In vivo* expansion of tissue could be an alternative solution. The aim of this study was to evaluate a 3-dimensional scaffold with high tensile strength and assess the possibility of expanding autologous epithelium in one single surgical procedure.

Materials and Methods:

Two slabs of collagen type I gels including a core of polycaprolactone (PCL) knitted fabric was placed in a mould with minced skin on top. Plastic compression was carried out by a technique previously described. The final construct was assessed *in vitro* and later subcutaneously in a rat model *in vivo*. Constructs were analysed up to 4 weeks after transplantation in respect to morphology, histology and mechanical properties.

Results:

We showed successful keratinocyte proliferation on top of the scaffold *in vitro* and *in vivo*. All samples where integrated in the surrounding tissue with capillary formation within the construct and reorganization of the collagen already after 5 days and where fully integrated in the surrounding tissue after 4 weeks. The construct kept its integrity, did not lose its high tensile strength and kept 61 % of its elasticity during the study period.

Conclusions:

By adding minced tissue to a collagen gel including a PCL-knitted fabric, cell expansion and re-organization of epithelium could take place without the need for conventional *in vitro* cell culturing. Our 3-dimensional scaffold can be handled surgically, has high tensile strength, high elasticity and could be used during a one-staged procedure in an ordinary surgical unit for tissue expansion. It also has the potential to be implemented for bladder augmentation.

P069 Controlled delivery and release of NGF loaded carriers as a potential Alzheimer disease treatment

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The Nerve growth factor (NGF) is a well-characterized protein and an essential contributor to neuronal differentiation. NGF has shown an immense pharmacological potential in treating several models of neurodegenerative diseases, such as Alzheimer disease (AD). Previous studies revealed that NGF is highly effective in the treatment of AD, as the protein stimulate survival and functionality of the cells and thus reduces cell degeneration and induces regenerative and neuroprotective effects. However, growth factors undergo rapid degradation, which leads to a short biological half-life, limiting their effectiveness as therapeutic agents. Recently, we have designed and fabricated highly-porous drug carriers based on nanostructured silicon. Our porous silicon (PSi) delivery system allows high loading efficacy of NGF and a sustained and prolonged release of the protein. Nanostructured PSi is characterized by several particularly appealing properties predestining it for design of tunable drug delivery systems, including high surface area, biocompatibility and degradability in a physiological environment. Different PSi nanostructures have been fabricated by anodic electrochemical etching of single-crystalline Si wafers and the synthesis conditions were adjusted to allow efficient loading of NGF by physical adsorption. The NGF release has been profiled demonstrating a sustained release for period of a month. We show that NGF entrapment within the PSi maintains its bioactivity and induces neurite outgrowth and profound differentiation (in several cell lines and culture) throughout the period of release within a single administration. This proof-of-concept leads us to study the therapeutic effects of our novel system on AD models. We aim to introduce the NGF loaded PSi therapeutic carriers using a biolistic-based approach to cell culture and tissue. Biolistics has emerged in recent years as a promising non-invasive route for delivering payloads into tissues for therapeutics and regeneration (Simonnet et al., 2005; Shefi et al., 2006; Zilony et al.,2013). This particle-mediated delivery was shown to be effective for delivery into target cells in internal layers of tissue, insensitive to the intrinsic permeability of the cell. Thus, it may open new possibilities in the neuro-regenerative medicine treatment.

P070 Chitosan-based medical device for improving functional recovery after radical prostatectomy: in vitro study

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Prostatic cancer is the most frequent cancer in males. Whereas the progress in early cancer detection and surgical removal has made significant improvement in patient survival, erectile disfunction often results after radical prostatectomy due to damage of the peri-prostatic nerve bundles.

The objective of this experimental work was to conduct an in vitro study with the aim to assess the regenerative and anti-cancer properties of a biomedical device consisting of chitosan (CS), which has already achieved a clinical use for the peri-prostatic nerve plexus protection and a patent (Application reference: 102016000070911).

CS is a biomimetic material of natural origin which has been proven to exert a pro-regenerative action on nerves. The BIOHYBRID project already succeeded to bring to the clinic a tubular medical device for the successful treatment of injuries to somaticnerves.

The **regenerative potential** of CS films was assessed through primary neuronal cultures derived from different ganglia: giugular and nodose ganglia originating from the vagus nerve, as model for the parasympathetic segment, stellate ganglion (cervicothoracic ganglion), as model for sympathetic ganglion and dorsal root ganglia located along the dorsal spinal roots which allowed to study the regenerative process on sensory somatic neurons. Different parameters have been tested: cell survival, neurite outgrowth and cell differentiation, proving that the material represents a permissive substrate for neurite regeneration.

As regarding **CS-anti-tumoral properties**, different human prostate cancer cell lines (PC-3, DU145, LNCap) were seeded on CS films and evaluated in terms of cell proliferation and morphology. The analysis conducted, after 1-3-5 days from plating, has allowed to demonstrate that the direct contact of the cell lines with the CS substrate determines not only a substantial change in morphology, but also a significant decrease in proliferation.

In the context of prostatic cancer therapy, erectile disfunction is one of the consequences which most undermine the quality of postoperative life. The application of new techniques and new materials in the field of regeneration of peripheral nerve fibers would result in minor inconvenience for patients and allow to extend the treatment also for other applications inoncology.

P071 Controlled delivery of recombinant neurotrophic factors from a nerve guidance conduit for enhanced peripheral nerve repair

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Tissue engineered nerve guidance conduits (NGCs) have shown promising results *in vivo* for peripheral nerve repair but their clinical application remains limited to the repair of small gaps (< 15 mm). We have previously developed a tubular collagen-based NGC and have demonstrated its capacity to functionally and morphologically repair 10 mm sciatic nerve injury in rats (1). However, its therapeutic efficacy might be improved for the repair of larger injuries by incorporating recombinant neurotrophic factors. Previous work in our group has successfully enhanced the therapeutic efficacy of collagen-based scaffolds for bone repair by the incorporation of recombinant growth factors (2). Building on this work, the aim of this study was to investigate the potential of the collagen-based NGC by the incorporation of recombinant neurotrophic factors. Specifically, nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF) were investigated due to their capacity to enhance axonal outgrowth and Schwann cell migration, and their potentially synergistic effect on nerve repair when used in combination.

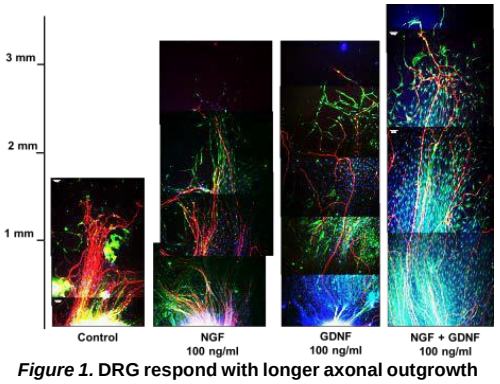

Figure 1. **DRG respond with longer axonal outgrowth (red) and further Schwann cell migration (green) to a combination of NGF and GDNF.**

NGF and GDNF were found to modulate differentiation of neural progenitor cells (NE4C cell line), and cytokine production, of ciliary neurotrophic factor and myelin associated glycoprotein, in Schwann cells (S42 cell line), in a synergistic and dose-dependent manner. The optimal dose of neurotrophic factors was determined using adult dorsal root ganglia explants (DRG), with a concomitant and delayed presence of NGF and GDNF in a 1:1 ratio rendering the longest axonal growth and furthest Schwann cell migration *(Fig. 1*). To control their release, poly(lactic-co-glycolic acid) (PLGA) microparticles were successfully fabricated in this study with a 68.5±6.5% neurotrophic factor encapsulation efficiency. PLGA microparticles encapsulating NGF and GDNF were incorporated into the NGC by soak-loading, resulting in controlled release of both neurotrophic factors over 28 days whilst retaining their synergistic bioactivity, confirmed by their capacity to induce the differentiation of neural progenitor cells.

In summary, controlled release of NGF and GDNF has the potential to improve the therapeutic efficacy of NGCs in a synergistic and dose-dependent manner, potentially providing an enhanced treatment for the repair of large peripheral nerve injury.

This research project has been funded by the Irish Research Council (GOIPG/2013/177).

P072 A gene-activated guidance conduit for peripheral nerve repair

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Peripheral nerve injuries have high incidence rates and autografts remain the gold standard treatment for the repair of large gaps (> 30 mm). However, this treatment is associated with donor site morbidity and neuroma formation. Tissue engineering nerve guidance conduits (NGCs) offer a potential alternative but are currently limited to the repair of small gaps (< 15 mm). We previously developed a tubular collagen-based NGC in our lab, and have demonstrated its capacity to support the functional and morphological repair of 10 mm sciatic nerve injury in rats (1). However, its therapeutic efficacy could be improved for the repair of larger gaps by incorporating genes encoding for therapeutic proteins. Previous work in our group has led to the development of a series of gene-activated scaffolds capable of localised gene delivery, showing success in bone and cartilage applications previously (2,3). Building on this work, the aim of this study was to develop a gene-activated conduit for peripheral nerve repair.

In this study, a non-viral cationic polymer gene delivery vector, polyethyleneimine (PEI), was used to optimize PEI-DNA complexes for gene delivery to Schwann cells (S42 cell line) and neural progenitor cell (PC12 cell line) using reporter genes encoding green fluorescent protein and Luciferase. Optimized complexes were capable of transfecting Schwann cells with 43% efficiency as reported by flow cytometry, while a transient gene expression profile lasting up to 28 days was identified in both 2D culture and in the NGC. Metabolic activity and DNA quantification assays showed minimal cytotoxicity. The optimized formulation of PEI-DNA at a charge ratio of 7 carrying 2 µg dose of DNA was then used to deliver genes encoding for therapeutic proteins nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF), which promote neuronal and Schwann cell growth in addition to cJun, a transcription factor involved in Schwann cell reprogramming. Transfection with these genes led to differentiation of neural progenitor cells, enhanced cytokine production by Schwann cells, with no polymer-associated cytotoxicity. Interestingly, cells transfected with cJun were capable of axonal outgrowth themselves but also induced axonal outgrowth in neighbouring non-transfected cells, indicating that transfection with cJun can have powerful autocrine and paracrine effects. Adult dorsal root ganglia explants transfected with c-Jun responded with enhanced Schwann cell migration and axonal outgrowth, compared to transfections with NGF or GDNF.

In summary, this study has led to the development of a gene-activated NGC capable of transient therapeutic gene delivery. The results highlight the potential use of therapeutic genes as bioactive molecules to enhance the regenerative capacity of NGCs for the repair of large nerve injuries.

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P073 Biomimetic apatite and activated iridium oxide formed on titanium as a protein carrier to study NSC behaviours

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Common methods for culture and expansion of neural stem cells (NSCs) are preparing engineered substrates with extracellular matrix (ECM) peptides/proteins for improving cell-matrix interactions. However, several studies demonstrate that substrates immobilized with laminin (LN) or poly-D-lysine present cell detachment or retraction due to burst release of immobilized molecules. In our study, we formed biomimetic apatite and activated iridium oxide on titanium as protein carrier to enhance protein retention, thus improving NSC behaviours.

For biomimetic apatite/LN, calcium phosphate coated titanium disc was immersed in DPBS solution containing LN to coprecipitate LN with apatite. For activated iridium oxide/LN, concentrated LN solution was dropped on activated iridium oxide coated titanium in clean bench. The water was allowed to evaporate leaving behind a residue of LN. PC12 and NSCs were used to evaluate the cellular responses to biomimetic apatite/LN and activated iridium oxide/LN in vitro. The cell attachment, cytotoxicity and proliferation were examined. The biomimetic apatite and activated iridium oxide retained LN at least for 7 days. The biomimetic apatite/LN and activated iridium oxide/LN facilitated attachment, biocompatibility and proliferation of PC12 and NSCs.

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P074 Bioactive collagen nerve conduit scaffolds for neural tissue engineering applications

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Peripheral nerve injuries affect more than one million people each year and often result in life-long disabilities due to lack of efficient therapeutic options. Currently available treatment options are associated with several drawbac. For addressing some of these problems, we have developed dual functional collagen nerve conduits (C-NCs) for triggering axonal regeneration in a rabbit sciatic nerve gap (> 40 mm) model. Dual functionality consisted in an embedded growth factor (GDNF) and aligned microfibers in the C-NC

GDNF, a potent growth factor for motor neurons, was engineered with collagen binding domain (CBD-GDNF) and expressed in eukaryotic cell. Resulting recombinant growth factor was loaded into C-NCs reinforced with aligned PLGA microfibers. As a control, unmodified GDNF was loaded into C-NCs. In vitro release kinetics was studied over 28 days followed by bioactivity assay of the released growth factors. Subsequently, biological performance of dual functional NC was tested *in vitro* using chicken embryonic motor neurons. Bioactive C-NC were further optimized for testing their biological performance in a rabbit sciatic nerve gap model. In the line of establishing this model, PFA perfused rabbit lower limbs were used for anatomical dissection and localisation of the sciatic nerve. Surgical procedures for operating the sciatic nerve were reviewed and key requirements were identified.

C-NC scaffolds showed sustained release for both GDNF and CBD-GDNF over 28 days. Interestingly, CBD-GDNF mediated slow and low release with significantly reduced initial burst release when compared to native GDNF. Bioactivity of released growth factor was maintained throughout the entire release period as demonstrated by neuronal differentiation of Neuro-2A cells treated with released growth factors. Topography of the microstructured PLGA scaffold in combination with bioactive stimulus determined the direction and extent of axonal outgrowth from spinal cord motor neurons *in vitro*. As desired, axonal outgrowth was perfectly (99%) in line with the aligned fibers. In case of randomly oriented fibers, axonal growth was scattered in all directions. The latter limitation seems to be a major hurdle for target oriented regeneration.

C-N C endowed with CBD-GDNF and aligned PLGA microfibers augmented axonal outgrowth and determined the direction. Thus, the developed device appeared to promote nerve regeneration over longer distance. Experiments are under progress to elucidate its biological performance in a rabbit sciatic nerve gap model.

P076 Collagen-fibrin engineered neural tissue for peripheral nerve regeneration

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Peripheral nerve injuries affect millions of people every year. Tissue engineering approaches in nerve regeneration aim to replace the gold standard therapy (autologous nerve grafts) and to improve results by bridging nerve defects with different kinds of conduits. In our study we present a simple and effective tissue engineering method for building an aligned cellular collagen-fibrin blend construct, promoting nerve repair. Collagen is the predominant ECM protein in normal nerve tissue and has been used previously to build cellular constructs to support nerve regeneration [1,2]. However, fibrin is a key ECM component found in Bands of Büngner at sites of nerve repair and is associated with induction of a pro-regenerative phenotype in Schwann cells [3]. The aim of this study was to enhance the regenerative performance of engineered neural tissue (EngNT) through the use of a novel matrix blend of fibrin and collagen. Results demonstrated that SCL4.1/F7 Schwann cells self-aligned within a tethered collagen/fibrin blend gel and, after removal of interstitial fluid, this resulted in a stable, tissue-like scaffold. In vitro studies showed that fibrin/collagen blend constructs promoted cell viability of SCL4.1/F7 Schwann cells as well as neurite outgrowth (NG108 cells). Initial *in vivo* tests in an 8 mm rat sciatic nerve model using rolled collagen/fibrin EngNT rods revealed significantly more axons in the mid-section of the tube and in the distal part of the nerve, compared to EngNT rods made from collagen alone. Summarizing, the established collagen/fibrin blend EngNT shows promising results as a novel luminal filler for peripheral nerve repair.

P077 Chitosan degradation products facilitates peripheral nerve regeneration

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Aims: Chitosan based artificial nerve grafts not only provide a good channel for nerve regeneration; its degradation products-chitooligosaccharides (COS) can also promote nerve regeneration, but the involved mechanism is still not very clear. This study is to provide the further experimental basis for mechanism study of chitosan based artificial nerve grafts facilitating nerve regeneration.

Methods: Chitosan based artificial nerve grafts were used to repair a 10mm sciatic nerve defect in rats, and the nerve defect was also bridged with silicone tube filled with COS or saline. Immunohistochemistry were performed to assess the nerve regeneration. The levels of IL-1α, IL-1β, IL- 6, IL-10, TNF-α and TGF-β were measured with a sandwich ELISA kit. HILIC analysis were conducted post-operation.

Results: Immunohistochemistry experiments revealed that COS stimulate Schwann cell (SC) proliferation during nerve regeneration. The proliferative effects of COS on SCs were further validated in the primary cultured SCs treated with COS. It was also found that the levels of inflammatory cytokines especially IL-6 and TNF-α changes quickly at the injury site, which was accompaniedby recruitment and disappearance of a large number of macrophages. This evidence suggests that chitosan degradation products--COS may be involved in the reconstruction of the microenvironments at the injury site by affecting inflammation status.

Impact of the investigation:

Collectively, our results revealed that COS promotes peripheral nerve regeneration by promoting SCs proliferation and improving microenvironments at injury site, providing a theoretical basis for the clinical application of chitosan based grafts in the future.

P078 Combination of hyaluronic acid hydrogel and wharton's jelly-derived mesenchymal stem cells for spinal cord injury repair

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Aims: Hydrogel scaffolds, which bridge the lesion, together with stem cell therapy represent a promising approach for treatment of spinal cord injury (SCI). Hyaluronic acid (HA) is widely used in tissue repair due to its biocompatibility, biodegradability and anti-inflammatory properties. In this study, we evaluated the neuroregenerative potential of HA hydrogel in combination with human Wharton's jelly-derived mesenchymal stem cells (hWJ-MSCs) in acute and subacute models of SCI.

Methods: Hydroxyphenyl derivatives of HA were modified with the integrin binding peptide RGD and crosslinked with horseradish peroxidase and hydrogen peroxide to form a soft hydrogel. HA hydrogel alone or seeded with hWJ-MSCs was applied into acute or subacute spinal cord hemisections. Animals were behaviourally tested and tissue regeneration was evaluated after 4, 8 and 16 weeks using histological and real-time qPCR analysis.

Results: Both injected and implanted HA hydrogels matched well with the surrounding tissue and filled the lesion cavity with no signs of adverse effects. The same axonal density as well as number of blood vessels were found after acute implantation or injection of the hydrogel. Subacute injection of HA hydrogel improved axonal ingrowth, and combination of the hydrogel and hWJ-MSCs further enhanced tissue regeneration. On the other hand, hydrogel treatment had no effect on the density of glial scar which was formed around the lesion as well as on the size of the lesion cavity when compared to the control empty lesion. Subacute injection of HA hydrogel with hWJ-MSCs increased expression of genes related to axonal growth and macrophages markers.

Conclusions: RGD-modified HA hydrogel promoted axonal sprouting, vascularisation as well as tissue bridging after SCI and represents a promising material and cell carrier for SCI repair.

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P079 The assesment of expression of ectoderm, mesoderm and endoderm markers in embyoid body-like cell aggregates formed from wharton's jelly mesenchymal stem cells using in regenerative medicine

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Regenerative medicine is an area of medicine with the potential to heal damaged tissues and organs. Stem cells have the ability of differentiation into many different types of cells and are a key component of regenerative medicine, as a new clinical application. Mesenchymal stem cells (MSC) isolated from Human umbilical cord Wharton's jelly (HUCWJ) have been shown to be able to differentiate into various cell types. As they are readily available, do not raise any ethical issues and showed higher differentiation potential compared to adult stem cells. Therefore, HUCWJ is a potential source of material that can be used in regeneration medicine. The objective of this study was to find if these cells could form cell aggregates similar to that formed by ESCs (embroid body-like and form three germ layers.

The Umbilical Cords were cut into small pieces and the explants were cultured in the presence of α-MEM containing 10% fetal bovine serum (FBS), 1% L-glutamine, 100 g/mL penicillin/ streptomycin. At passage 3rd, 1000, 5000 and 10.000 cells/ 20µL were cultured in hanging drops for 3 days. Then, they were incubated for additional 3 days in non-adhesive dishes. The cell aggregates were fixed by 4% paraformaldehyde and were incubated with human three germ layer, 3 color antibodies and the flowcytometry was done.

The data showed that the embryoid-body-like aggregates had little expression for ectoderm and endoderm markers and much expression for mesoderm markers.

These aggregates stay at the mesenchymal cell mass manner and showed a poor differentiation potential toward the ectoderm and endoderm.

P080 Nichoid substrates promote expansion of adult stem cells in the absence of animal-derived components

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Background: Stem cells-based therapies represent a promising strategy for the treatment of a variety of diseases because of trophic, immunomodulatory and regenerative properties of stem cells. However, the therapeutic applications require high numbers of stem cells and an accurate control of their differentiation during cell expansion. The most common protocols for in vitro culture of stem cells are conducted on bi-dimensional (2D) supports, with the addition of fetal bovine serum (FBS) in the culture medium. However, flat culture substrates don't resemble the three-dimensional (3D) environment to which stem cells are exposed to within their physiological niche. Moreover, addition of animal-derived soluble factors represents a concern for safe administration in patients. In the present study we set up a new protocol for the expansion of rat mesenchymal stem cells (MSC) on an innovative nanoengineered synthetic 3D substrate in the absence of animal-derived growth factors.

Methods: We isolated MSC from the bone marrow (BM) of healthy Sprague-Dawley rats. Cells were characterized for cell-surface antigen expression by FACS analysis and expanded in the absence of FBS and with the addition of human platelet lysate. We developed an innovative culture substrate, called "nichoid", nanoengineered via two-photon laser polymerization in a biocompatible photo-resin called SZ2080. Finally, we compared the culture of rat BM-MSC on bi-dimensional glass substrate and on the 3D nichoid substrate. In vitro cell growth and viability were assessed with the Alamar Blue assay.

Results and Discussion: We successfully expanded rat BM-MSCs in the absence of animal-derived factors on both substrates. Our results demonstrate that the nichoid substrate was suitable for adherence and growth of MSCs. Compared with cells cultured on glass substrates, cells expanded on the nichoid substrates showed a significantly greater growth rate. Thus, the nichoid substrate may be beneficial for expanding MSCs, even in the absence of animal-derived growth factors. By mimicking the physical constraints to cell migration present in the physiological stem cell niche, it could represent a tool to expand MSCs while maintaining stem cell properties in vitro. Our further studies are currently investigating the ability of the nichoid to preserve stemness marker expression during long-term culture of MSCs.

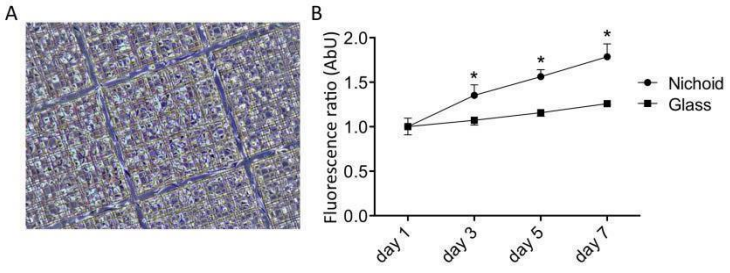

Figure 1. A, live image of rat BM-MSCs cultured on the 3D nichoid substrate. B, Alamar Blue assay showing an increased growth rate in nichoid-cultured MSCs as compared to glass-cultured cells. *p<0,01 vs glass.

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P081 Osteopontin-a and Osteopontin-b splicing variants are produced during BMSCs and HSCs co-culture in an in vitro bone marrow niche model

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In the bone marrow niche (BMN), several stromal and hematopoietic cell types interact in order to preserve their stemness and mutually guide the formation of the bone marrow (BM) for a lifetime. Osteoprogenitor cells, such as bone marrow-derived Mesenchymal Stromal Cells (BMSCs), Osteoblasts and Hematopoietic Stem Cells (HSCs) are among the protagonists of this niche, characterized by an intensive signaling through cell-cell contact and growth factor secretion. Although secreted growth factors and cytokines have been extensively studied in this context, the lack of a physical barrier dividing the niche microenvironment from the remaining BM makes it difficult to explain how mechanisms such as cell quiescence, stem cell pool regulation and HSCs transition to the proliferating/differentiating compartment is performed. Osteopontin (OPN) is a phosphorylated glycoprotein characterized by a wide range of possible splicing variants and post-translational modifications which has been reported to be involved in several of these processes, and recent reports suggest further involvements of this protein in the development of the leukemic niche and in the metastatization of other tumor types to the BM. Although several functions have been reconnected to OPN in different tissues, only few reports attempt to connect each variant of this protein to its function. In this work we detected the production of the OPNa and OPNb splicing variants after co-colturing of osteogenically differentiated BMSCs and HSCs in a BMN model (Porous microcarriers comprising Recombinant Peptide based on human collagen I, FujiFilm) and we evaluated the effect of recombinant OPNa and OPNb on HSCs in a methylcellulose assay. Human BMSCs were seeded on porous microcarriers and differentiated towards the osteogenic lineage in spinner flask bioreactors for 3 weeks. The microcarriers were subsequently transferred into a transwell system and human HSCs were injected in the construct and cultured for 1 week. Co-cultures were analysed by immunofluorescence staining for OPN detection. After enzymatic lysis of the microcarriers, HSCs were selected through magnetic beads selection and HSCs potency was assayed through methylcellulose assay. The remaining BMSCs were assayed for qPCR analysis of differentiation markers and compared to BMSCs monocultures. Different OPN variants were detected with primers flanking a differentially spliced region, and isoforms were confirmed through sequencing. Plasmids for OPNa and OPNb production were modified in order to express fusion proteins with 6 His tags that were isolated through affinity chromatography from the medium of transiently transfected hFOB1.19 cells. Matrix binding properties of the two splice variants were characterized upon incubation on a mineralized surface and effects on HSCs was tested after addition of the recombinant proteins to a methylcellulose assay. The results suggest OPNa and OPNb are produced during BMSCs-HSCs co-culturing, and may be involved in different processes in the BMN. This underlines the importance of the correct identification of the OPN variants involved in the BMN, suggesting that different variants play diversified roles in the BM environment and opening the way for more targeted strategies for addressing hematological malignancies and other congenital BM defects.

P082 Cellular and mechanical components synergistically enhanced ex vivo expansion in human hematopoietic stem/progenitor cells

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Effective ex vivo expansion of hematopoietic stem/progenitor cells (HSPCs) is a prerequisite for HSPC transplantation to treat hematologic malignancies and/or inherited diseases. In nature, HSPCs reside mainly in the bone marrow where they interact within a bone marrow niche, which regulates HSPC fate. In recent years, a synergistic effect in terms of expansion of HSPCs was reported from studies using recombinant growth factors and co-culture with neighboring cells. Nevertheless, the mechanisms involved in HSPC regulation remain undefined due to the limited availability of optimal ex vivo culture models that mimic the hematopoietic niche.

To address some of these unresolved problems, we carried out head-to-head comparisons of the expansion efficiency of co-cultures comprising MSCs or OBs with growth factors such as SCF, Flt-3, TPO, IL-3 and IL-6. Furthermore, we systematically employed combinations of hydrostatic pressure (HP; 20 kPa) to determine the optimal culture conditions for effective expansion.

The numbers of total nucleated cells (TNCs) were significantly increased when HSPCs were co-cultured with MSC or OB feeder layers, regardless of the feeder cell type, compared with HSPCs alone. Moreover, a significant increase was observed in the co-culture groups with HP (H_M_HP: 82.09 ± 5.72-fold; H_O_HP: 79.63 ± 0.82-fold) than in the initially seeded TNCs. The $CD34^+CD38^-$ proportion of co-culture groups (H_M and H_O) displayed significantly higher levels than HSPCs alone. In addition, HSPCs co-cultured with feeder cells subjected to HP (H_M_HP and H_O_HP) showed a significant increase in the number and fold expansion of $CD34^+CD38^-$ cells compared with other groups; however, no difference was observed between these two groups. Co-cultured MSC and OBs expressed substantially higher levels of hematopoietic niche markers (SDF1 and VCAM1) and NOTCH signaling genes (NOTCH1, HES1 and DLL1) compared with MSCs and OB alone. In particular, the application of 20 kPa HP induced significantly higher levels of all markers compared with other groups. Namely, these data illustrate that cellular and mechanical components of hematopoietic niche promote maintenance and expansion of HSPCs by up-regulating Notch-mediatedsignaling.

This study demonstrated that cell-cell interaction between HPSCs and MSCs or OBs enhances expansion of HSPCs. In addition, application of HP was found to significantly influence HSPCs expansion outcome and maintaining stemness. However, no significant differences were identified between MSCs and OBs. It is the first study which applies HP to the expansion of HSPCs. These results are expected to have an important impact on ex vivo expansion of HPSCs and eventually on the design of an efficient clinical-scale expansion system.

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P084 Introducing interstitial fluid flow in a bioengineered breast cancer perivascular niche

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Metastasis is the leading cause of death in breast cancer patients. Disseminated tumor cells (DTCs), escape the blood stream through the microvasculature and reside in the perivascular niche of distant organs, such as the bone. The niche microenvironment - including the extracellular matrix, endothelial and mesenchymal cells - regulates DTCs metastatic outgrowth. Although interstitial fluid flow (IFF) is a physiologically relevant mechanical signal, regulating intracellular signaling pathways in healthy and cancer cells, its effect on DTCs growth has been largely overlooked.

Our goal is to develop a 3D human in vitro model of bone perivascular niche to elucidate the effects of IFF on endothelial function and cancer cell growth. To establish the model, endothelial and bone marrow derived mesenchymal stem cells (BMSCs) are cultured in a 3D bone matrix and placed into a millifluidic device, where cells are exposed to fluid flow and shear stress. Cancer cells are then introduced into the model to mimic metastatic invasion within the bioengineered niche. Endothelial and cancer cells tagged with RFP and GFP reporter genes, respectively, enabled tracking of endothelial network formation and cancer growth in a non-disruptive fashion overtime.

Using a computational analysis, we determined the optimal fluid flow range (0.25-0.5µL/min) that allows exposing the cells to physiologically relevant fluid velocities (0.5-1µm/s). These results were validated using fluorescent microbeads perfused into the scaffold and tracked over time. When the co-culture of BMSCs and endothelial cells was perfused, we found that 0.25 µL/min increased vessel density, junction densities, number of endpoints but not the average vessel length. Additionally, IFF modulated proliferation and drug sensitivity of breast cancer cells cultured in the bioengineeredniche.

In this study, we describe a bioengineered model of bone perivascular niche where interstitial fluid flow, within physiological ranges, promotes endothelial sprouting and mediates cancerfunctions.

P085 A computational model of spreading stem cells, coupling mechanical deformation to nuclear membrane permeability to small solutes

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Cell morphology is intrinsically related to cellular functions, including proliferation, differentiation. Changes in the cytoskeletal architecture may induce modifications of the nuclear shape and architecture, which in turn govern the mechanisms of mechanotransduction. In previous works, we reported evidence of multipotency maintenance for mesenchymal stem cells cultured on three- dimensional (3-D) engineered niche substrates, nanofabricated via two-photon laser polymerization. We correlated maintenance of multipotency to a more roundish morphology of the cells cultured in the engineered niches, with respect to those cultured on conventional flat substrates. To interpret these findings, we developed a multiphysics model coupling nuclear strains to passive diffusion of small molecules across the cell (Nava et al., 2016a,b). Here, we present an improved model in which fully 3- D reconstructions of such cultured cells were based on live images of fluorescent cells labelled by Hoechst acquired through laser scanning confocal microscopy. Z-stack images of 10 cells were taken every 15 minutes along 3 hours after cell seeding. On each couple of 3-D scans, relevant to two consecutive measurements along the test, we ran an advanced code for 3-D Digital Image Correlation (DIC) apt to detect local displacements within the cell nucleus, and the strain field by differentiation (Fedele et al., 2013). The level of nuclear spreading resulted significantly dependent on the cell localization within the 3-D engineered niche substrates. In particular, an average change of the nucleus outer surface up to 14% was met on the flat surface surrounding the 3-D engineered niches, significantly larger with respect to cells lying in the 3-D niche (4%). We assumed the nuclear envelope permeability as a function of the local strain (provided by 3-D DIC data) and predicted the nuclear uptake of small solutes driven by passive diffusion. The model predictions driven by experimental data through image processing indicate that the higher the level of nuclear spreading, and the relevant nuclear membrane permeability, the higher the flux of small solutes across the membrane. Our results point toward nuclear spreading as a primary mechanism by which the stem cell translates its shape into a fate decision.

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P086 Controlled protein environments and patterned electrospinning can form artificial stem cell niches

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Introduction:

Regeneration of bone injuries could be significantly improved by a ready source of MSC's supplied by an artificial reservoir. Stem cells have the ability to self-renew almost indefinitely, presenting a significant opportunity in regenerative medicine. If a synthetic niche environment could be prepared that maintained a mesenchymal stromal cell population, this could enhance local bone tissue regeneration in medical devices. The behaviour of stem cells is highly complex, but it is partly influenced by their defined 3D environments or so-called niches. The chemical and morphological environment within these niches are equally important in influencing stem cell behaviour. These conditions play a key role in maintaining stemness whilst directing differentiation when required. To produce an artificial stem cell niche both structural & chemical environments were investigated usingMSCs.

Methods:

Patterned stainless steel collectors were design via CAD software and manufactured using selective laser melting). Biodegradable polymer was electrospun onto the collectors to produce scaffolds imprinted with the negative pattern. Proteins were deposited within the niches by adsorption. Primary rat Mesenchymal stromal cells (MSC) were seeded onto the electrospun scaffolds for 7 days and proliferation measured with Presto-blue. Fluorescence microscopy was used to image cells and biological deposits.

Results:

Well-defined artificial 3D niche environments were successfully incorporated into electrospun scaffolds using template technology. Fluorescence and the stain Sirius red showed the presence of proteins deposited within the niches. PrestoBlue indicated an increase in cellular metabolic activity over 7 days. Different MSC morphologies were observed within the niche depending on location and coating. Confocal z-stacks showed cells and extracellular matrix conformations growing within the niche.

Discussion:

The investigation found that MSC morphology was altered by both heparin and collagen coatings along with its position within the electrospun niche. The inclusion of additive manufacturing as part of the fabrication process allows the creation of intricate structures that can be designed to mimic certain aspects of the native stem cell niche. MSCs were identified in the niches, cell proliferation was observed and morphological differences in cell structures were recorded. The deposited protein did not affect proliferation and further research is ongoing to identify effects on differentiation. Targeted coatings of proteins combined with electrospun membranes containing niche structures were able to influence MSC behaviour.

P087 Biomaterials based modulation of the MSC secretome

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Intro:

Mesenchymal stromal cells (MSCs) are now increasingly being used in regenerative medicine due to their paracrine signalling properties rather than their differentiation potential. Bioactive factors secreted by MSCs can not only modulate the immune environment, but can also influence the pro-regenerative functions of progenitor cells such as those in bone and muscle. Since MSCs are responsive to their microenvironment, we investigated whether changes in scaffold pore size can influence the paracrine function of these cells.

Materials & Methods:

Alginate was modified using carbodiimide chemistry to present cell adhesive RGD peptides on the cell-material interface. Two types of substrates were prepared: (1) nanoporous hydrogels that encapsulate MSCs, and (2) macroporous cryogels where cells can infiltrate the interconnected structure. MSCs were isolated from bone marrow of Sprague Dawley rats, and used in passage 2-3. Cells were cultured on the two substrates for two days before collecting conditioned media. ELISA kits specific for rat VEGF, rat IGF, rat HGF, and rat FGF were used to analyse the cytokine secretion. The concentrations were normalized to cell number. RNA was isolated from the cells to analyse any signalling pathways involved.

Results:

Our data shows that MSCs cultured on macorporous scaffolds secreted significantly larger amounts of all growth factors analysed, compared to those in hydrogels. Moreover, the total protein secretion from MSCs in macroporous scaffolds was significantly higher than from those in hydrogels. RT-qPCR analysis showed that cells in macroporous scaffold had higher expression of AKT (1000 fold) and NFkB (200 fold) relative to MSCs in hydrogels. These data indicate that along with the known effects of the environment on MSC differentiation, changes in biomaterial microenvironment can also alter their paracrine function.

Conclusion and Implications:

Novel injectable hydrogels with advanced chemistries and physical properties are being reported often. Our data shows that injectable gels may not be the optimal carrier material for MSCs, and that using open porous scaffold may help maximize their regenerative potential. In the future, optimized biomaterials based on stiffness, pore size, and chemical properties may help to ensure that MSC based regenerative therapies provide effective outcomes.

P088 Delivering a proliferative niche for liver repair via KGF-treatment

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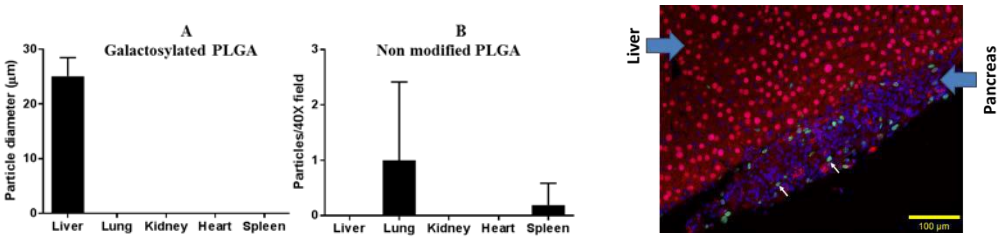
Introduction: According to recent national statistics, liver disease is the third leading cause of death in the UK in subjects aged under 75 with a 400% increase in mortality since 1970 [1]. Transplantation remains the only proven treatment for end stage liver disease. The aim of this work is to maximise liver tissue regeneration by enhancing therapeutic delivery of keratinocyte growth factor (KGF) using galactosylated polylactide-co-glycolide (PLGA). A proliferative hepatic niche will also enhance pancreatic islet cells engraftment, which is a new avenue for treating diabetes.

Materials and Methods: PLGA Galactosylation was achieved following *Yoon, J.J., et al* method [2]. The particles were then fabricated using double emulsion method in which, 0.1% (w/v) KGF was homogenised in polymer solution at 11000 RPM followed by a second homogenisation of the primary emulsion in 200ml of 0.3% PVA at 11000 RPM. The created MPs were vortexed overnight then collected via centrifugation and freeze dried before storing at -20°C. Biodistribution was studied in mice model following the injection of 1mg of particles via the hepatic portal vein. Organs where harvested 24 hours later followed by sectioning and quantification of the particles. Cell proliferation in the liver was assayed using BRDU proliferation assay.

Results: PLGA galactosylation was confirmed by NMR, data from biodistribution study showing a selective localisation of galactosylated PLGA particles into the liver compared to random distribution of particles in other organs (Figure 1). The particles were engineered to give controlled release kinetics for the payload. The release started with an initial burst followed by steady release for 2 weeks. Finally, KGF succeeded in achieving specific growth stimulation in the liver (Figure2).

Conclusion: This work provided engineered PLGA particles that can target the liver specifically to achieve selective growth factor delivery to the liver. KGF elicited specific growth stimulatory effect in the liver. This targeted effect is expected to improve cell therapy with MSCs via creating a favourable niche.



F**igure 1** Average particle count in 11 random fields

Figure 2 Microscopic image for liver from organ sections. Section stained with Brdu assay, Green dots refers to grwoth stimulation by KGF

P090 A SDF-1 tethered 3D ECM –based scaffold improved muscle progenitor cell migration

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The engineering of functional skeletal muscle tissue construct holds promise for the treatment of various muscular diseases and injuries to replace lost muscle. Decellularized scaffolds, composed of tissue-specific network of proteins complex and glycosaminoglycan, which help regulate many cellular behaviors. Natural extracellular matrix-based bioscaffolds can be recellularized to create potentially functional constructs as an emerging generation of tissue engineered replacements in regenerative medicine for tissue repopulation. We believe that a key step in the development of a construct for replacing of damaged muscular tissue is the ability to attract the blood vessel and specific stem cells to generate a 3-dimensional (3D) constitution of muscle tissue. In our present proof of principle studies, we developed a macroporeous 3D sponge from decellularized skeletal muscle extracellular matrix and examine its potential for recruiting the muscle derived-stem cells to damage tissue with immobilized SDF. Our *in vitro* results showed that MBS-SDF immobilized into muscle derived-sponge enabled rat muscle-derived stem cells (MDSC) have more migration, maintenance and proliferation compared with no SDF, Absorbed- SDF and EDC-SDF. Histological evaluation of transplanted scaffolds after one month revealed enhanced angiogenesis and MDSCs recruitment in MBS-SDF scaffold compared to no SDF and EDC-SDF. This research demonstrates the feasibility of fabricating 3D porous scaffolds from native ECMs and suggests the therapeutic potential of scaffold in the treatment of damaged muscle.

P091 Exploring the potential of the physico-mechanical environment and circadian timing in adult stem cell differentiation

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Without adult stem cells, mammalian tissues would not be able to maintain their natural homeostasis, as these cells play a vital part in the replenishment and repair processes, whereby old or damaged cells become replaced. With age, the regenerative capacity of tissues decreases significantly due to deterioration in number and/or function of tissue-specific adult stem cells and alterations in the properties of stem cell niches and the regulatory cues they receive.

One homeostatic mechanism shown to regulate adult stem cell and tissue physiology is the circadian clock, which anticipates and synchronises vital tissue functions to the cyclic needs of the organism (food/fasting, activity/rest cycles). Previous research has indicated that core clock proteins of the molecular clock mechanism, such as Bmal1, bind to the promoters of stem cell regulatory genes in an oscillatory manner and modulate their expression. In addition, several circadian cues have the ability to entrain stem cells. One novel entrainment cue is mechanical stimulation, however, whether or how mechanical cues entrain cellular and molecular clocks in various adult stem cells and their progenitors is unknown.

Our current hypothesis is that circadian entrainment mechanisms such as mechanical cues can be used to direct and control adult stem cell differentiation. To this end, our research aims to discover the most efficient mechanisms of entrainment in different types of adult stem cells, as well as signalling pathways and downstream stem cell genes regulated by circadian timing. The methodology will include the culturing of adult stem cells from various tissue sources, including both bone marrow and dental pulp derived mesenchymal stem cells, and subjecting them to different forms of mechanical stimulation using a unique uniaxial mechanical stretch rig. The most efficient type of coating protein used to optimise the system will also be investigated.

Understanding circadian entrainment mechanisms of adult stem cells will help design future strategies to rescue or delay the age-related changes seen in tissue function and stem cell differentiation potential.

P092 Formation of tendon organoid by a defined uni-axial mechanical loading in 3D bioreactor culture

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INTRODUCTION

Tendon is a mechanosensitive fibroconnective tissue but little is known about the impact of mechanical loading profiles on tendon derived cells and their ability to form tendon tissue. In this study, we have examined the molecular phenotypic effects of uni-axial and bi-axial mechanical stimulation on tendon-derived stem cells (TDSCs) isolated from both mice and human, and defined the optimal uni-axial mechano-profile for the fabrication of a three-dimensional tendon organoid in 3D bioreactor system that shows realistic micro-anatomy of tendon structure.

METHODS

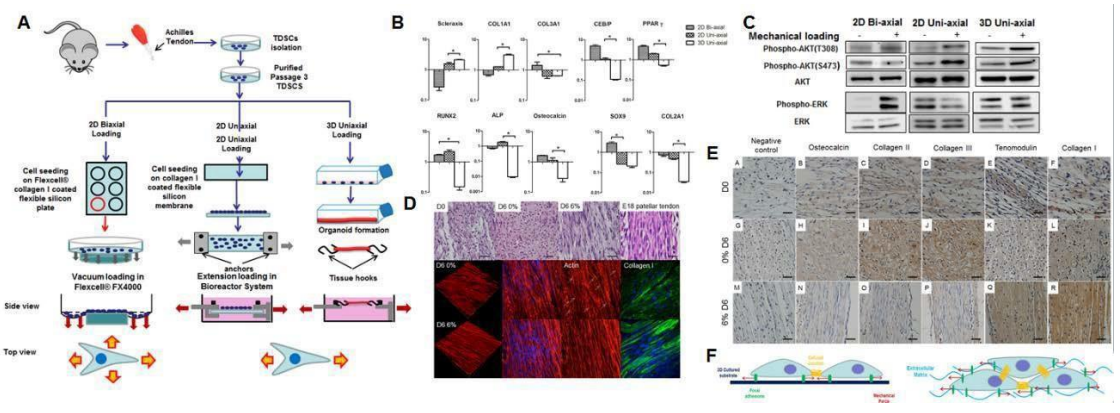
TDSCs were isolated from mice Achilles tendon then seeded on silicon substrate for 2D loading culture after purification. QPCR and western blot were used to assess the molecular phenotypic effects of uni-axial and bi-axial loading (6% strain, 8h/d for 6 days) on traditional monolayer cultured TDSCs. Tendon organoids were constructed and subjected to uni-axial loading with same regime, then the tissues were then evaluated by QPCR, western blot, confocal microscopy, immunohistochemistry and mechanical loading.

RESULTS

Our result showed that distinct mechanobiological and differentiation regulatory pathways were triggered by loading. In monolayer system, bi-axial loading inhibited tenogenic differentiation, and induced adipogenic, osteogenic and chondrogenic differentiation, whereas uni-axial loading induced tenogenic and osteogenic differentiation and inhibited chondrogenic differentiation of TDSCs. Interestingly, tenogenic specific differentiation was observed in tendon organoid subject to uni-axial loading. Moreover, ERK signal was induced by bi-axial loading, whereas AKT was involved in uni-axial mechno-transduction pathway. Tendon organoid subjected to uni-axial loading exhibited tendon-like morphology with organized collagen bundles and spindle shape cell nuclei similar to the patellar tendon of mice embryo at 18 days. Immunohistochemistry assessment indicated uni-axial stimulated tenogenesis of tendon organoid.

DISCUSSION

The fate that uni-axial loading on TDSCs in 3D organoid culture only induced tenogenesis may imply the importance of biomechanical environment and dynamic of biostructure in the bioreactor system. It reflects that the 3D organoid culture system more closely mimics the in vivo physiological loading in tendon tissue than the monolayer cultures. TDSCs in monolayer sense the mechanical stimulation through focal adhesions on the bottom of the cell body. In contrast, TDSCs in the 3D bioreactor cultures can sense the mechanical stimulation of the whole cell body through the cell-cell interaction and cell-ECM interaction within the tissue. Thus, the 3D bioreactor cultures provide a better environment of cell-cell interactions and regulation of fate commitment. In support of this, it has been shown that tendon development during embryogenesis requires a gradual transition from the one that is rich in cell-cell interactions to the one that is dominated by cell-ECM interaction. Based on the results reported here, we propose that the tenogenic-specific differentiation and tendon formation is the result of the 3D dimension of uni-axial stimulation on TDSCs in a defined biomechanical environment.



P093 A new 3D hybrid zonal scaffold for articular cartilage tissue engineering

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INTRODUCTION: Articular cartilage is a thin hydrated tissue, which covers articulating surfaces. It has an anisotropic, zone-specific structure, extending from the articular surface to the subchondral bone. The main challenge associated with cartilage tissue engineering is to generate functional tissue with zonal organisation similar to the native cartilage tissue. The aim of this project is to fabricate a hybrid 3D zonal scaffold to mimic the native variations of ECM organization between zones, enabling a better cartilage tissue formation.

METHODS: Bovine chondrocytes were used. Electrospinning technique established in the lab has been used to produce nanofibrous zonal-specific scaffolds. 2% poly (lactic acid) solution was used to obtain nanofibers. The microscale channels were formed by using micro-needle in Hyaluronic acid (HA) hydrogel. Various characterisation assays including live images to assess cell morphology and micro-channels, DNA content, DMMB to quantify Glycosaminoglycans (GAG) production have been conducted. The live-dead staining for cell viability and morphology, immunofluorescence imaging and Western blotting for collagen type II expression were conducted.

RESULTS & DISCUSSION: Nanofibrous scaffolds with aligned fiber (for superficial zone), random fibers (for middle zone) have been produced. The deep zone has been mimicked by micro-channels in HA hydrogel. The seeded chondrocytes on nanofiber scaffolds shown different morphologies and the channels has been demonstrated by optical coherence tomography clearly (Fig 1). Chondrocytes in superficial zone scaffold exhibited elongated morphology and low GAG production per DNA content; whilst middle zone scaffold produced random chondrocyte clusters and had a higher GAG production per DNA content. The random and deep zones scaffold had a higher GAG production per DNA content than superficial zone scaffold (Fig 2-4). Collagen type II expression showed the same GAG expression pattern in these zones, indicating that the hybrid scaffolds which combine nanofiber and HA hydrogel can regulate chondrocytes' metabolic activities and produce zonal-specific tissues.

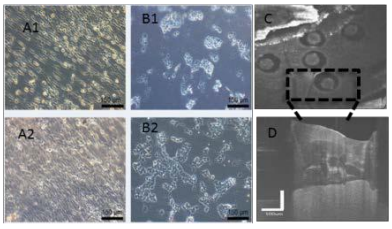

Figure 1: Chondrocytes morphology and orientation on different 3D zonal scaffolds on day 3 (Label 1) and day 7 (Label 2) culture. Scale bar: 150um A: Superficial zone scaffold B: Middle zone scaffold C: Surface view of Micro-channels (Deep zone) D :cross section for micro channels. Scale bar: 100um.

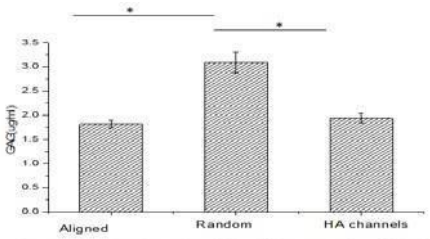

Figure2:Total amount of GAG released in the different 3D zonal scaffolds at day 7

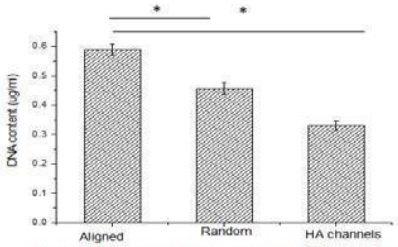

Figure3: DNA contents in the different 3D zonal scaffolds at day 7

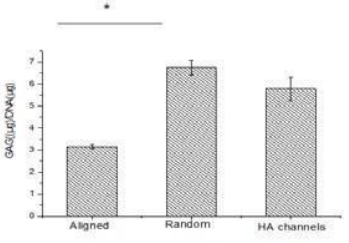

Figure 4:Normalisation of GAG content per DNA content in the different 3D zonal scaffolds at day 7

P094 Enhancement of Therapeutic Angiogenesis Efficacy by Combination of Plasmid-based PDGF-B Gene Therapy and Adipose Tissue-derived Stromal Cell Therapy in Mice

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Cell therapy using autologous progenitors is an intensively developing approach to promote tissue repair and regeneration. We and others have demonstrated potential impact of adipose tissue-derived stromal cells (ADSC) on angiogenic cell therapy for myocardial and limb ischemia. The main benefit of ADSC is that they can be feasibly harvested by a simple, minimally invasive method and also easily cultured. However, efficacy of cell therapy for ischemic diseases is restricted by viability of transplanted cells, which may be exposed to inflamed hypoxic environment of damaged tissue resulting in drastic reduction of their number and, thus, therapeutic effect. Since the initial success of cell therapy for ischemic diseases many attempts have been made to increase its efficacy. One of the promising approaches is a combination of gene and cell therapy by expression of growth factors in ischemic tissues to stimulate transplanted cells' survival and proliferation. The aim of this work was to study the feasibility of combined therapy using ADSC delivery with plasmid-based PDGF-B gene therapy to increase therapeutic angiogenesis efficacy. PDGF-B is important regulator of ADSC function which stimulates their proliferation, migration, inhibits apoptosis of these cells and increases their angiogenic potential. Using a hind limb ischemia model in C57BL/6 mice we assessed the efficacy of combination of ADSC with plasmid based PDGF-B gene therapy for blood flow restoration and angiogenesis in ischemic limb. Intramuscular administration of plasmid encoding PDGF-B gene immediately after hind limb modeling was followed by transcutaneous intramuscular injection of mouse ADSC 5 days later. Blood flow was measured by laser Doppler every 7 days during 21 days; at endpoint animals were sacrificed and skeletal muscle was evaluated for vessel density. Mice injected with PDGF-B plasmid followed by ADSCs transplantation showed significant increase in perfusion compared to plasmid injection or ADSC transplantation alone. These findings were supported by significantly increased CD31+/a-SMA+ vessel density in animals that received combined gene and cell therapy compared to single gene or cell therapy. Obtained results provide a basis for development of gene/cell therapy methods for patients with critical limb ischemia often resulting in amputations especially in diabetic subjects.

Study was supported by a Russian Science Foundation grant #16-45-03007

P095 The investigation of anion emitting cosmetic textile effect on the improvement of blood circulation in rat model

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Purpose: Beneficial psychological and physiological effects from exposure to negative ions have been reported in humans including sleep enhancement, increased growth, the potentiation of peripheral blood flow and body temperature, autonomic nervous system control, bio-activity, and inhibition of obesity. However, the mechanisms underlying these effects are not fully understood. The purpose of this study is to investigate the effect of anion in local flap micro-circulation improvement.

Methods: The experiments in this study were conducted in vivo on four groups of twenty Sprague-Dawley rats. Group A was control group. Group B was given indirect exposure of anion emitting cosmetic textile and Group C was given direct exposure of anion by apply on the flap. Group D was given both indirect and direct exposure of anion emitting cosmetic textile. During 2 weeks, the serial gross finding photo, thermal imaging, Laser-induced fluorescein fluoroscopy were performed. Local flaps were excised after 2 weeks and were evaluated for vascular state, angiogenesis, through RT-PCR and histologic findings.

Results: Twenty Sprague-Dawley rats were subjected. 2x8cm random cutaneous flap was elevated. Flap was placed in original location and was sutured by primary closure. We observed survival of flap and blood circulation. Gross photography and Laser-induced fluorescein fluoroscopy were compared with each other As a result, flap survival rate of the control group (A group) was 52% and 62% in the B group, 85% in the C group, 87% in the D group. Mean temperature of distal flap of the control group was 29.4℃and 29.6℃in the B group, 31.6℃in the C group, 33.1℃in the D group. Flap survival rates and flap temperature of C, D group which are directly exposed to cosmetic textile were highly measured and it show a statistically significant (<0.05). Histologically, more active proliferation of endothelial cell and vasodilatation were observed in the C, D group. The result of RT-PCR shows increased expression of CD31 and iNOS mRNA, which are associated with angiogenesis and vasodilatation in the experimental group.

Conclusion: In this study, anion emitting cosmetic textile may be used to significantly improve the microcirculation and survivor rate of local flap. It is thought that our investigation will be the fundamental of research to identify the positive effect of anion on human peripheral vascular circulation.

P096 Novel biomaterial-based approaches for therapeutic angiogenesis

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Background: Peripheral arterial disease (PAD) affects over 200 million people worldwide and can lead to limb ischemia, amputation of and mortality. By promoting regeneration of tissue damaged by ischemia poses a significant challenge. Therapeutic angiogenesis aims to deliver angiogenic factors to promote the formation of new blood vessels and thus treat ischemic tissues. Biomaterial scaffolds can be an effective way of delivering therapeutics to a target site, as well as utilizing physical characteristics to influence cell behaviour. Surface topography is known to influence cell alignment, morphology and differentiation and rough surfaces have been shown to effect cellular expression of biomarkers and growth factors. By producing substrates with tuneable surface characteristics cellular responses can be controlled.

Hypothesis: Hierarchical textured surface topographies promote the secretion of angiogenic growth factors for therapeutic angiogenesis.

Methods: To create hierarchically structured materials, microparticles were prepared from biodegradable poly-DL-lactide-co-glycolide (PLGA) using thermally induced phase separation (TIPS). Surface topographies were characterised using scanning electron microscopy, focused ion beam microscopy and X-ray NanoCT. The biological effect of surface topography on adipose derived mesenchymal stem cells (ADMSCs) was assessed using cell viability and proliferation assays and ELISAs to measure the secretion of angiogenic growth factors and a proteome profiler angiogenesis array. To validate the effect of the microparticles *in vivo,* a murine model of hind-limb ischemia was used. Unilateral femoral artery ligation was used to induce ischemia with microparticles injected at the occluded bundle. Laser doppler imaging was used to evaluate the blood flow in the hindlimb up to 21 days.

Results: The TIPS process results in a hierarchically structured porous and rough topography compared with control microparticles. Proliferation assays showed increased cell numbers found on TIPS microparticles after 10 days (1769 cells/particle) compared to polymer controls (868 cells/particle). Profiling secretion of angiogenic growth factors and ELISAs showed increased secretion of VEGF on TIPS microparticles after 10 days (1415.43pg/mL) compared to polymer controls (670.6 pg/mL). Microparticles were successfully implanted *in vivo* via injection and retained for 21 days at the implant site. Histology revealed evidence of blood vessel formation around the TIPS microparticles and laser doppler images show the revascularisation of the limb after 21 days.

Conclusions: In conclusion TIPS microparticles stimulated an increase in angiogenic growth secretion capable of stimulating angiogenesis *in vitro*. Microparticles were successfully administered via a minimally invasive technique to a pre-clinical model of PAD, with future work investigating the effects of cellularized microparticles *in vivo*. Findings from this study offer a novel approach for using biomaterials to simulate the secretion of angiogenic growth factors.

P097 An in vitro study of conductive nanofibrous composites of poly (ε-caprolactone) / chitosan/ Polypyrrole for myocardial regeneration

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Myocardial Tissue Engineering has emerged as a promising approach to replace or support an infarcted myocardial tissue and thus may hold a great potential to treat and save the lives of patients with heart diseases. The development of scaffold for biografts for the repair of myocardial tissue is particularly challenging but holds great potential. Electrospininng is one of the most effective methods for providing new, bio-mimicking natural matrices. On the other hand, the use of electrically conductive scaffolds can improve electrical signalling of cardiomyocytes in damaged myocardial tissue.

The aim of this study is to investigate the Polypyrrole (PPy) influence on biocompatibility of poly (ε-caprolactone) / chitosan nanofibrous scaffolds. The surface morphology, mechanical properties and electric properties of the scaffolds were characterized. The adhesion and proliferation cardiac progenitor cells were assessed on scaffold .After 3days the adhered cell morphologies and proliferation were observed by SEM and MTT assay. The results of MTT assay shown that the highest proliferation rate of cells was observed in conductive PCL/CS/PPy scaffolds compared to the PCL/CS .These results suggest that PPy improves the conductivity of PCL/CS nanofibers and increases the biocompatibility of PCL/CS. Therefore, novel electrospun PCL/CS/PPy blend to be a biomaterial may provide the development of contractile myocardial patch in myocardial tissue engineering.

P098 Enhancing endothelial adhesion and functionality by modifying surfaces for a biomimetic blood contact interface

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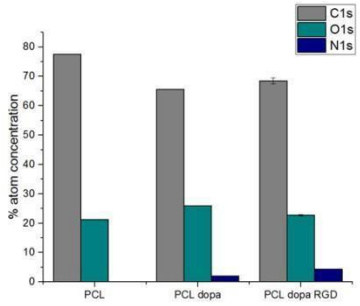
INTRODUCTION: The main challenge when designing implants that are in blood contact such as the membrane in a ventricular assist device (VAD), is to prevent the coagulation of blood at its surface. Nature however provides us with a perfect blood-tissue interface, the endothelium. When engineering such endothelium on top of artificial materials, the success depends on the stable adhesion of endothelial cells to the substrate and their functional state. It is known that by modifying surfaces, e.g. with specific peptides, cell adhesion can be stimulated (1). It has previously been shown that peptides can be immobilized onto various materials via a mussel inspired two step approach (2). To achieve long term stability and functionality of the engineered endothelium, intercellular communication with e.g. smooth muscle cells (SMCs) is essential (3). Our aim is therefore to create a 3D blood-vessel biomimetic with a monolayer of endothelial cells and SMCs below. For this, different adhesion peptides and combinations will be identified and used to locally control cell adhesion within the 3D construct.



METHODS: Nanofibrous scaffolds were produced by electrospinning of poly-ε-caprolactone (PCL). For subsequent peptide immobilization, dopamine was polymerized on the surface followed by binding of RGD peptides via Michael addition to the polydopamine layer.

RESULTS: Successful mussel inspired peptide immobilization was confirmed by XPS measurements. In Fig. 1 the increase of the surface nitrogen concentration indicates the dopamine coating and subsequent peptide immobilization.

Fig. 1: XPS elemental surface concentrations of untreated PCL nanofibrous scaffolds, after polydopamine coating and after RGD peptide functionalization confirms the success of the proposed immobilization, shown by increased percentage of nitrogen atoms on the surface (n=1 for PCL/PCL dopa and n=2 for PCL dopa RGD)

DISCUSSION & CONCLUSIONS: We were able to covalently immobilize RGD peptides on nanofibrous PCL substrates as shown by XPS. The functionalization has to be validated by in vitro cell attachment assays. This approach will allow us to steer the adhesion behavior of endothelial cells on peptide functionalized substrates. In the next step, we will thus immobilize different cell specific adhesion peptides and combinations thereof, to identify optimal conditions for targeted endothelial and smooth muscle cell adhesion.

ACKNOWLEDGEMENTS: This work is part of the Zurich Heart project of Hochschulmedizin Zurich.

P099 Lactate-releasing biomaterials as cardiac repair inducers

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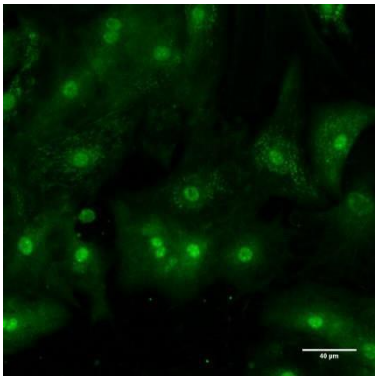
Lactate is known as a typical metabolite of the anaerobic glycolysis, commonly produced and secreted by cells. However, growing evidence suggest that lactate is not just a residual product but also a signalling molecule and a key factor in many processes, even able to influence epigenetic programs and gene expression in a dose dependant manner. Nevertheless, little is known on the effect of lactate on cardiac tissue and even less on how can this molecule be used as a tissue repair inducer after a myocardial infarction (MI).

Here in this work, we demonstrate for the first time that lactate is able to enhance cellular activity of cardiac fibroblasts while reducing the proliferation of these cells in a dose-dependent manner, which was confirmed by DNA quantification and the presence of mitomycinC as proliferation inhibitor. Collagen quantification experiments also revealed an increase in collagen production when different lactate concentrations are used. Cardiomyocytes are as well able to respond to the presence of lactate by reducing apoptosis and thus increasing long-term survival. Additionally, the expression of different receptors and transporter molecules involving lactate, such as MCT1, MCT4 and GPR81 (figure), has been demonstrated on cardiac fibroblasts as well as on cardiomyocytes by using immunofluorescence along with western blot techniques, demonstrating that cardiac cells are able to sense and even respond to different concentrations of lactate.



With all these new evidences, we design and fabricate a lactate releasing biomaterial based on Poly(lactic-co-glycolic acid) or PLGA polymer. This biomaterial has a unique configuration in which the main core is made of a microporous PLGA film as a high lactate releasing component, combined with electrospun PLGA nanofibers for a precise control of cellular environment within an ECM-like structure. We have demonstrated that this biomaterial is able to effectively release lactate in a biological activity range. In addition, this developed lactate-releasing biomaterial has demonstrated to be non-toxic to cardiac cells, and even more, induce the same effects previously described with soluble lactate. Moreover, the electrospun fibers content allows for the control of the morphology and orientation of the cells, resembling the anisotropic structure of the heart tissue. The migration of cells to this biomaterial has been assessed, as well as the enhancement of cardiac properties when cardiac fibroblasts and cardiomyocytes are cultured together for a synergistic effect on cardiac repair.

As a result, we demonstrate that lactate-releasing biomaterials can be a feasible strategy for cardiac tissue modulation and repair after a MI.

MINECO is acknowledged for funding (MAT2015-62725-ERC and MAT2015-68906-R).

P100 Potential use of decellularised human vein for coronary arterial grafting

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Background and Aims: The development of arterial-like vascular conduits is desirable to overcome the problem of early vein graft thrombosis, late vein graft thickening, and artificial grafts infection of autologous, xeno- and artificial graft conduit used for coronary surgery. One potential approach is the use of decellularised venous extracellular matrix scaffolds seeded. The aim of this study was to assess the suitability of decellularised human saphenous vein (hSV) for arterial grafting.

Methods and Results: Segments of surplus human saphenous vein (hSV) were decellularised with sodium dodecyl sulphate (SDS). The number of detectable nuclei in hSV by haematoxylin and eosin staining (H&E) staining was significantly reduced with 0.1-0.025% SDS ($n=10$, $p<0.05$). However, no significant reduction in DNA content was observed after the decellularisation protocol with any concentration of SDS ($n=10$, $p>0.05$). Culturing decellularised hSV for 14 days and assessing cell proliferation by BrdU incorporation revealed an absence of cell proliferation ($n=10$, $p < 0.02$). Elastin van Gieson (EVG) staining was performed to assess extracellular matrix composition and revealed no significant differences in elastin and collagen content with all concentrations of SDS ($n=10$, $p>0.05$), while reduced levels of α-elastin were detected with 0.1-0.025% SDS compared to control segments ($n=10$, $p< 0.05$). The biocompatibility of decellularised hSV segments was good, with viability and proliferation of three human cell types; adipose derived stem cells (hADSC); umbilical vein endothelial cells (HUVEC) and saphenous vein smooth muscle cells (hsvSMC). Decellularised hSV mechanical properties were suitable for grafting and the burst strength of decellularised hSV was not significantly reduced compared to intact hSV ($n=3$, $p>0.05$). Decellularised hSV was implanted into the carotid artery of pigs ($n=3$) and retrieved after 1 month. The lumen area post implantation increased in size as compared to pre-implantation and native artery lumen area. An endothelial lining was observed along the decellularised graft but the vast number of cells repopulating the graft were to be further analysed.

Conclusion: The decellularisation protocol was effective and did not affect the extracellular matrix and mechanical properties of veins. In addition, the resulting decellularised matrix showed high biocompatibility and no cytotoxicity when seeded with hADSCs, HUVECs and hsvSMCs. The *in vivo* implantation shows a promising results but further analysis is required.

P103 Raman spectroscopic analyses of bone-specific matrix quality produced by in vitro cultured jaw periosteal cells (JPCs)

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INTRODUCTION: The establishment of serum-free culture conditions have to be considered in order to achieve safe tissue engineering (TE) constructs. We compared in the present study the quality of bone-specific matrix produced by MSCA-1$^+$ cells isolated from the human jaw periosteum tissue and cultured under serum-free and –containing culture conditions by the label- free, non-invasive Raman technology.

METHODS: the MSCA-1$^+$ subpopulation was magnetically separated from the entire cell population derived from the human jaw periosteum and JPCs were osteogenically induced for 20 days under FCS-containing and xeno-free culture conditions. Raman spectra from 3 donors were measured from up to 100 random points within each cell culture dish after day 5 and 20 of differentiation. The quality of formed mineral crystals was assessed by calculating following Raman spectral ratios: hydroxyapatite/phenylalanine (961/1003 cm^{-1}), hydroxyapatite/amide III (961/1280 cm^{-1}), carbonate-to-phosphate (1075/961 cm^{-1}), and hydroxyapatite crystallinity (the inverse of full-width half maximum (FWHM) of the hydroxyapatite peak at 961cm^{-1}). The quality of the formed collagens was assessed by calculating the proline/hydroxyproline (853/872 cm^{-1}) and the nonreducible to reducible crosslink ratio (1658/1682 cm^{-1}).

RESULTS: MSCA-1$^+$ JPCs revealed higher levels of hydroxyapatite formation and higher mineral to matrix ratios under serum-free culture conditions, whereas higher carbonate to phosphate ratios and higher crystallinity were detected in JPCs cultured under serum-containing conditions. Concerning the collagen quality and production, we obtained significantly lower collagen production by serum-free cultured JPCs. Additionally, higher collagen maturity as revealed by significantly lower proline/hydroxyproline spectral ratios indicate higher amounts of cross-linked collagen under serum-free culture conditions.

DISCUSSION & CONCLUSIONS: Our findings demonstrate immature bone formation to a higher extend under FCS-containing culture conditions. However, analyses of the mechanical stability of the formed matrix should be correlated to the Raman measurements to validate the accuracy of this approach. Raman spectroscopy represents an optimal and intriguing technology to detect differences in bone matrix quantity of TE constructs.

P104 Development of zinc and strontium based phosphate glass scaffold as a material for bone tissue engineering

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Phosphate-based glass scaffolds with the formula of (P_2O_5)–(Na_2O)–(TiO_2)–(CaO)–(SrO) or (ZnO) have been made to examine its ability to work as scaffold material for bone repair. These were made in two different compositions according to previous work by sintering phosphate glass powder (figure 1), then further studies were done to examine mechanical properties by measuring the diametrical tensile strength and physical properties by measuring the porosity percentage. Another study was done to optimize seeding density technique of human mesenchymal stem cells (hMSCs) by coating the low attachment 24 well plate by (HEMA) polymer and coat the scaffolds with fibronectin and collagen 1 as both of them have been proved to enhance cell attachment to surfaces, this was followed by CCK biocompatibility study and Confocal images. The results showed that increase temperature in scaffold sintering procedure may lead to the decrease in scaffold porosity and that the height sintering temperature directly related with the diametral strength. Cellular studies revealed that coating scaffold may play positive role in biocompatibility and this was confirmed by the confocal images (figure 2). These results displayed the possibility of developing scaffold with accepted porosity and mechanical strength by sintering. Moreover, the results showed that coating the scaffold with collagen 1 may enhance cell attachment and also stimulate biocompatibility.

Keywords: scaffold, phosphate based glass, biocompatibility

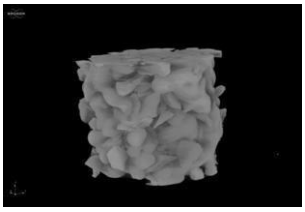

Figure1: phosphate based glass

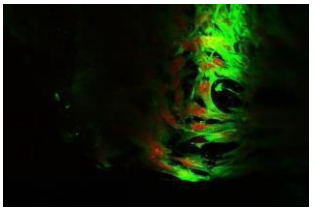

figure2: collagen coated scaffold

P105 Mesoporous bioactive glasses accelerate the scaffolds colonization by preosteoblast cells

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Macroporous scaffolds are becoming a very important tool in the field of bone regenerative therapies. These scaffolds can be designed as 3D substrates to fabricate tissue ex vivo, but also for in situ bone regeneration. In this last case, the success of the implant depends on its capability to attract osteoprogenitor cells towards the interior of the structure. In this work, we have prepared 3D macroporous scaffolds made of mesoporous bioactive glass (MBG)-polycaprolactone (PCL) composites, with the aim of evaluating the migration and colonization capabilities of pre-osteoblast cells. The experiments proposed in this work have allowed the evaluation of cell behaviour within the different levels of the scaffolds, i.e. from the initials source of cells towards the farther scaffold locations.

Experimental

PCL and MBG-PCL scaffolds have been prepared by robocasting, a layer by layer rapid prototyping method, by stacking of individual strati (Figure1a). MC3T3-E1 preosteblast-like cells were seeded on stratus 1. Thereafter strati 2 and 3 were gently stacked and proliferation, differentiation, cytotoxicity and cell morphology were studied for 30 days on each individual stratus.

Results and discussion

MC3T3-E1 preosteblast proliferated and differentiated much better onto MBG-PCL composites. In addition, the presence of MBG allowed the cell movement along its surface, reaching the upper stratus faster than in pure PCL scaffolds. The scaffolds colonization depends on the chemical stimuli supplied by the MBG dissolution and surface changes associated to the apatite-like formation during the bioactive process. Finally, scanning electron and fluorescence microscopy revealed that cell migration strongly depends on the layers interconnections, as they were used as migration routes to reach the farther scaffolds locations (stratus 3) from the initial cells source (stratus 1).

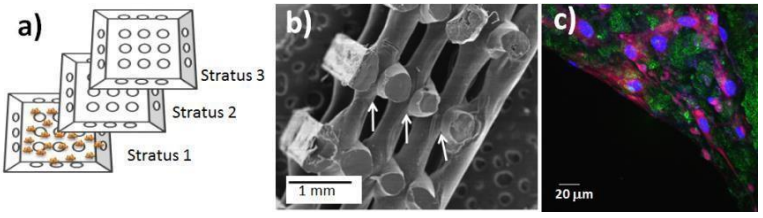

Figure 1. (a) Scheme of the experimental setup. (b) SEM image of an individual stratus. Arrows point to layers interconnections. (c) Laser scanning microscope images of PCL-MBG from Z sections of strati 2, showing cells moving upwards through layers interconnections.

These results indicate some of the factors to consider for accelerating regeneration processes under in vivo conditions.

P106 Protective effects of mechanical stimulation and extremely low frequency pulsed electromagnetic fields in human osteoblast exposure to cigarette smoke extract

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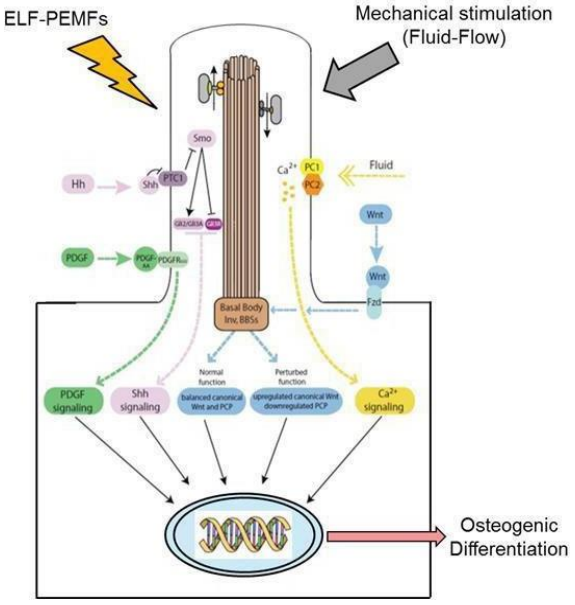
Progressive studies have demonstrated the deleterious effects of cigarette smoking on bone integrity. The best known negative consequences are loss of bone mineralization, increased incidence of fractures, delayed fracture healing and osteoporosis. Bone density is maintained by the balance between bone forming osteoblasts and bone resorbing osteoclasts. Bone is an exquisitely mechanosensitive organ, and its homeostasis depends on the ability of bone cells to sense and respond to external loads, mechanical, chemical and thermal stimulations. This is achieved via an immotile microtubule-based sensory organelle called primary cilia. Several studies have shown that primary cilia also act as mechanosensors in bone and play important roles in proliferation, differentiation and maintenance of bone integrity. We have shown previously that extremely low frequency pulsed electromagnetic fields (ELF-PEMFs provided by Somagen ®, Sachtleben GmbH Hamburg) exposure improved the viability and maturation of osteoblast. Clinical studies have shown that PEMFs increased bone mineralization and prevented bone loss. However, the role of PEMFs in preventing bone loss is not fully understood. Therefore, the project is aimed at investigating the effects of PEMFs and mechanical stimulations under shaking conditions in regulating the effects of cigarette smoke extract (CSE) in primary cilia structure and function during osteogenic differentiation. Our study revealed a significant reduction of primary cilia length in human osteoblasts exposed to physiological concentrations of CSE. The loss of cilia structure also correlated with decreased AP activity.



Restoration of primary cilia structure by ELF-PEMFs improved osteogenic differentiation of human osteoblasts exposed to CSE. Moreover, mechanical stimulation induced by fluid flow also markedly improved osteogenic differentiation of human osteoblasts exposed to CSE. Therefore, protection of primary cilia and its restoration by mechanical stimulations under shaking conditions and ELF-PEMFs could be proposed as an alternative therapy in improving bone function and enhanced fracture healing in smokers.

P109 Fragility fractures treated by minimally invasive plating augmented by IlluminOss® system combined with early weight bearing, the first cases

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Osteoporotic fractures will, in line with demographic developments, increase further in the coming years. This will result in concomitant raised health care costs and burden our health care system. Improvements can for example be realised by early(-ier) functional aftercare and therewith maintain independency of these elderly. In order to achieve early functional aftercare in the more fragile osteoporotic bone more stability of your construct is needed.

The IlluminOss® system, a minimally invasive patient-specific intramedullary fracture fixation system, has the qualities of providing this extra stability.

The IlluminOss® system is a light curable polymer-filled intramedullary balloon which forms to the bone instead of the fracture forms to the implant. After curation the polymer is easily drillable with excellent grip of your screws, not only on two thin osteoporotic cortices but along the whole intramedullary filled canal.

Hereby we present you the first 22 IlluminOss® augmentated cases published.

Patients were collected in two educational hospitals in the Netherlands between July 2015 and November 2016. Operations were performed by three trauma-consultants. IlluminOss® augmentation was added in cases with proven severe osteoporosis and/or failure of conventional treatment in combination with 'difficult' fractures. This is a retrospective caseserie of prospective collected data.

We treated 22 patients (table), 14 femur fractures combined with LISS-system, 6 humerus fractures combined with LCP system, 1 olecranon fracture combined with zuggurtung and 1 fibula fracture combined with LCP system. No hardware failure was obtained. Mean follow-up 8 months, consolidation at average 4,5 months. No superficial or deep infections. All patients returned to their previous mobility. Weigth bearing (femurs) was allowed after 6 weeks guided on pain scores, humerus, olecranon en fibula fractures had a functional aftercare.

Femur	Periprostetic	3
	Severe ostoporosis	10
	Intra-articular and metafysair # with osteoporosis	1
Humerus	Severe osteoporosis and failed conventional therapy	1
	Pathological fracture	1
	Subcapital fracture combined with severe osteoporosis	4
Olecranon	Severe Osteoporosis	1
Fibula	Severe Osteoporosis	1
Total		22

Combination of intramedullary support with the light curable polymer-filled balloon and conventional treatment gives excellent support in fragility fractures and/or after failed conventional techniques. This new method in our arsenal gives us more opportunities on early weight bearing with less loss of independency of this fragile population. Further research (randomised controlled trial) is needed to support our results.

P110 Thin degradable coatings for optimization of osteointegration associated with simultaneous infection prophylaxis

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The colonization of biomaterials with bacteria represents the main cause of implant-associated infections. Both an antibiotic prophylaxis and a faster osteointegration can be obtained by incorperation of bactericidal active metals in degradable CaP coatings. At present there is no reliable method on the basis of thermal spraying to get thin homogeneous layers containing silver, copper and bismuth in bacteriostatic / bactericidal concentrations. The aim of the proposal is the development and optimization of high-velocity suspension flame spraying (HVSFS) process for producing thin resorbable bioactive ceramics coatings on the basis of degradable calcium phosphates and bioactive glasses. In these layers bacteriostatic / bactericidal effective metals silver, copper and bismuth were integrated.

For HVSFS process initially, appropriate suspensions with finely dispersed sub-micron and nanoscale particles doped with silver, copper and bismuth were produced. Due to the finer microstructure suspension sprayed coatings differ significantly in terms of mechanical, physical and biological properties of conventionally sprayed coatings. We have shown that thin Ca/P-containing layers without doping can be prepared with the aid of the HVSFS coating process. With the help of such coatings a faster ingrowth and significantly improved mechanical stability after operation is expected. We have succeeded in establishing the necessary process for the HVSFS suspensions and in obtaining very finely structured, thin homogeneous layers. With the help of such coatings a faster ingrowth and significantly improved mechanical stability after operation is to be achieved. The materials without metal additive show no change in biocompatibility after deposition on a metal substrate. The incorporation of silver, copper and bismuth in degradable CaP coatings has been not studied until now. For preparation of suitable suspensions consisting of fine particulate materials with a doping of these elements, there are several alternative routes. Silver, copper or bismuth-containing thin coatings by HVSFS process can be produced. These layers do not change the biocompatibility of the applied raw materials and enable continuous release kinetics of metal ions. We study the release kinetics of the various ions and the effect of different cell cultures in vitro, and analysed the antibiotic effectiveness of the spray coatings. Assessment of the quantitative, time-dependent in vivo degradation of the layers of material and the quality of the osteointegration was performed by means of an established animal model in rabbits.

P112 Fluid flow enhanced osteogenic differentiation of human embryonic stem cell derived mesenchymal progenitors (hES-MP cells) using perfusion flow reactorsystem

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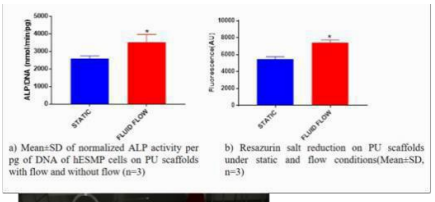
Abstract: Bioreactors can be used to apply fluid flow *in vitro* to scaffolds to improve diffusion and apply mechanical forces to cells. This work uses an autoclavable, modular perfusion bioreactor designed in house that is suitable for large scaffolds. We investigated the effect of fluid flow shear stress on osteogenic differentiation of human embryonic mesenchymal progenitors (hES-MP cells). hES-MP cells were cultured on polyurethane (PU) scaffolds (30 mm diameter × 5 mm thickness) with seeding density of 5×10^5 cells. The effect of flow was investigated by comparing with the scaffolds in static condition. Flow scaffolds were transferred to the bioreactor on day 3 and a constant flow rate of 4 mL/min applied using a peristaltic pump. Osteogenic induction media was also applied to both conditions at this time point. Alkaline Phosphatase activity (ALP) was used to evaluate the osteogenesis and resazurin salt reduction (RR) used to measure cell metabolic activity on day 10. A significant increase in RR in PU scaffolds subjected to flow than static confirms higher metabolic activity under flow condition. Higher flow also significantly increased ALP activity in the cells after normalizing to total DNA. These results confirm the responsiveness of hESMP cells to fluid flow and that scaffolds capable of filling large defects can be maintained *in vitro*. However, further work is necessary to optimize the flow rate and investigate different scaffold sizes, geometries, and materials that are more suitable for clinical applications.



a) Mean±SD of normalized ALP activity per pg of DNA of hESMP cells on PU scaffolds with flow and without flow (n=3)

b) Resazurin salt reduction on PU scaffolds under static and flow conditions(Mean±SD, n=3)

c)Perfusion bioreactors set up for studying fluid flow effect on osteogenesis

P114 Behaviour of a new formulation of fixing cement in joint prostheses with osteomyelitis: a rabbit model

Blanca Ibarra[1,4], Joaquin García[2], Galo Azuara[3], Miguel A Ortega[1,4], Ángel Asúnsolo[5], Mar Fernandez-Gutierrez[4], Natalio García-Honduvilla[1,4], Basilio Dela Torre[6], Julio San Román[4], Julia Buján[1,4]

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Joint prostheses improve quality of life but they can fail due to a long variety of factors, being the *Staphylococcus aureus* infection one of the most common and most severe one. The search for fixing bone cements with less reactivity on bone tissue, more capacity to release antibiotics and an adequate response in presence of infection is one of the main challenges nowadays. In this study we used New Zealand rabbits to evaluate the response of bone tissue by introducing a new formulation of experimental fixing cement (EC) that includes PLGA (Poly-lactic-co-glycolic acid) microspheres in presence of *S. aureus* infection. We observed different patterns when analyzing the degree of disruption of bone tissue. The conservation of bone histoarchitecture was significantly better in groups with the EC, both in control and infected groups as well as in the ones which also had antibiotics. In addition, the macrophage counting (RAM-11) was significantly higher in non infected samples with CC in comparison with the ones with CE, showing the infected groups with EC a better macrophagic response.

Keywords: regenerative medicine, osteomyelitis, PLGA, macrophagic response

ACKNOWLEDGEMENTS: This work was supported by grant from SECOT (2016/0055 C/SECOT).

P115 The use of an injectable scaffold for the reconstruction of critical-size mandibular defects in rabbits

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University of Glasgow, Glasgow, UK

Introduction. The reconstruction of critical-size bone defects in the maxillofacial region following trauma or cancer treatment remains a challenge. The gold standard for the reconstruction of such defects is autogenous bone graft. This is because bone possesses the three properties required for bone regeneration; osteogenesis, osteoinduction and osteoconduction. But harvesting grafts results in significant patient morbidity. As a result, several scaffolds have been developed to replace grafts. This project evaluated a novel injectable form of scaffold, together with Bone Morphogenetic Protein 7 (BMP-7) and mesenchymal stem cells (MSCs), in the regeneration of bone in critical-size mandibular defects in rabbits.

Materials and Methods. The scaffold construct was developed to induce bone formation within a pedicled muscle flap. A critical-size defect (20×15 mm^2) was created in the mandible of ten rabbits. The masseter muscle was adapted to fill the surgical defect and a combination of calcium sulphate/hydroxyapatite cement (CERAMENT™, SPINE SUPPORT), BMP-7 and MSCs was injected into the muscle (Figure 1). Bone regeneration was evaluated 3 months after surgery.

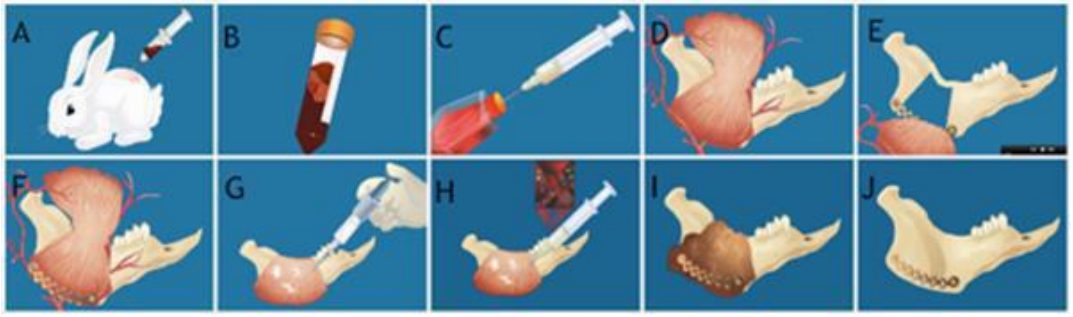

Figure 1 - Summary of the steps in the in-vitro and in-vivo research protocol.

Results. Limited areas of bone formation bridged the defect, despite new bone formation throughout the muscle and within the connective tissue. The bone was thicker in the bucco-lingual direction compared to the contra lateral (non-operated) side. Quantitative histomorphometric assessment showed that the average bone surface area was 21.2±6 mm^2, this was significantly greater than that of the contra-lateral side. The amounts of residual cement and soft tissue were 20±12% and 41±10%, respectively. Dynamic histomorphometry showed and average mineral apposition rate (MAR) of 1.92 µm/day.

Discussion/Conclusion. The findings demonstrated the potential use of local muscle flaps for injectable bio-cements loaded with BMP and seeded with MSCs to induce bone formation, but further development of this approach is required to improve the physical nature of the cement to allow a better diffusion of the material within the muscle tissue.

P116 Clinical translation of a tissue engineering product for bone healing: Bioprocess design, preclinical studies and human use

Daniel Chaverri[1], Daniel Vivas[2], Santiago Gallardo-Villares[1], Irene Oliver-Vila[2], Marian Vives[3], Ana Carreño[3], Margarita Blanco[2], Margarita Codinach[2], José Ramirez[3], Javier Pinto[1], Ruth Coll[2], Màrius Aguirre[4], Joan García-Lopez[2], Joaquim Vives[2], Fernando Granell-Escobar[1], Salvi Prat[3]

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Background

Deficits in bone remodelling activity due to osteonecrosis, complications after trauma injuries, and aging lead to pathologic conditions such as avascular necrosis of the femoral head, lumbar spondylolisthesis and pseudoarthrosis. The latter is a relatively frequent complication of fractures, in which the lack of mechanical stability and biological stimuli results in the failure of bone union, most frequently in humerus and tibia. Treatment of recalcitrant pseudoarthrosis relies on the achievement of satisfactory mechanical stability combined with adequate local biology. Herein we present the development of a Tissue Engineering (TE) product composed of Mesenchymal Stromal Cells derived from Bone Marrow (BM-MSC) in combination with bone particles, from conception up to its use in the clinical setting in two cases of atrophic pseudoarthrosis.

Materials and Methods

Feasibility of the treatment and osteogenic potential of the cell-based medicine was first demonstrated in an ovine model of critical size segmental tibial defect. Additional preclinical studies included the analysis of the cytogenetic and phenotyopic characteristics of the cell-based TE product following Good Laboratory Practice guidelines. Clinical grade autologous BM-MSC were produced following a Good Manufacturing Practice (GMP)-compliantbioprocess.

Results and Conclusions

A GMP-compliant bioprocess was developed for high titre cell expansion of BM-MSC in approximately 23 days. Cells were successfully loaded on natural bone scaffolds at 0.3-1x10^6 viable BM-MSC/cc. Preclinical studies confirmed the identity, purity, osteogenic capacity and genetic stability of BM-MSC, thus demonstrating the safety of the cellular component as well as in combination with bone matrices. Clinical results were successful in one case, with pseudoarthrosis resolution, and inconclusive in the other one. The first patient presented atrophic pseudoarthrosis of the humeral diaphysis and was treated with osteosynthesis and TE product resulting in satisfactory consolidation at month 6. The second case presented a recalcitrant pseudoarthrosis of the proximal tibia and the Masquelet technique was followed before filling the defect with the TE product. This patient presented a neuropathic pain syndrome unrelated to the treatment that forced the amputation of the extremity three months later. In this case, the histological analysis of the tissue formed at the defect site evidenced neovascularisation but no overt bone remodelling activity. We conclude that the use of expanded autologous BM-MSC to treat pseudoarthrosis was demonstrated feasible and safe, provided that no clinical complications were reported, and early signs of effectiveness were observed.

P117 Preparation and properties of stromal cell-derived factor-1-loaded gelatin/nano-hydroxyapatite composite scaffold

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Repair of large bone defects is a considerable challenge in orthopaedic surgery. The chemokine stromal cell-derived factor-1 (SDF-1) has been shown to recruit bone marrow stromal cells to injured tissue and is suggested to be a promising candidate for in situ bone tissue engineering. In this study, gelatin/nano-hydroxyapatite composite scaffolds with SDF-1 (GGHAS) were prepared by mixing gelatin, genipin, hydroxyapatite nanoparticles (PGAHA) and alginate microspheres containing SDF-1. PGAHA was synthesized by a co-precipitation method in presence of sodium polyglutamate. SDF-1-loaded alginate microspheres were prepared by a coaxial air-flow droplet generator, followed by ionotropic gelation with calcium ions. Scaffolds were characterized with respect to morphology, porosity, water uptake, degradation rate and in vitro cell compatibility. The effect of SDF-1 released from GGHAS on the migration of bone marrow stromal cells was assessed using a transwell system. The result of X-ray diffraction confirmed that PGAHA was hydroxyapatite. The PGAHA had a rod-like shape with a diameter of about 15-30 nm. The alginate microspheres had a uniform diameter of about 250 um. GGHAS had a porous structure with high porosity of 83% and water absorbency of 580%. Cytotoxic tests demonstrated that the extract of the GGHAS scaffold promoted the proliferation of bone marrow stromal cells. In vitro chemotaxis assay showed that the optimal concentration of stromal cell- derived factor-1 for migration of bone marrow stromal cells was 500 ng/mL. Moreover, three- dimensional chemotaxis migration assays exhibited that a higher degree of bone marrow stromal cells migrated to the SDF-1-loaded scaffold than scaffold without SDF-1. Thus, GGHAS could serve as a scaffold for bonerepair.

P118 New strategies for the evaluation of knee osteonecrosis treatments: an in vitro model

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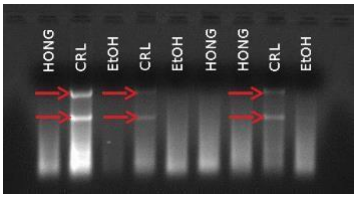
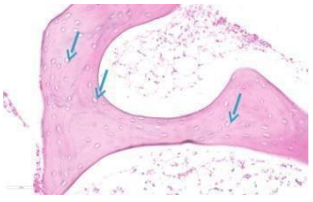
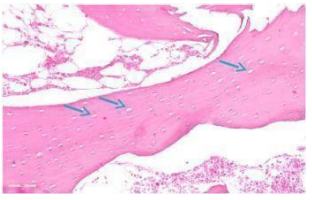
Osteonecrosis (ON) affects mainly the femoral head and the knee, and the management of this disorder is complex and frequently not effective. Many experimental animal models have been proposed to study new approaches to prevent and treat hip ON. On the contrary, little or nothing is reported in literature about *in vitro* and *in vivo* models of knee ON, except for a paper describing ON induction through freezing and thawing in liquid nitrogen (Hong 2013). Given the increasing interest in developing more and more reliable *in vitro* tests and in the perspective of further *in vivo* studies on the treatment of knee ON, we tested the feasibility of a long term bone tissue culture as a model reproducing ON *in vitro,* comparing two different methods of ONinduction.

Rabbit distal femoral condyles, harvested at euthanasia from an uncorrelated study not affecting the health of bone or joints, underwent ON induction. A first group was subjected to three cycles of 4 minutes each of freezing in liquid nitrogen and thawing at 37°C (HONG), while a second group received an intraosseous injection of pure ethanol (EtOH). The control group (CRL) was left untreated and all bone specimens were cultured up to 28 days. Alamar blue viability assay was performed at time zero and periodically after the treatments, while the weight of the samples was measured at time zero and after 28 days. At the end of experimental time, specimens were collected for paraffin histology and analysis of RNA yield andintegrity.

Viability at time zero was high and comparable among samples. After 24 hours, the viability decreased sharply and never recovered over the 28 days. Conversely, the control group maintained a stable viability. No variation in weight was observed. The RNA yield from ON samples at 28 days was reduced. Moreover, while the RNA obtained from the control group was of high-quality, the samples from ON induction groups showed poorer A260/280 and A260/230 ratios and were highly degraded. Histological assessments of both ON groups showed signs of necrosis, in particular the presence of empty lacunae, in comparison to control group that preserved the normal bone structure.







In summary, data collected so far showed the feasibility and effectiveness of both methods to induce ON in a model of long term bone culture. This will allow to test *in vitro* potential treatments and in particular tissue engineering approaches. The opportunity to reproduce successfully ON in *in vitro* models will be also fundamental for the evaluation and selection of treatments before animal testing, in accordance with the principle of the three Rs (replacement, reduction, and refinement).

P119 Influence of fiber spacing on mechanical properties of lattice structured titanium bone grafts: A three-dimensional finite element study

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Bone injury owing to trauma or osteosarcoma often calls for the application of bone substitutes or bone grafts. Recently, isoelastic, metallic 3D-printed porous bone grafts have shown promising results for this purpose. However, numerous parameters related to the design of 3D-printed bone grafts require investigations. Therefore, this study is aimed at developing a finite-element (FE) based numerical framework in order to investigate the influence of lattice architecture parameters on the effective mechanical properties of the 3D-printed bone grafts. The fiber diameter of the Ø7mm×9mm Ti_6Al_4V bone grafts was 0.6mm. The fiber spacing varied from 0.3 mm to 0.7 mm. The lay deposition angle was 0°/90°. The CAD model was developed in SolidWorks and was meshed with 10-node tetrahedral elements in Ansys FE software. Virtual compression testing was performed using Ansys, wherein, the bottom surface was fully constrained and a gradual displacement was applied at the top surface. The effective Young's modulus of the bone grafts were obtained from the predicted force-displacement curve for each of the virtual compression testing. The FE-predicted Young's modulus of the bone grafts ranged between 5GPa – 17GPa (Fig. 1), exhibiting its isoelastic property. A gradual decrease in the effective Young's modulus of the bone grafts was observed with an increase in the fiber spacing, similar to the trend reported by Li et al. [1] in their experimental work. The quantitative deviations in the Young's modulus of the bone grafts may be attributed to the differences in mechanical properties of 3D-printed dense Ti_6Al_4V blocks and cast Ti_6Al_4V blocks. Nonetheless, the qualitative corroboration provided confidence in the developed numerical framework and its applicability in designing bone grafts for site-specific applications.

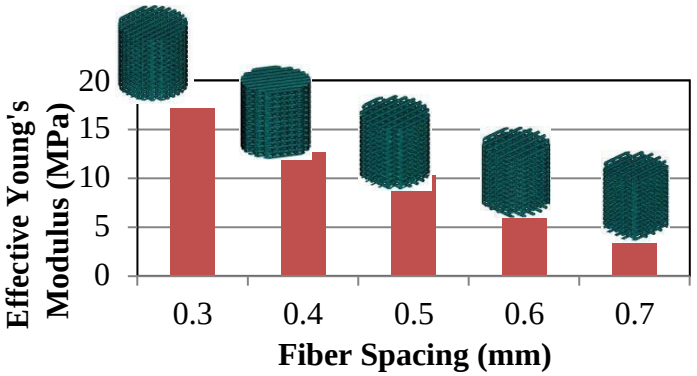

Fig.1: Influence of Fiber Spacing on Effective Young's Modulus of the Bone Graft

P120 Image-based histological evaluation of scaffold-free 3D osteoblast cultures

Sebastian Eggert[1], Jutta Tuebel[1], Peter Foehr[1], Lara A. Kuntz[1], Andreas Obermeier[1], Carmen Marthen[1], Christian U. Große[2], Rainer Burgkart[1]

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Motivation: The analysis of tissue network characteristics and cell distribution using immunohistochemical and histochemical methods is widely used by the tissue engineering community. Hereby, imaging analysis still relies heavily on manual evaluation methods, also known as semi-quantitative analysis, which are time-consuming and to a certain degree subjective. For this reason, automated imaging processing methods have an enormous potential to increase sample processing and to reduce the variation that is caused by an individual evaluation. The object of this project was to develop a suitable evaluation method in terms of histological analysis for mechanically compressed samples of scaffold-free, human osteoblast-derived 3D histoids after long-term culture (50 weeks).

Materials and Methods: Samples (n=3 each) with a diameter of 6 mm were compressed by 20%, 40% or 60%, respectively and evaluated by histology using the Van Gieson staining. Images were then analyzed by an automated approach based on the open source software framework ImageJ and the plug-in Angiogenesis Analyzer to evaluate the quantitative degree of tissue damage within the histoids with respect to their compression value. The raw images (Figure 1A) were converted into binary images followed by the analysis of the network organization of the skeleton (Figure 1B). The network parameters i) number of nodes and ii) strut length between two nodes were used to conduct a strut analysis and calculate the parameter iii) Node-to-Free ratio (N/F ratio), which is defined as the ratio between the number of nodes and number of endpoints.

Results and Conclusion: The N/F ratio revealed a median value of 1.29 for the control, whereas the values for the mechanically compressed samples decreased to 0.97 for 20% compression, 0.85 for 40%, and 0.86 for 60% (Figure 1C). Furthermore, the performed strut analysis demonstrated a decrease in the connected branches and junctions of the matrix structure with increasing compression rate. However, no significant differences could be observed due to a reduced group size in this pilot study; thus an increased number of samples is necessary for further evaluation of this method. In conclusion, the designed imaging processing approach was successfully established and future histological analysis will greatly benefit from this integrating automated, software-based evaluation method. Therefore, we will use this method for high-throughput analysis of our patient specific 3D osteoblast-derived cell cultures.

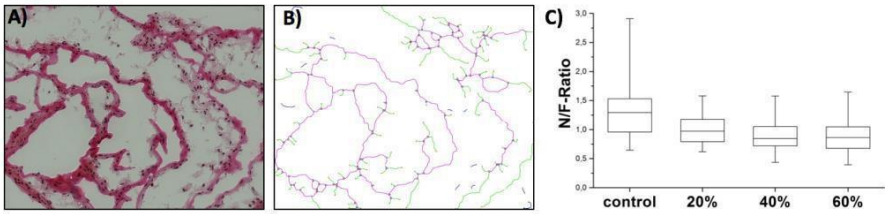

Fig. 1: Raw images (A) after staining (Van Gieson), results of the network organization (B) using the software tool ImageJ, and evaluation of the Node-to-Free ratio between all samples (C).

P121 In vivo results of the osteogenic potential of elastin-like recombinamers and citrate microarchitectured hydrogels with tailored matrix stiffness

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Stem cells differentiation is known to be strongly affected by signals provided by the scaffold they are seeded on. Among the myriad of properties characterizing a specific scaffold, matrix stiffness has been shown to be enough to drive in vitro Mesenchymal Stem Cells (MSCs) differentiation both in 2D and 3D.

Using Elastin-like recombinamers (ELRs) containing RGD as starting materials, hydrogels were produced by crosslinking with citric acid, an essential molecule for proper in vivo mineralization of bone3, our target tissue

Herein we report the in vivo implantation of ELRs-citrate hydrogels, so the osteogenic potential of samples demonstrate that the fibrilar structure of the hydrogel promotes bone formation without adding cells or any kind of growth factor.

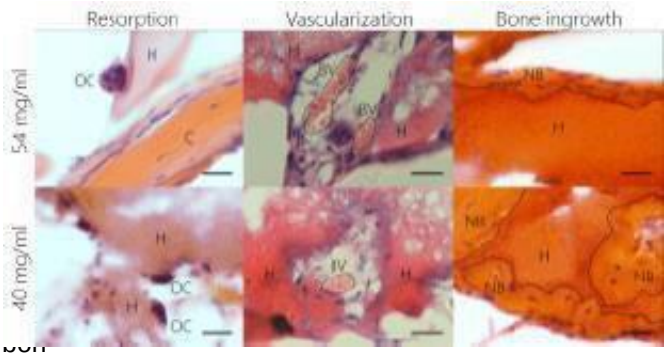
EXPERIMENTAL METHODS

ELRs were crosslinked with citric acid and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide at polymer concentrations of 54 mg/ml (osteoid-mimicking hydrogels) and 40 mg/ml (samples with low Young's modulus).

In vivo implantation. Two 3.5-mm calvarial defects per animal were created on mice. 40 mg/ml and 54 mg/ml hydrogels were implanted. Void defects were used as negative control. At 1 and 2 months post-implantation, new bone formation was assessed by Micro-computed Tomography (µCT) and histology.

RESULTS AND DISCUSSION

Both types of hydrogels supported the formation of new bone, compared to void defects. In vivo, 40 mg/ml matrices led to improved regeneration in comparison to 50 mg/ml ones, as revealed by µCT and Goldner's Trichrome staining. Overall, the amount of new bone formed was found to be higher in the case of hydrogels with the lower mechanical properties.



The mechanical properties of the hydrogel seem not to have a great effect on the bone formation, as the hydrogel with lower young modulus showed the larger bone formation. This is due to that this hydrogel is less dense and allows cell penetration and more bone formation inside the gel. In summary, herein described hydrogels have been proven to be an interesting approach to achieve in situ bon regeneration.

MINECO is acknowledged for financial support (MAT2012-38793 and MAT2015-68906-R

P122 Hypoxia-mimicking porous calcium/ phosphate coated silk scaffolds with controllable cobalt ion release for bone tissue engineering

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Low oxygen pressure (hypoxia) plays an important role in stimulating angiogenesis; there are, however, few studies to prepare hypoxia-mimicking tissue engineering scaffolds. Silk fibroin (SF) is a natural polymer with great permeability to oxygen and water, good mechanical properties, low immunogenicity and good cell adhesion and growth characteristics. Ionic cobalt (Co) is established as a chemical inducer of hypoxia-inducible factor (HIF)-1α, which induces hypoxia-like response. The aim of this study was to develop hypoxia-mimicking a nanocomposite scaffold based on silk/calcium phosphate (Ca/P)/Co by freeze-drying and investigate if the addition of Co (2+) ions would induce a cellular hypoxic response in such a tissue engineering scaffold system. The highly porous scaffold possessed appropriate chemical and physical structure as confirmed by FTIR, XRD and SEM analysis and the cellular effects of scaffold on the proliferation, differentiation, bone and endothelial related gene expression of mesenchymal stem cell (MSC) were systematically investigated. The results showed that low amounts of Co (<5%) incorporated into scaffolds had no significant cytotoxicity and that their incorporation significantly enhanced bone and endothelial related gene expression in MSCs, and also that the silk Ca/P/Co scaffolds support MSC attachment and proliferation. Our results indicate that incorporating cobalt ions into silk Ca/P scaffolds is a viable option for preparing hypoxia-mimicking tissue engineering scaffolds and significantly enhanced angiogenesis. The hypoxia-mimicking silk Ca/P/Co scaffolds have great potential for bone tissue engineering applications by combining enhanced angiogenesis with already existing osteogenic properties.

P123 Novel synthesized magnesium alloy with improved bio-corrosion behavior for bone regeneration applications

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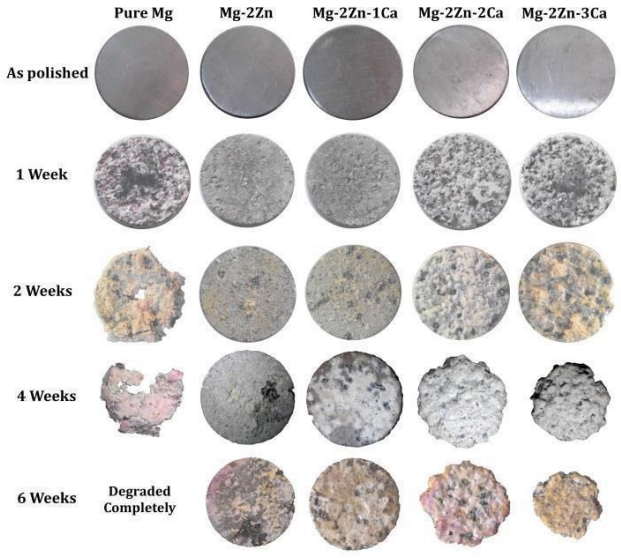
Biodegradable magnesium (Mg) implants are one of the attractive materials among other conventional non-degradable implants such as titanium, stainless steels and cobalt-chromium alloys due to their well degradation behavior without toxicity effect. The mechanical properties of Mg alloys are closed to that of natural bone and therefore by reducing stress-shielding in between Mg alloy and bone tissue can further help bone healing. Beside tuning the degradation rate of the implanted scaffold, to assure providing enough space for new generated bone tissue, the degradation product has to be considered as well when designing in organic implantable alloy. Considering the latter mention factor the aim of the present research was synthesizing a new type of biodegradable Mg alloys comprising zinc (Zn) and calcium (Ca) as alloying elements. While the amount of Zn element was fixed at 2 wt. % changing Ca concentration from zero to 1, 2 and 3 wt. %. The corrosion behavior of Mg-2Zn-xCa (x = 0, 1, 2 and 3 wt. %) alloys was studied by potentiodynamic polarization (PDP) examinations and interestingly Mg-2Zn-1Ca showed better result in comprising of Mg-2Zn-2 and 3 Ca alloys. Scanning electron microscopy (SEM) confirmed that the severe corrosion observed for the pure Mg sample and Mg-2Zn-2 and 3 Ca alloys. Importantly, for heat treated Mg-2Zn-1Ca ternary alloy, the presence of $Ca_2Mg_6Zn_3$ intermetallic phase improved the corrosion resistance (E_{corr}=-1.57 V and i_{corr}=195 µA/cm^2).

In order to find the biocompatibility of synthesized Mg alloys, indirect MTT viability test performed according to ISO 10993-5:2009 standard with Adipose derived Mesenchymal Stem Cells (ASCs). The viability results indicate that Mg alloys have no significant toxicity effect on ASCs viability; however the viability increased in Mg-2Zn-1Ca group. Also, direct ASCs culturing on the surface of Mg alloys represented good attachment and proliferation.

New synthesized biodegradable Mg-2Zn alloy containing 1 wt. % Ca, which is the most abundant element in natural bone tissue, showed considerable improvement in term of degradation product in compare to those of other conventional in organic scaffolds. The corrosion behavior of pure Mg was significantly improved by addition only 2 wt. % Zn and 1 wt. % Ca, which besides providing high mechanical properties makes it a suitable candidate for bone regeneration applications.



Biodegradation behaviour of synthesized Mg alloys in DMEM media at 37C°

P124 Endothelial progenitor cell response to nanocrystalline silicon substituted hydroxyapatite coated 3D macroporous Ti6Al4V scaffolds with adsorbed VEGF

M.J. Feito[1], D. Fernández-Villa[1], D. Pedraza[2], M.C. Serrano[4], H. Alfonso[5], D. Monopoli[5], D. Arcos[2,3], I. Izquierdo-Barba[2,3], M. Vallet-Regí[2,3], M.T. Portolés[1]

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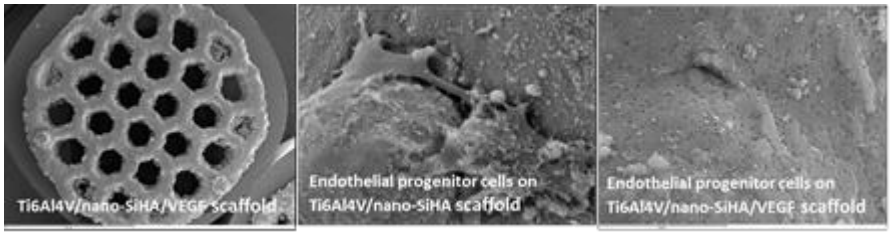
INTRODUCTION: Due to their excellent mechanical properties, metallic implants (i.e. Ti6Al4V) are the most widely used in bone surgical repair [1]. Hydroxyapatite coating on the metallic materials enhances the bond between human bone and metallic implant surface [2]. Porous metallic scaffolds are designed for bone regeneration in order to promote cell adhesion, proliferation and neovascularization, which accelerates the osseointegration process [1]. Vascular endothelial growth factor (VEGF) is involved in both angiogenesis and osteoblast maturation [3] and has been recently adsorbed on nanocrystalline silicon substituted hydroxyapatite (nano-SiHA) disks to improve the adhesion and proliferation of endothelial progenitor cells (EPCs) [4]. In the present study, nano-SiHA coatings have been prepared on macroporous Ti6Al4V scaffolds, followed by VEGF adsorption, to promote EPC adhesion and proliferation on these metallic implants.

METHODS: 3D macroporous Ti6Al4V scaffolds were prepared by electron beam melting. Nano-SiHA (nominal formula $Ca_{10}(PO_4)_{6-0.4}(SiO_4)_{0.4}(OH)_{2-0.4}$) coatings were prepared on Ti6Al4V scaffolds by the dip-coating method. VEGF (2.5 µg/500 µl) was adsorbed for 24h on the scaffold surface. EPCs [5] were cultured on Ti6Al4V/nano-SiHA/VEGF scaffolds ($3x10^5$ cells/scaffold) for 5 days to evaluate cell adhesion, proliferation and differentiation. Cell morphology was observed by SEM.

RESULTS & DISCUSSION: Nano-SiHA coated 3D macroporous Ti6Al4V scaffolds allowed EPC adhesion and proliferation, preserving their characteristic morphology. VEGF improved EPC adhesion and proliferation on these scaffolds. Structures suggesting the presence of fenestrae, typical of endothelial cells [6], were observed in EPCs cultured on Ti6Al4V/nano-SiHA/VEGF scaffolds.



CONCLUSIONS: The obtained results suggest the potential utility of these nanocrystalline Si-substituted HA coated 3D macroporous Ti6Al4V scaffolds with immobilized VEGF for bone repair and tissue engineering by promoting angiogenesis.

P126 The rapid induction of bone formation by the human recombinant transforming growth factor-β_3 in the non-human primate papio ursinus

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The soluble osteogenic molecular signals of the transforming growth factor-β (TGF-β) family are the molecular bases of the induction of bone formation. The mammalian TGF-β_3, pleiotropic member of the family, controls a vast array of biological processes including the induction of bone formation but in primates only. To the contrary, the TGF-β isoform does not initiate the induction of bone formation in rodents and lagomorphs. Our laboratories have studied the induction of bone formation by coral-derived macroporous calcium phosphate-based bioreactors when combined with doses of recombinant hTGF-β_3. Harvested specimens from the *rectus abdominis* muscle on day 15 after heterotopic implantation showed the induction of fibrin-fibronectin rings within the macroporous spaces that provided the structural anchorage for hyper chromatic cells, interpreted as differentiating osteoblastic cells re-programmed by hTGF-β_3 from invading myoblastic and/or pericytic differentiated cells. Harvested tissues on day 15 display *Runx2* and *Osteocalcin* expression significantly up-regulated as compared to untreated coral-derived macroporous bioreactors, correlating to multiple invading cells differentiating into the osteoblastic phenotype. Bioreactors pre-loaded with recombinant human Noggin, a BMP antagonist, show down-regulation of *BMP-2* and other profiled *osteogenic proteins*' genes resulting in minimal if any induction of bone formation on day 30, 60 and 90. Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) evaluated the expression' profile of *BMP-2*, *BMP-3*, *BMP-4*, *BMP-6*, *BMP-7*, and *TGF-β_1*, *TGF-β_2* and *TGF-β_3* in tissue generating bioreactors as well as in the adjacent surrounding *rectus abdominis* muscle. On day 30, 60 and 90, 125 and 250 µg hTGF-β_3/ treated bioreactors showed prominent induction of bone formation. 250 µg hTGF-β_3 showed significant induction of bone formation at the periphery of the implanted macroporous biomimetic matrices only. On day 30, treated macroporous constructs showed the substantial induction of bone formation across the macroporous spaces; on day 60, 250 µg hTGF-β_3/treated bioreactors showed up-regulation of *TGF-β_1* with a six fold increase *vs*. *TGF-β_1* expression in adjacent muscle of untreated constructs that however do initiate the spontaneous induction of bone formation. In hTGF-β_3/treated bioreactors, both the adjacent muscle and the macroporous construct show *BMP-2* up-regulation, correlating with the temporo/spatial rapid induction of bone formation at the periphery of the bioreactor only. To the contrary, hTGF-β_3/treated calvarial defects show inhibitory molecular pathways with *OP-1*, *Osteocalcin*, *Runx-2*, and *ID2* and *ID3* down-regulation set as to avoid encroaching inductive activities of a plurality of morphogens to negate cerebral compression by uncontrolled osteogenesis. Reconstituted hTGF-β_3 implanted in mandibular defects of *Papio ursinus* induces however substantial bone formation with generation of both buccal and lingual corticalized mandibular plates by day 30 post implantation.

P127 Stimulation of human embryonic stem cell-derived mesenchymal progenitors cultured on to porous magnetic microcarriers

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University Of Sheffield, Sheffield, UK

Introduction

Microcarriers have the potential to overcome the limitations experienced with 2D cell culture, which become evident when the vast quantity of cells required for therapeutic treatments are considered[1]. Among the many advantages their high surface to volume ratio[2], and the ability to scale-up with ease, then this makes them attractive prospects for bioreactor technology. The stress induced during dynamic cell culture due to fluid flow can influence osteogenic differentiation, which when combined with porous microcarriers could hold the potential to produce differentiated cells in a 3D environment. Herein we assess the feasibility of dynamic cell culture, by applying an external magnetic field to responsive microcarriers.

Methods

Magnetically responsive microcarriers were produced in batch, using a dual curing microfluidics setup, which utilised UV and heat. Particle surfaces were treated with acrylic acid to improve cell adhesion. Human embryonic stem cell-derived mesenchymal progenitors (hES-MP) were cultured on particles, and tested for their biocompatibility. A modified linear actuator was used to provide stimulus to cultured particles for 30 minutes, daily, for up to 7 days. The results were evaluated by measuring the ALP Alkaline Phosphatase (ALP), and DNA activity with respect to a static plate. Microcarriers were characterised using SEM to investigate the particle and pore size distribution.

Results

Size distribution of imaged microcarriers confirmed their porous and monodispersed nature. Particle-cell viability test after 2, and 9 days showed hES-MPs were successfully anchored to the porous micro-carriers, and continued to proliferate. Additionally, they were stained and imaged to investigate cell-particle interaction.

Discussion

Initial results indicate porous microcarriers are suitable for dynamic cell culture, and a suitable degree of response can be achieved by using commercial magnets. With regards to cell stimulation, several variables are in the process of being tested such as; duration and speed of stimulation to determine optimum conditions.

P128 Novel beta titanium alloy Ti36Nb6Ta is a potential material for bone implants

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Physicochemical properties of artificial materials for implantation influence cell adhesion, proliferation and differentiation. Moreover, biomechanical properties are important for healing and for the stability of the implant in the body. In our study, we have tested novel beta titanium alloy Ti36Nb6Ta compared to standard medical titanium alloy Ti6Al4V using physicochemical methods, nanoindentation as well as cell adhesion, proliferation and differentiation tests.

Discs from both Ti6Al4V and Ti36Nb6Ta were tested using X-ray photoelectron spectroscopy (XPS) using ESCA Probe P (Omicron). A nanomechanical instrument Hysitron TI 950 TriboIndenter™ was used for measurement of mechanical properties of Ti6Al4V a Ti36Nb6Ta and a diamond Berkovich tip as an indenter probe. Continuous measurement mode (CMX) was used to obtain depth profiles of the mechanical properties of the modified titanium alloy.

The discs were seeded by pig mesenchymal stem cells at a density of $60\times10^3/cm^2$. Metabolic activity was evaluated using CellTiter 96® AQueous One Solution Cell Proliferation Assay, cell proliferation using Quant-iT™ ds DNA Assay Kit. Cell spreading area was calculated from photomicrographs after immunohistochemical staining with monoclonal antibody beta-actin, and secondary antibody conjugated with AlexaFluor®488. Cell differentiation was evaluated by alkaline phosphatase assay. In addition, we have evaluated gene expression of osteogenic markers osteocalcin and type I collagen by real-time reverse transcription-PCR (qRT-PCR). For statistical evaluation, One-way ANOVA followed by Student-Newman-Keuls Method was used. For qRT-PCR, the nonparametric Kruskal-Wallis Test and Dunn's Multiple Comparison Test were used.

The full range XPS spectrum of Ti6Al4V alloy proved the presence of Ti, Al, O in a sample, but V was not proved. In Ti36Nb6Ta alloy, Ti, Nb and Ta as well as O were present. The higher reduced storage modulus and indentation hardness we measured on the Ti6Al4V. Ti36Nb6Ta alloy showed reduced storage modulus approximately 48% lower and indentation hardness approximately 44% lower than Ti6Al4V sample.

Metabolic activity was significantly higher on titanium alloy Ti6Al4V compared to the beta titanium alloy Ti36Nb6Ta on days 7 and 14. The spreading area of mesenchymal stem cells was similar on both alloys. Moreover, no differences in alkaline phosphatase assay, as well as the expression of type I collagen, and osteocalcin genes were observed. However, we have observed higher expression of type I collagen compared to osteocalcin on both alloys.

Both beta titanium alloy Ti36Nb6Ta and titanium alloy Ti6Al4V Ti36Nb6Ta supported mesenchymal stem cells' adhesion, proliferation and osteogenic differentiation. Novel beta titanium alloys Ti36Nb6Ta is a promising material for bone implantation.

The project was supported by the Czech Science Foundation: grant No. 16-14758S, and by the Ministry of Education, Youth and Sports NPU I: LO1309.

P129 Evaluation of a scaffold-free 3D osteoblast culture using biomechanical loading and histological characterization

Peter Foehr[1,2], Sebastian Eggert[1,2], Jutta Tübel[1], Andreas Obermeier[1], Lara A. Kuntz[1], Carmen Marthen[1], Belma Saldamli[1], Christian U. Große[2], Rainer Burgkart[1]

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Motivation: Tissue engineered bone or bone-like tissues can be realized by a 3D cell culture approach. Therefore, either a scaffold-based or a scaffold-free technique can be used. It is highly desired that bone or bone-like 3D cell cultures increase their biomechanical strength over time in order to be able to withstand external loading. In this study we used a scaffold-free 3D culture, derived from primary human osteoblasts, cultivated for >56 weeks. To evaluate possible damaging effects, unconfined compression tests were applied in a pilot study, followed by a histological evaluation.

Materials and Methods: Primary human osteoblasts were harvested from the femoral heads of three donors undergoing total hip replacement. 3D cultures with cylindrical shape and a diameter of 6 mm were used to determine the correlation between mechanical loading levels and histological outcome. Besides an uncompressed control group, two destructive compression levels (40% and 60% strain) were applied. The unconfined compression consisted of a preloading phase of 10% strain followed by a relaxation time of 60 s. For the main loading a compression ramp was used to load and unload the specimen within 1 s. Later, the specimens were analysed using H&E staining (as an overview) and Van Gieson Trichrome staining (to inspect the fibrous-like structures). Based on the Remmele score the characterization of the intact nuclei was also conducted. Finally, to confirm the osteogenic phenotype an alkaline phosphatase substrate assay was performed.

Results and Discussion: Histological analysis of the matrix was separated in a peripheral area and a central area of each specimen. Both areas showed morphological differences/damages compared to the control group (Figure 1). Both 40% and 60% compression were leading to 19.5% and 22.7% damaged nuclei, respectively. Between the two compression groups no significant differences were observed. Even though the specimen groups were small, we can conclude that the compression level had a major impact on both the matrix and the cells, wherein a destruction was already observed at 40% compression. As a next step we are planning to investigate the effect of lower compression levels of 5% to 20%. The results of the control group will also be used in further studies when slight mechanical loading will be applied during the cultivation process.

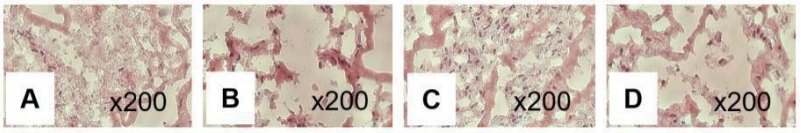

Figure 1: H&E staining of the peripheral area (A=Control, B=60% compression) and the central area (C=Control, D=60% compression).

P130 Guided bone regeneration with heterologous material in foot surgery: a pilot study

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Background: Bone healing after trauma or elective foot surgery is often compromised due to various reasons, and complications such as delayed union, malunion or nonunion can occur. Autologous bone grafts harvested from the patients are the gold standard in reconstructive foot surgery, since they form new bone by osteogenesis, osteoinduction, and osteoconduction. However, their use is associated with donor-site morbidity. Heterologous materials are readily available and obviate the risk associated with bone-graft harvesting. Nonetheless, the use of heterografts raised concerns of immunologic reactions, infections, slower or incomplete incorporation. To enhance the healing rate guided bone regeneration with a resorbable collagen membrane can be used, since the membrane works as a fine filter that allows the passage of pluripotent and osteogenic cells to the bone defect site and excludes the influx of fibroblasts and epithelial cells, collagen facilitates blood clotting and also increases the proliferation rate of osteoblasts. There are currently only few comparative studies on this issue, although heterologous materials have become widespread.

Purpose/Aim: The purpose of this study was to evaluate the speed and completeness of graft incorporation and the possible side effects of heterologous materials in reconstructive foot surgery, and compare it to autologous graft.

Methods: A retrospective study was conducted between January 2014 and January 2015. A total of 20 patients with various etiologies who needed reconstructive foot surgery were randomly chosen, 10 of them had reconstructive foot surgery with autologous grafts and 10 with xenografts of porcine origin and heterologous collagen membranes. The results were assessed on standard radiographs. Time to recovery and time to full weight-bearing wasmeasured.

Results: No clinical complications were evident during or after surgery. Postoperatively there were no infections, no induced immune responses and no nonunions or malunions at the operation site in both groups of patients. The average time of full incorporation of the autologous group was comparable to the heterologous group.

Conclusion: The heterologous material proved to be biocompatible, bioresorbable and osteoconductive when used as a bone substitute for reconstructive foot surgery of various etiologies. This pilot study shows the possibility of interchangeable use of guided bone regeneration with collagen membrane and heterograft instead of autograft, with the clear benefit of avoiding the well-documented complications at the donor site. Larger scale studies are needed for definite conclusions.

P132 Composite microspheres of glucose-crosslinked gelatin and nano-hydroxyapatite in photo-crosslinked poly(trimethylene carbonate) matrices

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Introduction. In bone tissue engineering applications, gelatin microspheres have been used for the encapsulation and delivery of osteoinductive compounds. Such microspheres are generally crosslinked using chemicals like glutaraldehyde to prevent them from dissolving. This may give rise to toxic side effects when applied *in vivo,* due to the presence of unreacted crosslinker. Here we report on the preparation of gelatin- and composite gelatin/nano-hydroxyapatite (nHA) microspheres using glucose as a crosslinker. In addition, photo-crosslinked poly(trimethylene carbonate) (PTMC) networks containing these microspheres were prepared as scaffolding materials for bone tissue engineering.

Materials & Methods. To prepare gelatin- and gelatin/nHA microspheres, gelatin was dissolved in water at 80 °C in the absence or presence of nHA respectively. To crosslink the gelatin, glucose was then added shortly before stirring the resulting mixture into soy bean oil at 80 °C. This was then cooled to 15 °C after which cold acetone was added. The resulting dispersion was filtrated and washed with acetone. Photo-crosslinked PTMC films containing microspheres were prepared by dissolving photo-crosslinkable PTMC into propylene carbonate. Then 10 vol.% (relative to PTMC) of either gelatin or gelatin/nHA microspheres and Lucirin TPO-L photo-initiator were added. A film of the resulting mixture was cast and photo-crosslinked using UV light. The photo-crosslinked films were extracted using propylene carbonate/ethanol mixtures. The microspheres were characterized by thermogravimetric analysis, optical microscopy and dissolution experiments in demineralized water at 40 °C. Strips of the photo-crosslinked films were subjected to tensile experiments.

Results. The gelatin- and gelatin/nHA microspheres had an average diameter of 150 and 190 µm, respectively. Using TGA, it was determined that the composite microspheres contained 35 wt.% nHA. The dissolution experiments showed that the crosslinking of the microspheres was successful: while non-crosslinked gelatin dissolved within 1 hour, the microspheres were swollen and remained spherical. Even though only a low loading (10 vol.%) of microspheres was used, the tensile mechanical properties of photo-crosslinked PTMC films containing gelatin and gelatin/nHA microspheres depended on the type of microsphere incorporated. The stiffness of films containing composite microspheres was significantly higher (3.3±0.1 MPa) than that of films with gelatin microspheres (2.6±0.1 MPa), no significant difference was found in the ultimate tensile strength, elongation at break and toughness.

Conclusions. We have shown that gelatin and composite gelatin/nHA microspheres can effectively be crosslinked with glucose. These microspheres could be used as filler in photo-crosslinked PTMC, modulating its mechanical properties. We are currently assessing photo-crosslinked PTMC films containing the gelatin- and composite microspheres for use in bone tissue engineering applications.

P134 Nanoroughness in titanium boost the cells interaction and bone formation

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Titanium is the main metal studied for application as bone implants, obtained as a porous structure it can provide good mechanical properties and enhanced biological interactions. Production methods of porous metallic materials are based on powder metallurgy (PM), because it allows the manufacturing of parts with complex shapes and dimensions close to the final (near- net shape), with pores. The pore production by space-holder technique constitutes of mixing organic compounds with metallic powder, that when removed by heat treatment, prior structures are kept in place. The main target of this study was to analyse the surface features of commercially pure titanium (cpTi) porous implants, and the bone interaction. Samples of titanium were prepared by powder metallurgy (PM) with addition of different natural polymers (cornstarch, rice starch, potato starch and gelatin) at proportion of 16wt%. In aqueous solution the hydrogenated metallic powder (TiH_2) and the polymer were mixed, homogenized and frozen in molds near net shape. The water was removed in kiln and the polymer by thermal treatment in air (350ºC/1h) before sintering in high-vacuum (1300ºC/1h). The biological evaluation was performed by *in vivo* test in rabbits. Sintered bodies were characterized by Scanning Electron Microscope (FEG-SEM) and their interaction with bone tissue were characterized by Confocal Laser Microscopy. The investigated samples showed interconnected pores with high surface roughness in nanoscale. All implants osseointegrated, the pore microarchitecture and its interconnected network allowed bone ingrowth in all pore sizes. The nanoroughness surface propitiated the attachment of osteoblast cells. The morphology of pore edges showed polygonal
- like morphology that is attributed to the crystallographic planes which had grown during the sintering, by the anisotropy of the titanium surface energy. The obtaining of porous materials with addition of natural polymers in powder metallurgy process, results in an outstanding structure for osseointegration, in macroscale the bone ingrowth is increased by the high porosity, and in the nanoscale, the nanoroughness propitiated a friendlier surface for cell attachment, improving the bone implant contact area.

A high technology of nanoroughness metallic surface can be obtained by a low cost process using natural polymers, and the results showed that bone interaction can be improved bythat.

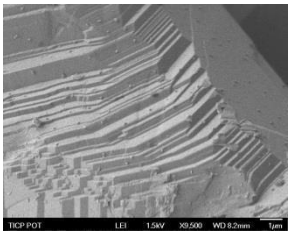
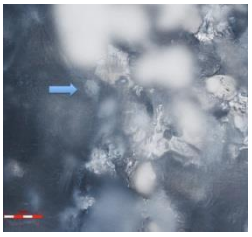
(A)  (B) 

Fig.1. A) Nanoroughness at surface. B) The arrow indicate the osteoblast cell, an it extensions attached on the nanorough surface.

P135 Radiopaque biodegradable polymeric composites for in vivo monitoring of TE products by X-rays imaging

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Introduction: Radiopaque properties enable *in vivo* monitoring of implants and occurring changes in their morphology, detect cracks and defects, etc.[1] Improvement of radiodensity of polyesters can be improved by use of contrast agents.[2,3] The aim of the study was to investigate the influence of widely used contrast agents, barium sulfate and iohexol, on degradation process of polyesters.

Methods: Materials used in following experiments were prepared by solvent casting technique, dried in air at room temperature and then in a vacuum dryer. Materials were processed by Fused Deposition Modelling (FDM) method. Characterisation of composites were performed before and after incubation in phosphate-buffered saline (PBS). Thermal analysis, microscopic observations and calculation of mass loss and water absorption were investigated to characterise materials during degradation. Micro computed tomography and cytotoxicity assay of materials were also performed.

Results: The obtained composites had much higher radiopacity than pure polymer. The addition of contrast agents did not cause considerable changes in properties of polyesters. There was no cytotoxic effect observed after the usage of contrast agents in contact with murine fibroblast cell line L929.

Summary: The proposed contrast agents can be successfully used in non-invasive approach for *in vivo* monitoring of implanted TE products and correlation these data with tissue regeneration processes.

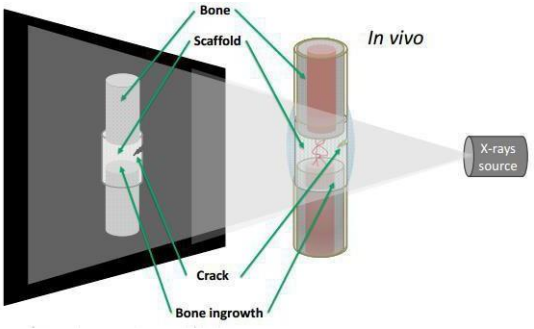

Fig. 1. The idea of the usage of contrasting composite material for *in vivo* X-rays imaging.

Acknowledgements: This study was supported by the National Center for Research and Developments in Poland (STRATEGMED1/ 233624/4/NCBR/2014, project MENTOREYE)

P137 Purification of an osteogenic peptide isolated from marine fish frame and application of the 3D printed polycaprolactone scaffold for bone regeneration

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The fish frame that generated from fishery processing is composed of amounts of the protein, calcium and other minerals. The objectives of this study were to investigate the osteogenic effects of the peptide from marine fish frame, *Johnius belengerii*, as well as the molecular mechanism underlying the peptide's effect in the pre-osteoblast, and the osteogenic effect on fabricated 3D scaffolds. Using consecutive purification by liquid chromatography, a potent osteogenic peptide FFP is composed of three amino acids, Lys-Ser-Ala (KSA, MW: 304.17 Da). The fish frame peptide (FFP) promoted cell proliferation, alkaline phosphatase (ALP) activity, mineral deposition and levels of the osteoblast differentiation phenotype markers in MC3T3-E1 pre-osteoblast. To elucidate the mechanism underlying the osteogenic effect of the FFP on the mitogen-activated protein kinases (MAPKs) and Smad pathways. The FFP was significantly induced the phosphorylation of MAPKs and Smad 1/5/8. In addition, we designed scaffolds consisting of the biodegradable polymer (polycaprolactone; PCL) and FFP fabricated by three axis plotting system for bone regeneration. The effect of the FFP/PCL scaffolds on various mechanical properties and characteristics including the morphology image, FT-IR analysis, and tensile properties were investigated. Moreover, the in vitro biocompatibilities of FFP/PCL scaffolds were examined using MC3T3-E1 pre-osteoblast. At the results, the FFP/PCL scaffolds show significantly higher cell proliferation, mineral deposition and mRNA expression of the osteogenic markers than the PCL scaffold. Consequently, the FFP has a potential pharmacological substance for bone metabolism. Moreover, FFP/PCL scaffold suggests further investigation a potential biomedical engineering field due to promotion of the osteogenesis.

P138 Differential chemotactic responses in primary and stem cell-derived osteoblasts

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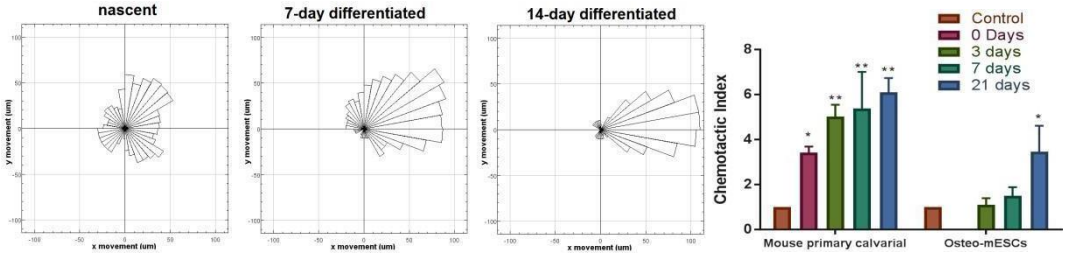
Comparison of primary and stem cell-derived osteoblasts has revealed differences in morphology, expression of bone-specific proteins and genes, as well as different responses to inflammatory signals. These differences have important implications on the use of stem cell-derived osteoblast in cellular therapies and research models.

The work herein studies chemotactic differences in mouse primary calvarial osteoblasts (mPCs) and osteogenically differentiated mouse embryonic stem cells (osteo-mESCs) revealing vastly different responses to PDGF-BB; a potent chemoattractant for osteoblasts. Both cell types were cultured under osteogenic conditions for up to 21 days and ability to migrate towards PDGF-BB was assessed via Boyden assay and by adherent chemotaxis assay in conjunction with timelapse microscopy.



***Left**: Rose plots showing distance and direction of mPC migration in response to PDGF-BB over 24 hours. **Right:** Chemotactic index of mPCs/osteo-mECSs in response to PDGF-BB throughout osteogenic culture. mPCs cultured in growth media (nascent) or osteogenic media for up to 21 days.* =significant difference to control, p<0.004; ** =p<0.0001.*

Migration of mPCs towards PDGF-BB increased following culture in osteogenic media for up to 14 days, indicated by directional movement to the right towards PDGF-BB stimulus (Figure 1, left). This result was not observed in osteo-mESCs. Chemotactic index (CI) assessed by Boyden assay also increased in mPCs following 3 days of osteogenic culture, and further increased up to 21 days (figure 1, right). In osteo-mESCs CI value was only markedly increased after 21 days of osteogenic culture, and still remained similar to nascent primaryosteoblasts.

Representative, 3D models of the bone microenvironment are required to study bone cell chemotaxis. Future work in this project will involve developing a 3D model to study chemotaxis based on decellularized bone matrx, osteoblasts and PDGF-BB releasing polymer beads. Hologrpahic optical tweezers will be used to precisely postion cells in relation to the PDGF-BB release source, and accurately track their migrarion. This model will assess osteoblast chemotaxis in a 3D setting that mimics the native cell microenviornemnt and allows the comparison of primary and stem cell-derived osteoblasts.

P141 Spongy scaffold containing dimethyloxallylglycin accelerates angiogenesis and osteogenesis in rat calvarial defect

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Vascularization is an imperative event during osteogenesis that promotes successful reconstruction of large bone defects. Hypoxia inducible factor-1a (Hif-1a) plays an essential role in angiogenesis-osteogenesis coupling. Dimethyloxalylglycin (DMOG) small molecule regulates the stability of Hif-1a at normal oxygen tension through mimicking hypoxia which in turn accelerates the angiogenesis. Hence, this study is aimed to develop a novel construct by seeded-adipose derived-mesenchymal stem cells (AD-MSCs) onto spongy scaffold containing DMOG to induce angiogenesis and regeneration of critical size calvarial defect in rat. The spongy scaffolds were synthesized with the combination of gelatin, sodium alginate and tri-calcium phosphate, with and without DMOG. The DMOG release pattern was evaluated using UV-spectroscopy. The effect of DMOG delivery on ADMSCs was subsequently investigated in terms of adhesion, viability, osteogenic and angiogenic-related genes under in vitro condition. Immunohistochemistry and histological analysis of cell-scaffold constructs were also performed following transplantation in rat calvarial defect. The results showed that DMOG was released in a sustained manner over 10 days from spongy scaffolds. Scanning electron microscopy (SEM) images and MTT assay demonstrated the attachment and viability of ADMSCs in the presence of DMOG, respectively. Moreover, osteogenic activity of ADMSC including alkaline phosphatase activity and calcium deposition was significantly increased in DMOG scaffold. CT imaging and H&E staining indicated enhanced bone formation and angiogenesis in DMOG-loaded scaffolds group. It is concluded that spongy scaffolds containing DMOG with the ability of angiogenesis could be utilized to enhance bone regeneration of large bone defects.

Keywords: Angiogenesis, Small molecule, Critical size defect, Mesenchymal stem cells.

P142 A large animal caprine model for critical mandibular bone defects

Jeffrey Janus, Kasperbauer Jan, Claudia Gutierrez, David Schembri Wismayer, Heidi Lehrke, Yaszemski Michael

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Background: Currently the standard of care for the surgical treatment large (greater than 4cm) segmental bone defects of the mandible is complex and morbid, often involving free flap surgery. To date, few models of mandibular defects adequately replicate the volume of bone, forces of mastication, intraoral exposure, and plating systems seen in patients. Here we present a large animal caprine (goat) translational to address these parameters.

Methods: Five adult Saanen goats underwent a transcervical 4 cm mandibulectomy including a dentulous segment of the left mandibular body. Periosteum was excised and the resultant intraoral communication was closed. Two titanium 3.0mm locking plates and screws were used to fixate the defect. Oblique x-rays were taken before and after the removal of the bone segment, and then prior to sacrifice at 12 weeks. The goats were sacrificed at 12 weeks and the operative site was excised with adjacent bone (10 cm total). After fixation in 70% ethanol and dehydration, specimens were embedded in polyester resin, cut to a thickness of 100µm and prepared with EXAKT grinding machine to 50 micrometers. Slides were then stained with SRBS and H&E, and then examined by a blinded pathologist, who measured the defects and made commentary on presence or absence of bony continuity. The radiographs were examined by a blinded radiologist.

Results: All five goats survived the 12 week study period without infection, obvious signs of pain, or disability. All were able to eat a regular diet within 24 hours of the operation. There were no instances of plate fracture or hardware exposure. Radiographic evaluation of the segment at the 12 week period showed evidence of nonunion in all five cases. Histologic evaluation showed no evidence of any bony continuity at any section level. Bony callous formation did result in some defect approximation, but an average nonunion distance of 1.75cm remained for all animals ($p<0.05$).

Conclusions: This Caprine model for segmental mandibulectomy defects affords a safe, effective, and consistent translational critical defect model to address the use of regenerative solutions in a translational setting. The model replicates oral contamination, bone volume, masticatory forces, and hardware use similar to that seen in human beings.

P143 Craniofacial bone regeneration with native periosteum: A caprine model Jeffrey Janus, Jan Kasperbauer, David Schembri Wismayer, Heidi Lehrke, Michael Yaszemski *Mayo Clinic, Rochester, MN, USA*

Background: Currently the standard of care for the surgical treatment large (greater than 4cm) segmental bone defects of the mandible is complex and morbid, often involving free flap surgery. As we explore regenerative applications to address this problem, the use of autologous periosteum deserves robust consideration.

Methods: Ten adult Saanen goats underwent a transcervical 4 cm mandibulectomy including a dentulous segment of the left mandibular body. In the five controls, periosteum was excised; in the experimental group, the periosteum was left in the wound. In all goats, the resultant intraoral communication was closed. Two titanium 3.0mm locking plates and screws were used to fixate the defect. Oblique x-rays were taken before and after the removal of the bone segment, and then prior to sacrifice at 12 weeks. The goats were sacrificed at 12 weeks and the operative site was excised with adjacent bone (10 cm total). After fixation in 70% ethanol and dehydration, specimens were embedded in polyester resin, cut to a thickness of 100μm and prepared with EXAKT grinding machine to 50 micrometers. Slides were then stained with SRBS and H&E, and then examined by a blinded pathologist, who measured the defects and made commentary on presence or absence of bony continuity. The radiographs were examined by a blinded radiologist.

Results: All 10 goats survived the 12 week study period without infection, obvious signs of pain, or disability. All were able to eat a regular diet within 24 hours of the operation. There were no instances of plate fracture or hardware exposure. Radiographic evaluation and histologic evaluation of the control specimens showed evidence of nonunion in all five cases ($p<0.05$). In contrast, bony reconstitution across the defect was seen, histologically and radiographically, in all five animals where periosteum remained ($p<0.05$). Bone volume restoration was on the order of 70-80% in just 12 weeks ($p<0.05$).

Conclusions: This caprine model demonstrates the remarkable power of native periosteum to regenerate craniofacial bone. Continued exploration of this tissue for osteogenesis could very well lead to use in human beings to address craniofacial defects.

P144 Bacterial components staphylococcus pyogenes lipoteichoic acid enhanced the osteogenic differentiation potential of periodontal ligament stem cells

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[Abstract] Purpose: Lipoteichoic acid (LTA), a major cell-wall virulence factor of Gram-positive bacteria, is one of the key toxins that affects the development of periodontitis. Our previous studies have shown that periodontitis could be cured by periodontal ligament stem cells (PDLSCs). However, the interaction between LTA and PDLSCs remains uncertain during the process of periodontal regeneration. Thus, this study is aiming at investigating how LTA affects the biological functions of PDLSCs. Methods: Human PDLSCs were treated with different concentrations of LTA (0.1-10 μg/ml), then effects of LTA on the functions of HPDLSCs including proliferation, apoptosis, osteogenic and adipogenic differentiation were evaluated. CFSE and Annexin V were adopted respectively to detect the proliferation capacity and apoptosis rate. The osteogenic differentiation potential was compared among each group using ALP(Alkaline phosphatase) staining and AR-S staining (Alizarin red-staining). In addition, adipogenic differentiation capacity was assessed by Oil-red staining. Besides, osteogenic and adipogenic differentiation-related genes were studied via real-time PCR. Results: LTA inhibited the proliferation capacity of HPDLSCs through accelerated the apoptosis rate, which showed a LTA- dose-dependent effect. However, high concentration of LTA (10 μg/ml) improved the capacity of early osteogenic differentiation with an enhanced ALP staining at day 4 and day 7, whereas the late osteogenic potential with stable AR-S staining showed no significant difference among each group. And along with the increasing concentration of LTA, the adipogenic differentiation of HPDLSCs was elevated. Conclusion: High concentration of LTA could inhibit the proliferation capacity, accelerate the apoptosis rate and adipogenic differentiation, along with the unchanged late osteogenic differentiation of HPDLSCs, which display a negative effect during the process of PDLSCs-mediated periodontal regeneration.

[Key word] Lipoteichoic acid; HPDLSC; Proliferation; Apoptosis; Differentiation

P145 Single-dose local administration of parathyroid hormone (1-34) with octacalcium phosphate collagen composites enhances bone regeneration in a rodent critical-sized calvarial defect

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[1]Tohoku University, Sendai, Japan, [2]TOYOBO Co. Ltd., Osaka, Japan

Octacalcium phosphate (OCP) and collagen composite (OCP/Col) has achieved stable bone regeneration without cell transplantation. After preclinical study, the sponsor-initiated clinical trial has been recently conducted to commercialize the material. This study was investigated bone regeneration by OCP/Col with single local administration of teriparatide (parathyroid hormone 1-34) solution. OCP/Col was prepared by mixing sieved granules of OCP and atelocollagen for medical use and the disk was molded. After creation of a rodent critical sized calvarial defect, OCP/Col or OCP/Col with dripped teriparatide solution (1.0 µg or 0.1 µg; OCP/Col/PTHd1.0 or OCP/Col/PTHd0.1) was implanted into the defect. After implantation of four, eight, and twelve weeks, the in vivo micro-CT analysis was performed. Six defects of each group were fixed 12 weeks after the implantation, and radiographic, histological, and histomorphometrical examinations were performed. Radiographic examinations including micro-CT analysis indicated that the radiopaque figures of the defect treated with OCP/Col with teriparatide (OCP/Col/PTHd) occupied wider range than that of OCP/Col at every experimental period. And the radiopaque figures increased with time, and repaired the defect. Histological finding demonstrated that almost the defect of OCP/Col/PTHd groups was filled with newly formed bone. Histomorphometric examination indicated that the percentage of newly formed bone in the defect (n-Bone%) of OCP/Col/PTHd groups was significantly higher than that of OCP/Col, whereas no significant differences of n-Bone% was detected between OCP/Col/PTHd1.0 and OCP/Col/PTHd0.1. These results suggest that OCP/Col with single local administration of teriparatide enhance bone regeneration in a rodent calvarial critical sized bone defect.

P146 The rat femur chamber: development of a new and versatile model for the observation of changes of the microcirculation inside tissue engineering constructs in femoral defects

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Despite substantial scientific progress autologous bone grafts are still the gold standard for the regeneration of skeletal critical size defects, as they are osteoconductive as well as histocompatible. Tissue engineering (TE)-constructs for the reconstruction of bone defects by utilization of different combinations of scaffolds, pluripotent and/or osteogenic cells and growth factors are a possible alternative.

Until now, TE-constructs are size limited, as their initial supply with oxygen and nutrition depends on diffusion. For the development process of TE-constructs with a clinical relevant size, in vivo models that allow the observation and manipulation of the growing vasculature inside a TE-construct are mandatory. Recently a femur-window in mice was developed by our group. Although this model could successfully used to analyze the vascularization of TE constructs, it is restricted to the observation of small constructs, due to the limited animal size. To overcome these limitations we developed a femur chamber at the rat as a new animal model for the in vivo evaluation of TE-constructs. Due to the substantial increase in animal size, this model allows the creation of larger defects and the use of a larger observation-window. Furthermore, it is not only restricted to the observation of avascular constructs. The scale of the rat allows the development of vascularized TE-constructs that can be anastomosed with the animal circulation. Utilizing the model of the rat femur chamber it is possible to observe the growth and development of the microcirculation inside these constructs over a period of at least 20 days.

The rat femur chamber was made from stainless steel and integrates an observation window for intravital fluorescence microscopy. The observation chamber is fixed with four bonescrews at the femur and serves as a fixation of the femoral defect, the observation windows has a diameter of 1cm. The femoral defect was standardized to 5x3x3mm, which meets the criteria of a critical size defect. Additionally the chamber offers enough space for the integration of a vessel, which can be anastomosed with the femoral artery and therefore offers an additional supply with oxygen and nutrients.

The observation chamber was well tolerated by the animals; they behaved normal, gained weight and showed no limitations by the chamber. The chamber offered a stable fixation of the defect, capable to resist a normal physiological load; the animals strained the hind limb fully within 24h after surgery.

We successfully developed an animal model that allowed the evaluation of angiogenesis and osteointegration of TE-constructs implanted in a critical size defect. By using an observation chamber and intravital microscopy observations of the developing microcirculation at multiple time points we could show changes in the early vascularization in TE-constructs and analyze the impact of a prevascularisation on the developing microcirculation inside the constructs. This integration of a vascular graft into the construct allows the analysis of constructs, which are closer to the clinical situation and offer a reasonable alternative to the autologous bone graft.

P147 Enhanced osteogenesis of human mesenchymal stem cells in mesoporous magnesium silicate/polycaprolactone/wheat protein composite scaffolds

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Successful bone tissue engineering using scaffold is primarily dependent on the scaffold properties: biocompatibility, highly inter-connected microporosity and mechanical integrity. In this work, we propose new composite scaffolds consisting of mesoporous magnesium silicate (m_MS), polycaprolactone (PCL), and wheat protein (WP) manufactured by salt-leaching technique.

Experimental groups were set based on the component ratio: (1) 0%WP (PCL: m_MS: WP = 70: 30: 0); (2) 15%WP (PCL: m_MS: WP = 55: 30: 15); (3) 30%WP (PCL: m_MS: WP = 40: 30: 30). Human mesenchymal stem cells (MSCs) were seeded on to the scaffolds with osteogenic differentiation media. Various biological analyses such as DNA contents, ALP activities, gene expressions of osteogenic markers (BMP2, BSP, RUNX2, and OPN) and beta-galactosidase staining were examined.

Our previous data confirmed that the increasing amount of WP improved the surface hydrophilicity of m-MS/WP/PCL composites. 30%WP groups showed higher proliferation rate than other groups (Fig. 1a). Amounts of ALP normalized by corresponding DNA contents in 15%WP and 30%WP groups were significantly higher than in 0%WP group up to day 7, but no significant differences were identified between all groups at day 14 (Fig. 1b). The expressions of osteogenic markers (MP2, BSP, RUNX2, and OPN) in 15%WP groups were higher than in other groups (Fig. 2).

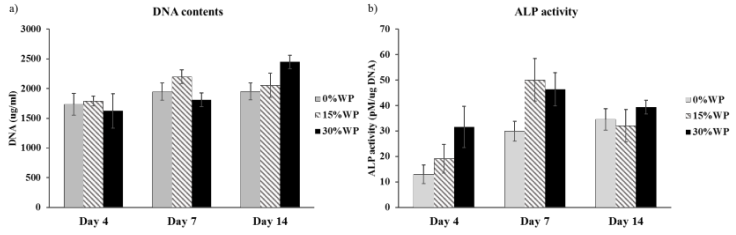

Fig. 1. Expressions of (a) DNA contents and (b) ALP activities for up to 14 days

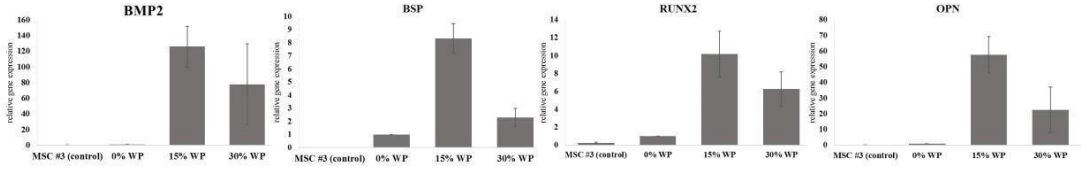

Fig.2. Relative gene expression levels of osteogenic markers at day 28

From this study, we concluded that m-MS/WP/PCL scaffold has a potential of affecting osteogenic differentiation depending on the component ratio. Moreover, further investigations are still needed to confirm optimal component ratio of m_MS, PCL and WP.

Acknowledgements: This work was supported by the National Research Foundation of Korea (NRF) Grant (NRF-2014K2A2A7066637, NRF-2015M3A9B6073642).

P148 Application of alginate microbeads as a carrier of bone morphogenetic protein-2

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Recombinant human bone morphogenetic protein-2 (rhBMP-2) is used to enhance bone regeneration. However, the bone regeneration ability of BMP-2 depends on the delivery vehicle. In this study, we used alginate microbeads as a delivery vehicle for rhBMP-2, and confirmed that alginate microbeads are useful to deliver rhBMP-2 and alginate microbeads with rhBMP-2 promote bone regeneration. Release ability of alginate beads was evaluated using BMP-2 ELISA between alginate microbeads with rhBMP-2 and collagen sponges with rhBMP-2 in 1, 2, 4, 7, 10, 14 and 21 days. Alginate microbeads rapidly secreted rhBMP-2 within 4 days and then secreted more constantly than collagen sponge did thereafter. Effect of bone regeneration in vitro was evaluated using ALP (alkaline phosphatase)-assay using canine adipose tissue-derived mesenchymal stem cells in 1 and 2 weeks. In 1 week, the amount of released ALP is similar between alginate microbeads with and without rhBMP-2. However, in 2 weeks, released ALP was more detected in alginate microbeads with BMP-2 than those without BMP-2. To confirm effect of bone regeneration in vivo, mice were injected with alginate microbeads containing rhBMP-2 into subcutaneous tissue. 4 weeks later after injection, Bone regeneration effect of rhBMP-2 was measured by micro-computed tomography and histological examination. We confirmed formation of bone in subcutaneous tissue in mice. We conclude that alginate microbead can act as an injectable delivery vehicle of rhBMP-2 to induce boneregeneration.

Keyword : Recombinant human bone morphogenetic protein-2; Alginate microbead; Bone regeneration; Delivery vehicle.

P149 Nanotopographical modification and electrical stimulation on titanium to promote osteogenesis

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Introduction: Surface nanostructured materials have the ability to enhance protein adsorption and cellular activities. Anodised titania nanotubes provide a nanoengineered biomimetic environment to promote osteogenesis and bone cell mineralisation. Electrical stimulation, on the other hand, offers excellent control on cell growth, orientation, and intracellular calcium level. Electrical signals have the capability to induce intracellular calcium concentration which helps in bone remodelling and mineral deposition.

Methodology: Titanium foils (0.25 mm thick, 99.5% purity, Alfa Aesar) were cut into a disc shape using a fibre laser cutting machine. The titanium disc was sonicated in degreasing solution (ethanol, propanol and deionized water) followed by air-dried. In the customised anodisation setup, titanium discs served as an anode, while an inert platinum foil (0.1 mm thick, 99.99% purity, Alfa Aesar) was used as a cathode. Saturated colomel electrode served as a reference electrode. 1M H3PO4 with 0.3wt% HF was used as an electrolyte for the anodisation. Applied voltage was in the range of 10-20 V, the anodisation was set to run for 1 hour at room temperature. Surface morphology of the specimens were characterised using Scanning Electron Microscope (SEM). The anodised and pure titanium specimens were then sonicated and finally sterilised before conducting electrical stimulation and biocompatibility tests.

Results: Surface characterisation via SEM showed titania nanotubes with uniform pore diameter across the anodised titanium disc. Larger pore diameter can be seen at an elevated voltage, i.e. 50 nm on 10 V and 100 nm on 20 V, as shown in Figure 1.

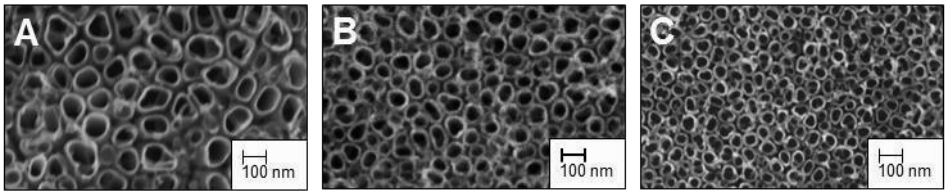

Figure 1. Top view SEM image of the nanotubes. Self-organised nanotube structures with uniform pore diameters can be observed. Anodising voltage of (A) 20 V, (B) 15 V, (C) 10 V across the titanium disc, together with optimum anodising parameters. Average nanotube pore diameter of (A) 100 nm, (B) 70 nm, (C) 50 nm can be observed across the anodised specimens.

Human mesenchymal stem cells seeded onto pure titanium specimen showed an increase in cell number at day 7 as compared to tissue culture plate as experimental control. Electrical stimulation on human mesenchymal stem cells seeded in customised bioreactor showed higher metabolic activity, more aligned cell directions, and increased calcium concentration.

P154 Cellular responses on polycaprolactone based mesoporous magnesium silicate scaffolds depending on wheat protein ratio and mechanical stimulation

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Bone has complex porous structure with meso and macro pores, where bone cells are under dynamic mechanical stress, also. In bone tissue engineering(BTE), manufacturing bone mimic structured scaffold was the one of critical challenges. In this study, we investigated the potential of fabricated scaffolds in BTE depending on wheat protein composition and mechanical stimulation.

The composite scaffolds were fabricated by 3-dimensional bioplotting system. The components were: polycaprolactone(PCL), mesoporous magnesium silicate(m-MS), and wheat protein(WP). Three groups were set: W0(PCL:m-MS:WP=70:30:0), W15(PCL:m-MS:WP=55:30:15), and W30(PCL:m-MS:WP=40:30:30). MG-63 cells were seeded onto the scaffolds and cultured for up to 7 days. Mechanical stimulation was engaged at day 3, 4 and 5 (72 hrs.) by a perfusion bioreactor(ACBF-100, AnyCasting Ltd, Korea). Various measurements with typical tools were performed: contact angles, Scanning Electron Microscopy(SEM), Energy Dispersive X-ray spectroscopy (EDX), and DNA assay.

The mesoporous structure and existence of WP were confirmed by SEM and EDX. The results of contact angle measurements indicated that the addition of WP could provide favourable environments to the cells as it made contact angle smaller (Fig. 1). However, our test showed contrary results overall. Without mechanical stimulation WP showed negative effect on proliferation. In each group gradual increase in proliferation was observed. However, the initial attachment of cells, at day 2, was so poor (Fig. 2). Even mechanical stimulation failed to increase proliferation rates. Moreover, the rate was significantly decreased in W30 group, at the end.

Generally, hydrophilicity has been known essential for cell attachment and proliferation. However, we could see that smaller contact angle did not always play favourable effects on cell attachment and following proliferation, at least under the conditions set in this study.

Further studies with other types of cells and scaffolds are highly recommended for concrete conclusion for cellular responses with three dimensional scaffolds.

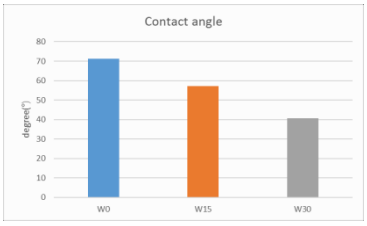

Fig.1. Contact angle

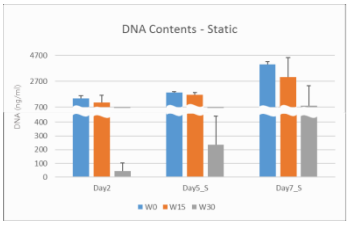

Fig.2. DNA Contents (Static)

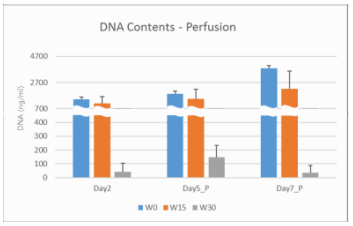

Fig.3. DNA Contents (Perfusion)

Acknowledgement: This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea (NRF-2014H1C1A1073148) and by the NRF Grant (NRF-2014K2A2A7066637)

P156 Integrated experimental-modelling approach for the osteogenic differentiation of umbilical cord blood mesenchymal stem cells for bone tissue engineering applications

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Introduction

Tissue engineered bone graft production with the use of cells, scaffolds and osteoinductive signals has the potential to meet the increasing need for grafts in orthopaedic and maxillofacial surgery. Stem cell-seeded constructs promise to achieve high quality grafting with efficient osteointegration, while also addressing problems such as graft rejection. Umbilical cord blood mesenchymal stem cells (UCB MSCs) represent an ideal cell source due to their high proliferation capacity and enhanced immunosuppressive properties. To achieve this aim, robust, reproducible and automatable bioprocesses are needed. Model-based control and optimisation can facilitate bioprocess control, model validation and model-based applications. Herein, we present an integrated modelling-experimental approach to describe the osteogenic differentiation process of umbilical cord blood MSCs.

Materials & Methods

A first-principles mathematical model links gene expression of osteonectin and osteocalcin, intracellular metabolite levels, and cell cycle heterogeneity of mesenchymal stem cells by means of population balance equations. The model has been implemented and solved in gPROMS (PSE Ltd, UK). Global sensitivity analysis revealed the most important parameters of the model, which we then regressed from triplicate experimental data. MSCs have been isolated from umbilical cord blood taking into account the relevant ethical approval, expanded in αMEM Glutamax-I (ThermoFisher) and differentiated for three weeks in medium with 10^{-7} M dexamethasone. Cell numbers for preliminary parameter data have been obtained by DNA quantification with QuantIT PicoGreen assay (ThermoFisher, UK) and gene expression data have been obtained by means of quantitative real time RT-PCR (KapaBiosystems, UK). Intracellular metabolite data were obtained with the use of GC-MS metabolomics analysis.

Results

The model predicted total cell counts and gene expression levels at all differentiation stages. Cell numbers significantly increased during the second and the third week ($p<0.0001$). According to the model and experimental results, osteonectin gene expression increased between day 7 and day 14 ($p=0.027$) indicating a transition from the undifferentiated to the osteoblast state, and remained constant after day 14. Osteocalcin levels started to increase at day 10, stabilising toward the end of the differentiation (day 21) where experimental results showed that its relative expression was higher than at day 14 ($p=0.003$). Finally, experimentally identified intracellular metabolite levels throughout the differentiation period matched with the model predictions (within the 95% confidence intervals) for all time points checked.

Conclusion

Population balance models can accurately predict expansion and osteogenic differentiation of cord blood MSCs. This model can find applications in the development of tissue-engineered bone grafts for the efficient and safe healing of critical sized bone defects.

P157 Effective bone regeneration using PCL/HA hybrid microspheres

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The needs for microspheres made of biodegradable polymers have gradually increased with the developments of bioactive delivery systems, tissue engineering, and regenerative medicine. As microspheres used in bone tissue engineering, poly(ε-caprolactone) (PCL) is one of the most attractive polymers, since it has excellent mechanical properties, flexibility, and easy processability as well as good biocompatibility and slow biodegradation rate. However, PCL itself has limited cell affinity and stem cell differentiation potential, which would restrict its wider clinical applications for bone regeneration. One of the promising ways of solving these limitations is to hybridize PCL with cell-compatible polymers such as hyaluronic acid (HA). Hyaluronic acid is a naturally occurring non-immunogenic glycosaminoglycan and plays a significant role as a facilitator of osteogenic differentiation and as a migration-stimulating agent for mesenchymal stem cells. In this study, PCL/HA hybrid microspheres were fabricated by a spray/precipitation method using a double nozzle spray. The microsphere sizes were controlled by adjusting gas flow rate and polymer concentration. Human bone marrow stem cells (BMSCs) were seeded on the prepared PCL/HA microspheres to demonstrate the feasibility of utilizing these microspheres for bone regeneration. From the *in vitro* osteogenesis using BMSCs, the PCL/HA microspheres showed the effective osteogenesis of bone marrow stem cells compared to the PCL microspheres without HA. From the *in vivo* animal study using rats, the PCL/HA microsphere groups also showed faster bone regeneration than the groups of control (blank) or PCL microspheres. From the results, we recognized that the PCL/HA microspheres can be applicable as bone fillers for tissue engineering applications.

P158 Development of bone tissue mimetic polymer mesh scaffold via human lung fibroblast-derived ECM and inorganic mineral

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Extracellular matrix (ECM) confers a natural microenvironment instructive for the regulation of cellular behavior and function. Various cell-derived extracellular matrices (CDM) have been used to promote osteogenic differentiation of both preosteoblasts and bone marrow mesenchymal stromal cells. Biodegradable polymer mesh scaffold is also supposed to provide appropriate microenvironments to support cells adhesion, migration and differentiation. Recent experiences direct us to develop producing polymeric composite with bioactive inorganics to enhance the osteogenic properties. In this study, we fabricate a bone tissue mimetic 3D constructs via introducing human lung fibroblasts-derived ECM (hFDM) and apatite crystals onto the surface of the PLGA/PLA mesh scaffold. We investigate their effect on osteogenesis of human umbilical cord blood-mesenchymal stem cells (UCB-MSCs) *in vitro* and on new bone formation *in vivo*. UCB-MSCs were cultured in the scaffolds and subjected to osteogenic differentiation for 3 weeks. The results show that alkaline phosphatase (ALP) activity, mineralization, and osteogenic marker expression were significantly improved with UCB-MSCs cultured in the hFDM- apatite mesh scaffolds compared to the control and fibronectin-coated one. In addition, rat calvarial bone defect model is used to examine the feasibility of current platform for bone regeneration. While hFDM-coated mesh scaffolds groups exhibit a significant increase of newly formed bone and in particular, hFDM-apatite mesh scaffold show the best bone healing as confirmed via micro-CT and histological observation. This work proposes a new platform that can provide more advanced biomimetic 3D environment for MSCs osteogenesis *in vitro* and bone regeneration *invivo*.

P161 Injectable hybrid system for strontium local delivery to promote bone regeneration

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In what concerns bone tissue regeneration strategies, injectable bone substitutes are very attractive since they can be applied with minimally invasive surgical procedures and can perfectly fill irregular defects created in cases of trauma, infection or tumor resection. These materials must combine adequate mechanical properties with the ability to induce new bone formation. Incorporating strontium (Sr) in bone substitute biomaterials may be a strategy to achieve high Sr concentrations, not in a systemic but in a local environment, taking advantage of the osteoanabolic and anti-osteoclastic activity of Sr, for the enhancement of new bone formation. In this context, the aim of the present work was to evaluate the response of a Sr-hybrid injectable system for bone regeneration, designed by our group, consisting of hydroxyapatite microspheres doped with Sr and an alginate vehicle crosslinked in situ with Sr, in an *in vivo* scenario. Two different animal models were used, focusing our study on both bone regeneration and inflammatory response upon material implantation. A critical-sized bone defect model and an air- pouch model were used, where non Sr-doped similar materials (Ca-hybrid) and empty defects were used as control. Sr-hybrid system led to an increased bone formation in both center and periphery of a critical sized defect compared to a non Sr–doped similar system, where new bone formation was restricted to the periphery. Moreover, an increase in $F4/80^{+}/CD206^{+}$ cells in inflammatory exudates was observed upon Sr-hybrid implantation comparing to the control in a rodent air-pouch model. The characteristic M2 macrophage phenotype observed is known to promote angiogenesis and tissue repair. Most importantly, the hybrid system provided a scaffold for cell migration and tissue ingrowth and offered structural support, further improving effective local bone formation, suggesting that this might be a promising approach for bone regeneration, especially in osteoporotic conditions.

P164 Ultrasonic foaming based development of a hetero-macroporous scaffold for bone tissue engineering applications

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INTRODUCTION: Among many physical parameters of a scaffold, pore size and porosity are the important determining factors for successful bone regeneration. Interconnected pores ranging from 100-1000 μm are essential for cellular and vascular invasion (1). Whereas, pores smaller than 20 μm are required for cellular development and orientation (2). Out of a multitude of scaffold fabrication methods, only a couple of methods such as gas foaming, rapid prototyping and salt leaching are capable of generating macroporosity, specially in synthetic polymers and ceramics (3). However, techniques have been rarely proposed to generate macroporosity in natural polymeric scaffolds. In the present work, we have present a gelatin/carboxymethyl chitosan (CMC)/n-HAP composite hetero-macroporous scaffold using a combined approach of ultrasonic-foaming and freeze drying method. The formulation was crosslinked using 0.15% glutaraldehyde.

METHODS: The composition Gelatin-CMC-nHAp was ultrasonically agitated and freeze-dried to generate hetero-macroporous scaffold. The scaffold was characterized for their physico-chemical properties using FTIR, XRD, SEM and UTM. The scaffolds were biologically characterized using mesenchymal stem cell differentiated osteoblasts for their viability, proliferation, mineralization and RT-PCR based osteogenic gene expression profile. Adhesion and proliferation rate of human endothelial cells was assessed on both hetero- macroporous and non-macroporous scaffold.

RESULTS & DISCUSSION: The hetero-macroporous scaffold showed augmentation in properties like swelling, porosity, pore size distribution, osteoconductive and osteoinductive behaviour in comparison to non-sonicated freeze dried gelatin-CMC-nHAp scaffolds. A higher expression of differentiated osteogenic genes (collagen type I, osteocalcin, ALP and RUNX2) was also observed in the hetero-macroporous scaffold. Live–dead staining and alamar blue assay showed an increased adhesion and proliferation rate of endothelial cells on hetero- macroporous scaffold.

CONCLUSIONS: Our study demonstrated that ultrasonically agitated scaffolds enhanced the cellular viability, proliferation and functionality of osteoblasts. This probably could be due to hetero-macroporosity, which allows efficient diffusion of nutrients and waste products into and out of the scaffold. Additionally, the ultrasonication evenly distributed nHAp throughout the scaffold, which significantly increase the osteoconductivity and osteoinductivity of thescaffold.

ACKNOWLEDGEMENTS: Department of Biotechnology, Govt. of India and IIT Kharagpur are acknowledged for their generous funding.

P167 Balancing bone resorption and overzealous bone growth in lumbar interbody fusion with RhBMP-2 in the sheep model - a question of dose and/or concentration?

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Question

Is there a dose- and/or concentration-dependent effect of rhBMP-2 on bone resorption and overzealous bone growth in anterior lumbar interbody fusion (ALIF)?

Methods

Thirty-six Merino sheep underwent right-anterior lumbar interbody fusion at L1-L2 and L3-L4 with the addition of a polyetheretherketone (PEEK) cage either filled with one of four different concentrations/doses of rhBMP-2 (interventional groups: 4.0 mg/ml, total dose of 4.0 mg; 2.0 mg/ml, total dose of 2.0 mg; 1.0 mg/ml, total dose of 1.0 mg; 0.5 mg/ml, total dose of 0.5 mg) or in the control group filled with an absorbable collagen sponge (ACS) or left empty. A pedicle-screw system was implanted in all surgical levels. Thin-cut CT image were taken directly postoperatively, after 3 months, 6 months and 12 months to assess bone resorption, cage subsidence and migration (indirect marker of bone resorption), and overzealous bone growth.

Results

In comparison with the control group, rhBMP-2 groups showed a higher fusion rate at 3 (72% vs. 13%), 6 (90% vs. 30%) and 12 (95% vs. 70%) months CT scans. Overzealous bone growth was detected at the right ventral circumference of the vertebral body as sign of the direct operative access. No ectope ossification was detected in all groups. The incidence of bone resorption as well as cage migration and cage subsidence as indirect marker of bone resorption were higher in the BMP-2 groups.

However, no clear dose-concentration-dependency of these adverse effects could be established. The side effects were less in the BMP-2 group treated with 0.5mgrhBMP-2.

Conclusion

In this animal model the application of rhBMP-2 in different concentrations/doses showed much better fusion rates compared with the control group. These results could be shown in the 0.5mg BMP-2 group with clear reduction of adverse effects. Higher doses of BMP-2 doesn't cause a benefit in fusion rate but an increase in side effects like cage migration and subsidence as maker of higher bone resorption.

No inflammation reaction or systemic side effects were detected in the BMP-2 group.

P168 Histological analysis of bone regeneration with different doses of rhBMP-2 in an ovine lumbar interbody fusion model

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The objective of this study was to investigate the effects of different doses rhBMP-2 on bone healing in an ovine lumbar interbody fusion model.

Methods

In this study 22 sheep underwent two level lumbar interbody fusion using a ventrolateral approach with secondary dorsal fixation at L1/2 and L3/4. After randomization in one level a PEEK-cage was implanted filled with one of three doses rhBMP-2 (0,5mg; 1mg; 2mg) delivered on an ACS. The other level received an empty PEEK-cage or ACS filled cage. Animals were sacrificed after 3 and 6 months and decalcified histology was performed. This included histomorphological analysis as well as histomorphometry of the tissues within the cage.

Results

At 3 months after surgery the groups treated with rhBMP-2 showed higher amounts of bone tissue within the cage. At 6 months the amounts of bone tissue increased in all groups, but were still lower in the groups without growth factor.

At 3 months there was only one active osteolysis in the cage/ACS. 7 of 8 segments of the rhBMP-2 groups had a compromised bone structure around the implant. These areas were filled with fibrous tissue and fibrocartilage. This finding was not detected in the groups without rhBMP-2 at 3 months.

At 6 months most of the segments with an empty cage or cage/ACS showed a chronic inflammation. Predominant cells were macrophages and giant cells. The groups treated with rhBMP-2 showed only a few mild chronic inflammatory reactions.

Discussion

The well-known dose dependent effect of rhBMP-2 on bone healing could also be recognized in our study. Attention has to be payed for the proinflammatory properties of the growth factor. Consistent with other studies we found 2 strong inflammatory reactions, each one in the lowest and highest dose group. Also the potential for causing transient bone resorptions, according to the results of others, was demonstrated. At 3 months 7 of 8 segments treated with rhBMP-2 showed compromised peri-implant bone. Osteoblasts, but not osteoclasts, were seen in the periphery of these areas. It can be concluded that there where bone resorptions which already merged into an increased osteoblastic activity. Usually resorptions occur between 2 and 12 weeks and are followed by a period of increased osteoblastic activity. This finding wasn't recognized at 6 months anymore.

Striking is that at 6 months most of the segments without rhBMP-2 showed a compromised bone structure around the implant with a mild to mainly moderate chronic inflammatory reaction. This cannot be attributed to the growth factor. Also the ACS is degraded at 6 months and is unlikely a possible explanation. Therefore, the cage as a reason must be considered and it has to be questioned whether PEEK is the optimal material for interbodycages.

P169 Liposomal delivery of BMP-2 and bisphosphonates from polymethyl methacrylate (PMMA) bone cements

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Introduction: Aseptic loosening is the primary cause of orthopaedic implant failures, accounting for 46% of the 8,923 hip revisions performed in 2015. The poor implant osseointegration observed clinically, is due to a combination of stress shielding, micromotion and increased osteoclastic activity as a result of an inflammatory response to implant wear particles. This study aimed to utilise a patented liposomal delivery system for bone cement, to deliver osteogenic materials locally to enhance early implant osseointegration.

Methods: Human recombinant BMP-2 (rhBMP-2; 1-100 ng/mL) and a bisphosphate (zoledronate, ZA; 1-100 µg/mL) were passively loaded into liposomes containing phosphatidylserine. The total drug encapsulation was quantified using an ELISA (rhBMP-2) and HPLC (ZA). Bone marrow derived mesenchymal stem cells (BMMSCs), and a MG63 (pre-osteoblast-like) cell line were treated with rhBMP-2, Z A or control liposomes (1 µg/mL-1 mg/mL) or free rhBMP-2/ZA for 3, 9, 15, 21 and 35 days. Cell viability, proliferation and osteogenic differentiation were assessed compared to basal media and osteogenic media controls. Human osteoclasts were derived from peripheral blood mononuclear cells and used to assess the effect of ZA liposomes on osteoclast viability and ability to resorb dentine.

Results: Stable liposomes encapsulating ZA and rhBMP-2 were made, which had low cytotoxicity. rhBMP-2 liposomes enhanced the osteogenic differentiation of BMMSCs when treated in osteogenic media, however lacked efficacy in basal media. Control and rhBMP-2 liposomes increased mineralisation in MG63 cells. Osteoclasts treated with ZA liposomes had a reduction in the amount of dentine resorption. Osteogenic liposomes were loaded into polymethyl methacrylate (PMMA) bone cements with minimal effect on mechanical properties.

Conclusions: Osteoclast activity was reduced by ZA liposomes, however, rhBMP-2 liposomes were no more effective than empty liposomes in promoting bone mineral deposition and MSC differentiation, without additional osteogenic stimulation. Liposomes containing bisphosphonates could be a promising therapy for improving the longevity of implants by preventing aseptic loosening around cemented joint replacements.

Acknowledgements: This work was supported by a research grant from the Dunhill Medical Research Trust.

P170 An Injectable, Bioactive Material for Osteoporotic Bone Regeneration

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Current research in bone tissue engineering has thus far been focused on developing materials for bone defects rather than bone diseases. Because of this, biomaterial treatment for osteoporosis is relatively underdeveloped. There is an imbalance in osteoporotic bone between bone formation by osteoblasts and bone resorption by osteoclasts. Thus, we posit osteoporotic bone requires a bioactive scaffold that can maximize osteoblast differentiation to build new bone while simultaneously minimizing osteoclast resorption via reactive oxygen species (ROS). This is a dual osteoinductive and antioxidant effect that no clinical material currently achieves. The Ameer lab has previously developed a pH and temperature responsive macromolecule, PPCN, that is liquid at room temperature and gels at body temperature, is antioxidant, biocompatible and biodegradable. **Herein, we show that three different routes of small molecule functionalization (crosslinking of strontium ions, covalent conjugation of cyclic RGD or β-glycerophosphate) can retain the thermoresponsive behavior of the material while inducing osteodifferentiation of both hMSCs and MC3T3s with no further osteogenic supplementation.** Prior research has relied on osteogenic media and/or PEG and alginate-based gels. However, here we propose a material that not only has greater utility than PEG and alginate, but can alone support the survival and differentiation of cells critical to the development of new bone. We perform concurrent theoretical modeling of this responsive, “smart”, material to predict its behavior across different ion concentrations, pH ranges and temperatures. Our goal is for PPCN to advance the field of personalized osteoporotic fracture therapies, to accelerate healing in a variety of bone sites, and consequently, aim to further investigate its potential for localized delivery of osteoporotic drugs and stem cells.

P171 Quiescence preconditioned human multipotent stromal cells adopt a metabolic profile favorable for enhanced survival under ischemia

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A major impediment to the development of therapies with mesenchymal stem cells/multipotent stromal cells (MSC) is the poor survival and engraftment of MSCs at the site of injury. We hypothesized that lowering the energetic demand of MSCs by driving them into a quiescent state would enhance their survival under ischemic conditions. Human MSCs were induced into quiescence by serum deprivation (SD) for 48h. Such preconditioned cells (SD-hMCs) exhibited reduced nucleotide and protein syntheses compared to unpreconditioned hMSCs. SD-hMSCs sustained their viability and their ATP levels upon exposure to severe, continuous, near-anoxia (0.1% O2) and total glucose depletion for up to 14 consecutive days *in vitro*, as they maintained their hMSC multipotential capabilities upon reperfusion. Most importantly, SD-hMSCs showed enhanced viability *in vivo* for the first week post-implantation in mice. Quiescence preconditioning modified the energy-metabolic profile of hMSCs: it suppressed energy-sensing mTOR signaling, stimulated autophagy, promoted a shift in bioenergetic metabolism from oxidative phosphorylation to glycolysis and up-regulated the expression of gluconeogenic enzymes, such as PEPCK. Since the presence of pyruvate in cell culture media was critical for SD-hMSC survival under ischemic conditions, we speculate that these cells may utilize some steps of gluconeogenesis to overcome metabolic stress. These findings support that SD preconditioning causes a protective metabolic adaptation that might be taken advantage of to improve hMSC survival in ischemic environments.

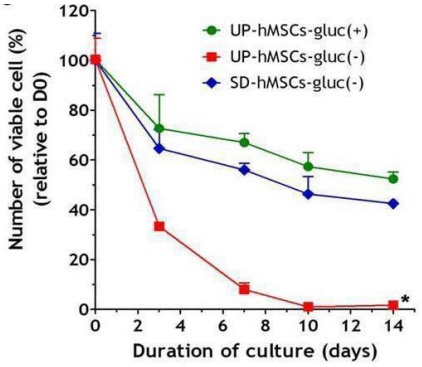

Figure 1: Time-course of cell viability assessed during the ischemic period *in vitro* assessed after Hoechst 33342 and Propidium Iodide staining and analyzed by flow cytometry.

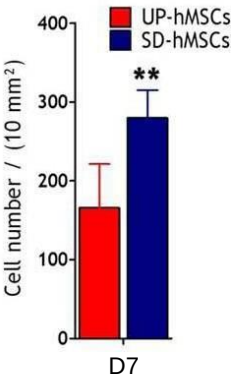

Figure 2: Anti-hβ2-MG immunostaining quantification on cell-constructs containing either Unprecondtioned hMSCs (UP-hMSCs) or SD-hMSCs after 7 days of ectopic implantation in nude mice.

P172 Developme~~nt~~ of injectable BMP-2 delivery materials using collagen-I based recombinant peptide microspheres

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Introduction

Bone morphogenetic protein-2 (BMP-2) adsorbed to collagen sponge is used in clinics as a bone graft; however the supra-physiological amounts of BMP-2 and the burst release have been associated to adverse effects observed[1]. In this study, we have developed Collagen-I Based Recombinant Peptide (RCP) microspheres included in *in situ* gelling hydrogels for sustained delivery of BMP-2 for successful bone regeneration.

Experimental Methods

Collagen-I Based Recombinant Peptide (RCP, commercial product of Fujifilm known as Cellnest™) micron-sized spheres with different size and porosity were produced by emulsification and subsequently crosslinked either by hexamethylenediisocyanate (HMDIC) or by dehydrothermal treatment (DHT). These spheres were then combined with alginate or thermosensitive hyaluronic acid[2] to form *in situ* gelling hydrogels. *In vitro* BMP-2 release from the various sphere types and hydrogels was studied by ELISA. Selected hydrogels containing three different doses of BMP-2 were injected to Sprague-Dawley rats subcutaneously and bone formation was followed up to 10 weeks by microCT. In addition, real time release from the hydrogels in the living animals was imaged using a fluorescently labelled BMP-2.

Results and Discussion

From a variety of different sized and crosslinked spheres, one type of microsphere was selected based on its preferable BMP-2 release profile, showing a small burst release comprising 20% of the initial BMP-2, followed by a sustained release. The *in-situ* gelling formulation of this sphere with alginate further eliminated the burst release and provided a prolonged release for several weeks *in vitro*. The same formulation containing 10 µg and 3 µg BMP-2 induced ectopic bone formation in rats accompanied by vascularization and cell infiltration. In addition, a desirable *in vivo* release profile was observed, where BMP-2 was retained at the injection site for at least 4 weeks, the time required for bone formation.

Conclusion

Novel *in situ* gelling hydrogels containing alginate and BMP-2 loaded RCP spheres demonstrated prolonged release of BMP-2 both *in vitro* and *in vivo*. Based on its thixotropic behaviour and observed bone formation *in vivo*, this hydrogel formulation is a promising biomaterial to be used in a bone regeneration therapy.

Acknowledgments

This work was funded by European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n° 607051.Thermosensitive hyaluronic acid was kindly provided by David Eglin.

P173 Preparation and characterization of scaffold from PCL-IGDP-Collagen and PCL-IGDP-Alginate for bone tissue regeneration

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Three-dimensional (3D) printing as an effective technology has shown considerable potential in tissue regeneration. Poly(ε-caprolactone) (PCL) 3D scaffolds including other materials have been highlighted in the biomedical fields. In this study, a novel method to combine PCL 3D scaffold with marine fish *Paralichthys olivaceus* collagen (Col) or sodium alginate (Sa) and intestine gastro-intestinal digests peptide (IGDP) from abalone *Haliotis discus hannai* for tissue-engineered bone was developed. Four 3D scaffolds of PCL modifications were examined: (1) PCL/Col, (2) PCL/Sa, (3) PCL/IGDP/Col, and (4) PCL/IGDP/Sa, in addition to PCL-only and PCL/IGDP. Mouse mesenchymal stem (MSC) cells were seeded onto the PCL 3D scaffold of pore size 421 - 463 µm, and after 1, 2, and 4 weeks of *in vitro* culture, MSC-scaffolds have investigated the proliferation, ALP content, and calcium deposited. Results showed that the surface-treated PCL/IGDP, PCL/Col, PCL/Sa, PCL/IGDP/Col, and PCL/IGDP/Sa 3D scaffolds had higher protein adsorption and proliferation than did the PCL-only 3D scaffold and the ALP contents in PCL/IGDP/Col and PCL/IGDP/Sa 3D scaffolds were higher than those seen in the PCL-only, PCL/IGDP, PCL/Col, and PCL/Sa 3D scaffolds. RT-PCR showed that ALP and OSC mRNA levels were remarkably elevated in PCL/IGDP/Col and PCL/IGDP/Sa 3D scaffolds versus the PCL-only, PCL/IGDP, PCL/Col, and PCL/Sa 3D scaffolds. The use of PCL/IGDP/Col and PCL/IGDP/Sa to improve the osteoblastic responses in the cell growth and bone tissue regeneration. In the *in vivo*, the 3D scaffolds were implanted in the rabbit tibia. The 3D scaffolds were harvested and dedicated for measurement of mechanical properties and microcomputed tomography (µCT). It was found that the PCL/IGDP/Col were strong induce osteogenic differentiation in the rabbit tibia. These findings of stimulated biological responses *in vitro* and *in vivo* suggest that the PCL/IGDP/Col and PCL/IGDP/Sa 3D scaffold were expected to provide adequate mechanical strength, and therefore were the promising material for use in tissue implants and enhance bone regeneration.

P174 Sustained release of BMP-2 from porous bead with unique leaf-stacked structure for effective bone regeneration

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Tissue engineering technique based on cells, scaffolds, and bioactive molecules to regenerate damaged tissues/organs have been extensively investigated. In recent years, bioactive molecules-loaded matrices which can induce the host stem cells or progenitor cells into target site have been have been gained much attention, due to their simple application in the body and effective target tissue/organ regeneration. It is well-known that the sustained release of the bioactive molecules leads to the maximized function of them, in terms of bioactivity. To introduce the bioactive molecules on certain matrices, surface modifications based on physicochemical (surface) modifications are commonly utilized. However, the potential toxicity of chemical residues used for the modifications and difficult processes are considered as major huddles for clinical applications. Therefore, the main aim of this study was to develop a porous bead with leaf-stacked structure (LSS bead; Fig. 1) which may allow sustained release of bioactive molecules even without any complex modifications. The morphology, BMP-2 release profile (using ELISA kit and rhodamine-conjugated BMP-2), osteogenic differentiation behaviour of human periosteum-derived cells (*h*PDCs), and new bone formation (mandibular defect, miniature pig) of the BMP-2-loaded LSS bead were compared with micron-sized porous bead (MP bead) and dense bead (D bead). It was recognized that the unique leaf-stacked structure of the beads can provide sustained release of the BMP-2 over 30 days, probably due to the repeated adsorption/desorption of the bioactive molecule during release period, and thus the sustained release of BMP-2 from the LSS beads can allow more effective osteogenic differentiation of *h*PDCs and new bone formation than the other beads. From the findings, we also suggest that the LSS bead may be a potential carrier system of a variety of bioactive molecules for clinical applications.

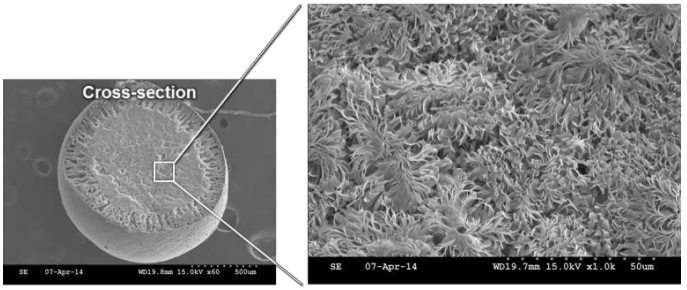

Fig. 1. SEM photographs showing the cross-sectional morphology of the LSS bead.

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Korea (HI13C1596).

P175 Fabrication and characterization of BMP-2-immobilized guided bone regeneration membrane with leaf-stacked structure

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Bone regeneration is one of the most important biological processes to maintain sound health. In general, the rapid appearance of fibrous connective tissues in bony defect during the healing process which lead to the incomplete bone formation in the injury region is considered as a major limitation. To solve this problem, guided bone regeneration (GBR) membranes which can prevent connective tissue infiltration into the bony defect, but allow penetration of essential substances (i.e., nutrients and oxygen) into the defects, and thus lead to sound bone regeneration has been adapted in clinical practices. In this study, we developed a unique GBR membrane with leaf-stacked structure (LSSM) which may meet the essential requirements of GBR membrane as well as allow sustained release of bioactive molecules (i.e. BMP-2) without any additional modification procedures. The morphology, mechanical property, wettability, permeability, BMP-2 release profile, osteogenic differentiation behaviour of human periosteum-derived cells (*h*PDCs) of the BMP-2-loaded GBR membrane were investigated. And also, the new bone formation of the GBR membrane was compared with commercialized products (Collaguide® and Osteoguide™) using SD rats (calvarial defect model). It was observed that the fabricated GBR membrane allow higher loading amount of BMP-2 and their longer release period (~37 days) compared with the Osteoguide™ (~6 days), probably due to much greater surface area (to BMP-2 adsorption) and repeated adsorption/desorption of the BMP-2 during release period through the unique structure. For *in vivo* animal study, we also recognized that the sustainedly released BMP-2 and leaf-stacked structure (similar to bone mineral structure; bottom surface of the membrane) of the GBR membrane can provide appropriate environment for new bone formation (Fig. 1).

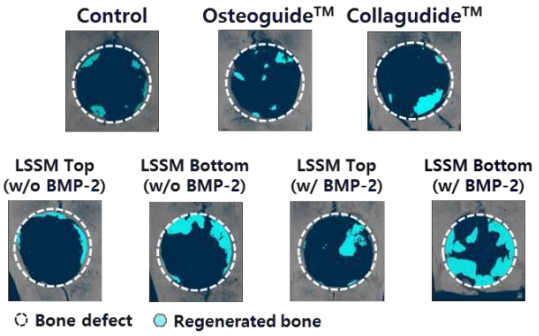

Fig. 1. μ-CT images of calvarial defect showing the bone regeneration behaviour.

This work was supported by grants of Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT and Future Planning (NRF-2015R1D1A1A01056713).

P177 Internalization peptides containing C-Terminal domain of Connexin 43 has no osteogenic effect on human bone marrow mesenchymal stem cells *in vitro* and on bone regeneration *in vivo*

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Bone tissue consists of bone matrix mainly two types of bone cells: osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells). Gap Junctions Intercellular Communication (GJIC) are regions on the surface of adjacent cells that facilitate intercellular communication through proteins called connexins. Different connexins are expressed by bone cells, however, connexin 43 (Cx43) is expressed in larger amounts, specially by osteoblasts and osteocytes and this connexin plays an important role on osteoblastic differentiation [1,2]. Two internalization peptides containing the C-terminal (CT) domain of Cx43 (an important regulatory domain) and a PDZ domain (a Cx43 CT-binding motif) have been reported in the literature showing in vivo better preclinical results in tissue regeneration such as wound healing [3,4]. However, there is no data regarding the effect of these peptides onosteogenesis and bone regeneration. For that, we performed different assays to evaluate the effect of these internalization peptides containing the CT of Cx43 with or without the PDZ domain on human bone marrow mesenchymal stem cell cells (hBMSC) towards the osteogenic phenotype. We delivered peptides directly in the culture medium (every two days) at 60 µM and 120 µM and performed the analysis after 1, 3 and 7 days. These concentrations have already showed to modulate wound healing response *in vivo* in a favourable way [2,3].We assessed the metabolic activity by Alamar Blue test; modulation of the osteogenicmarkers*RUNX2*, *COL1A1*, *OPN*, *ALPL* by real-time qPCR and activity of Alkaline Phosphatase (ALP). Our results suggest that these peptides do not alter metabolic activity, they do not modulate the osteogenic gene markers and they have no influence on the ALP activity in hBMSC*in vitro*. Moreover, to clarify the role of these peptides in bone regeneration, we performed an *in vivo* test using an experimental model of bone calvaria defects in mice (n=5 for each peptide and n=3vehicle, used as control). After 4 weeks, we analyzed the following parameters of the regenerated tissue: bone volume, mineral content and density by micro computed tomography using the program MicroView ABA 2.2. We did not observe any differences in bone regeneration in the presence of the peptides. Our data suggest that internalization peptides containing CT domain of Cx43 have no effect on the differentiation of hBMSC*in vitro* and during the bone regeneration *in vivo* using calvaria lesion model in mice.

P179 Novel supercritical CO_2 processed composites for bone applications: the road from manufacturing to cell culture and animal experiments

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Tissue engineered grafts are still used significantly less in contrast to autologous or allogenic bone grafts. However, with bone transplants there are problems like the morbidity of the harvesting site and limited availability. Therefore new, easier to use bone substitutes are needed in the treatment of large bone defects.

Composites comprising poly(L-lactide-*co*-ε-caprolactone) with 50wt-% of β-tricalciumphosphate were foamed by using supercritical CO_2 and cut into discs. Porosity and pore size of the composites were analyzed by µ-CT analysis. *In vitro* testing was done by culturing human adipose stem cells (hASCs) in the composites in osteogenic medium up to 21 days. As a control, commercial chronOS® Granules were used. Cell viability was assessed by Live/Dead staining. Osteogenic differentiation was analyzed using different methods, such as alkaline phosphatase activity assay and immunocytochemical staining of osteogenic marker proteins. *In vitro* analyses were conducted at 7-, 14- and 21d time points. Thereafter, composites with 50wt-% of β-tricalciumphosphate were implanted in rabbit distal femur defects. Empty defects were used as control. Histological staining and µ-CT analysis were conducted 4-, 12- and 24 weeks after implantation as 6 rabbits were sacrificed per time point.

Porosity of the composites was 63-65% with average pore size of 380-440µm. The novel composites supported the viability of hASCs up to 21 days (Figure 1). Furthermore, hASCs differentiated towards osteogenic lineage when cultured in the composites. In the rabbit model, host tissue was able to grow into the porous composites already in 4 weeks and histological staining showed that at 24 weeks calcified bone had formed inside the porous composites in defects.

To conclude, these flexible and user-friendly supercritical CO_2 –processed composite scaffolds show great potential as a new bone substitute material for treating bone defects.

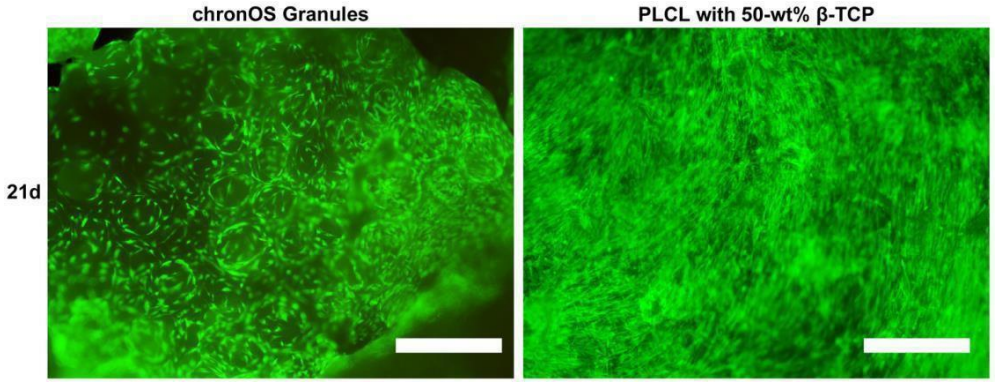

Figure 1. Live/Dead staining of hASCs in scaffolds at 21d. Scalebar 500µm.

P180 3D nanocrystalline silicon substituted hydroxyapatite scaffolds with adsorbed VEGF for bone regeneration by promoting angiogenesis and osteogenesis

L. Casarrubios[1], N. Gómez-Cerezo[2,3], S. Sánchez-Salcedo[2,3], M.C. Serrano[4], M.J. Feito[1], D. Arcos[2,3], M. Vallet-Regí[2,3], M.T. Portolés[1]

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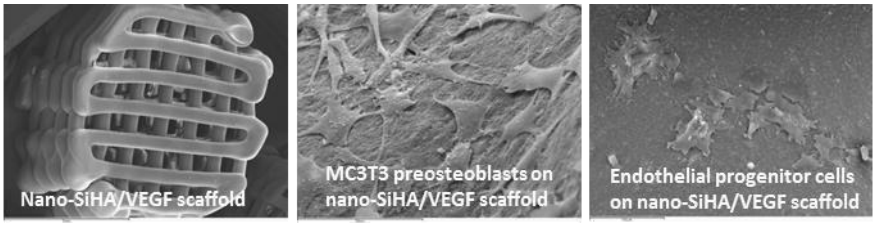
INTRODUCTION: The design of scaffolds for bone tissue engineering involves the optimization of biomaterials that support osteoblast differentiation and induce a rapid neo-vascularization for adequate oxygen and nutrient supply. Nanocrystalline silicon substituted hydroxyapatite (nano-SiHA) presents enhanced bioreactivity and could improve the osteogenic effect of Si [1,2], delaying osteoclast differentiation and resorptive activity [3]. Vascular endothelial growth factor (VEGF) has been recently adsorbed on nano-SiHA disks improving the adhesion and proliferation of endothelial progenitor cells (EPCs) [4]. Since VEGF is involved in both angiogenesis and osteoblast maturation [5], in the present study 3D macroporous nano-SiHA scaffolds with high interconnectivity and adsorbed VEGF were evaluated for preosteoblast differentiation and EPC colonization.

METHODS: 3D macroporous scaffolds based on nano-SiHA, nominal formula $Ca_{10}(PO_4)_{6-0.25}(SiO_4)_{0.25}(OH)_{2-0.25}$, were prepared by robocasting (EnvisionTEC GmbH Prefactory® 3D BioplotterTM, Germany). After scaffold characterization by SEM, VEGF (2.5 µg/500 µl) was adsorbed for 24h on the scaffold surface. Either MC3T3 preosteoblasts (ATCC) or EPCs [6] were cultured on nano-SiHA/VEGF scaffolds ($3x10^5$ cells/scaffold) for 5 days to evaluate adhesion, proliferation and differentiation of these cell types. Cell morphology was observed by SEM. Alkaline phosphatase was measured as an osteoblast differentiation parameter.

RESULTS & DISCUSSION: 3D macroporous nano-SiHA scaffolds allowed MC3T3 and EPC adhesion and proliferation, preserving their typical morphology. VEGF adsorbed to the scaffold improved osteoblast differentiation and EPC proliferation.



CONCLUSIONS: The obtained results suggest the potential utility of these 3D nanocrystalline Si-substituted HA scaffolds with immobilized VEGF for bone repair and tissue engineering by promoting angiogenesis and osteogenesis.

P181 Evaluation of the effectiveness of xeno-free media for human MSC osteogenesis in 2D and poly(ε-Caprolactone) scaffolds

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Animal sera is widely used and are a conventional supplement in *in vitro* cell culture for MSC proliferation and differentiation. However, serious shortcomings have been noted such as potential contamination, diseases transmission, and poorly undefined components. For these reasons, xeno-free media have recently been developed to overcome the limitation of animal sera but these have usually been developed for their ability to support cell proliferation and little is known about osteogenic differentiation in these media. The objective of this work was to evaluate the effectiveness of different Xeno-free media for on proliferation, expansion, and differentiation of human bone marrow mesenchymal stem cells (hBMSCs), with the long-term purpose of culturing in Xeno-free formula as a pre-culture in clinical applications for cleft palate repair. For monolayer experiments hBMSCs were seeded in 24-well plates at 1 x 10^4 cell/cm^2 in a different commercial media 'Stemulate TM Pooled Human Platelet Lysate' (COOK) (5% HPL), and 'Human Mesenchymal-XF Expansion Medium' (MILLIPORE) (XF2), 'Stem X VivoTM Xeno-Free Human MSC Expansion Media' (R&D; USA), (XF1), 'PeproGrow-1 Serum-Free' (PEPROTECH; UK) (SR-1), to compare with gold standard medium 10%FBS (as a control). All media were supplemented with beta-glycerophosphate 10 Mm (βGP) and ascorbic acid-2-phosphate 50 μM (AA), furthermore, osteogenic media was additionally augmented with dexamethasone (Dex) 10 nM. Metabolic activity was assessed by resazurin reduction assay at different time points, 'Quant- iTTM picoGreen®® (Invitrogen, life technologies, USA) was used to measure cell number. Cell proliferation was measured by 'Click-iT^{Tm} Plus EdU Flow Cytometry' (Invitrogen, ThermoFisher Science, USA). Initial osteogenic differentiation of hBMSCs was assessed by alkaline phosphatase (ALP) activity. For 3D experiment electrospun polycaprolactone (PCL) was used as a scaffold. hBMSCs were seeded at density of 2 x 10^5 in a different commercial media (5% HPL, 10% FBS, and XF2). hBMSCs growth and metabolic activity in both 2D culture and 3D PCL scaffold increased significantly over time in XF2 compared to all other groups. Of the Xeno-free media, XF2 supported significantly higher metabolic activity and DNA count on day 7. Furthermore, the new DNA synthesis as measured by EdU flow cytometry was much faster in XF2. The reason for this may be because XF2 contained of 2% human serum which is considered to contain more relevant nutrients for hBMSCs compared to bovine serum. When osteogenic supplements were added ALP activity was significantly higher in XF2 even in 'non- osteogenic' culture medium (NOCM) XF2. In conclusion XF2 is highly supportive of osteogenic hBMSCs and may be a functionally efficient pre-clinic medium for proliferation, expansion, and differentiation for bone tissue engineering including cleft palate repair.

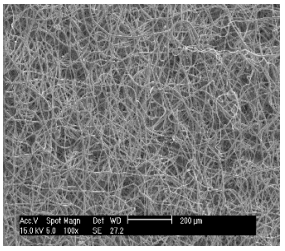
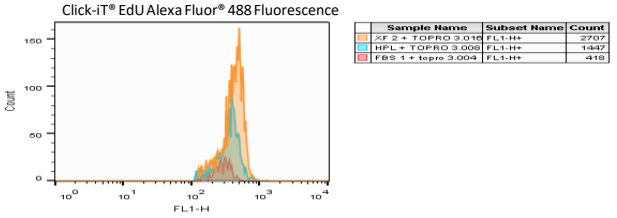


Fig. 1: SEM of a PCL demonstrating the non-alignment



P182 Evaluation of safety and feasibility of novel tissue engineered bone for large segmental bone defects using autologous mesenchymal stem cells

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Management of large segmental bone defect is challenging and often associated with donor site morbidity. In unfavourable etiologies such as infection and congenital pseudarthrosis of tibia (CPT), the chances of union are very less. With the current treatment, grafts take a long time to integrate and heal. Hence, an alternative approach for faster integration of the transplant that will hasten the bone union is essential. This study attempts to treat the diaphyseal segmental bone loss using autologous bone marrow mesenchymal stem cells (MSCs) on a custom made triphasic hydroxyapatite silicate (HASi) scaffolds in children. Five patients, (one post-osteomyelitis and four CPT) with mean age of 5.8 years (1 – 11 yrs) and mean defect size of 3.7 cm (3-4.5 cm) were recruited in the study. Mesenchymal stem cells were isolated from bone marrow and expanded in a Current Good Manufacturing Practice facility. The cells were characterised for MSC markers by flow cytometry. A cell density of 1 x 10^5 cells per sq cm were seeded and primed for osteogenic lineage on HASi scaffold prior to transplantation. The cell-seeded constructs were assessed for viability by live dead assay, cell adhesion and infiltration by scanning electron microscopy (SEM) and secretion of alkaline phosphatase by flow cytometry, immunofluorescence and gene expression. After establishing safety related to stem cell transplantation, union of graft (*in vivo*) at both ends of the bone was assessed using radiographs. The secondary efficacy outcome of the graft was assessed by computerized tomography (CT), bone mineral density using dual energy X-ray absorptiometry and measurement of alkaline phosphatase levels at pre-op, 2 weeks and 2 months post-operatively. The cells were more than 90% positive for CD 90, CD 73, CD 105 (positive markers) and <3% positive for CD 34, CD 45 and CD 14 (negative markers). Cell viability over scaffold was more than 98% prior to transplantation. The SEM score revealed good to excellent scores based on cell morphology and adhesion, cell-cell and scaffold interaction. The secretion of alkaline phosphatase on the cell-seeded scaffolds ranged from 10-65% on day 7 and 14. This was corroborated by gene expression analysis. Radiographs and CT show good incorporation of graft at either ends with partial scaffold resorption and *de novo* bone formation. The first two patients have completed 1.5 and 2 years of follow-up respectively and have become fully functional. The third patient at 1 year follow-up had infection at the site of transplantation followed by the removal of the graft. The fourth and fifth patients have completed 1 year follow-up with graft incorporation at either ends. Currently, they are ambulating using clamshell orthosis as per congenital pseudoarthrosis protocol. All patients have increased levels of alkaline phosphatase. We have assessed the safety and feasibility of a novel tissue engineered bone for large segmental bone loss. The two conditions recruited are notorious for a high failure rate with conventional treatment. No adverse event pertaining to stem cell transplantation was observed till date. Long term follow-up of these patients are required to comment on the efficacy of this strategy against the gold standard treatment.

P183 Generation and differentiation of scaffold-free self-organizing microspheres from different MSC niches: potential in tissue engineering and disease modeling

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Aims: The aims of the current study are to characterize and compare human (h)MSCs from healthy and osteoporotic patients on one hand and ovine (o)MSCs on the other hand from three different origins each. Using a tissue engineering model of alginate beads and scaffold-free self-organizing 3D microspheres, we investigated their osteogenic and chondrogenic differentiation potential for use as a model of disease and regeneration in a microenvironmental niche.

Methods: MSCs from bone marrow, adipose tissue and fat marrow were characterized via their differentiation towards adipo-, chondro-, and osteogenic lineages, surface marker expression, proliferation and immunomodulatory capacity. MSCs were encapsulated into alginate and scaffold-free self-organizing 3D microspheres from MSCs were generated on agarose. Differentiation towards osteogenic lineage was confirmed via ALP and Alizarin Red S staining in cryosections and monolayer cultures. The calcification process was evaluated by measuring optical density (OD) and free phosphate ions in the supernatants at the end of the osteogenic differentiation process. Differentiation towards chondrogenic lineage was confirmed via Alcian Blue staining in cryosections and monolayer cultures.

Results: All investigated MSCs expressed typical MSC surface markers, differentiated towards the three lineages and self-organized into scaffold-free 3D microspheres. The mineralization of all investigated MSCs was confirmed via specific stainings, increased values OD and free phosphate ions. The chondrogenic differentiation was confirmed via Alcian Blue staining. All bone marrow derived MSCs showed immunomodulatory capacity by suppressing lymphocytes proliferation.

Conclusions: We report an optimized characterization and comparison of oMSCs and hMSCs from three different niches. MSCs from osteoporotic and healthy patients showed similar characteristics. OD measurement serves as an early indicator of calcification and values of free phosphate ions demonstrate that oMSCs possess ALP activity. Scaffold-free 3D microsphere technique offer *in vivo*-like conditions, permitting the investigation of ECM functions, like MSC cross talk with other cell types in a microenvironment. Encapsulating microspheres into alginate beads can optimize MSC seeding efficiency and therefore improve tissue engineering in regenerative medicine. MSC-derived bone- and cartilage-like organoid cultures can serve as an *in-vitro* model to address investigation of musculoskeletal problems, such as degeneration, infection, drug effects and mechanical studies, as personalized therapies in regenerative medicine. Moreover, this model can be extended by adding other cells orchestrating bone remodeling or cartilage degeneration, like osteoclasts, immune cells or endothelial cells, creating an individual *in-vitro* disease and regeneration niche model.

P185 Bio-activation of poly(ester-urethane) scaffolds to enhance cell recruitment and bone regeneration

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INTRODUCTION: For bone tissue engineering, a small number of studies combined osteoconductive scaffolds, osteoinductive growth factors, and osteogenic cells since multi-component approaches involved many questions that remain to be solved. Within the aim to develop scaffolds capable of recruiting cells, the present study focuses on the bio-activation of poly(ester-urethane) (PCLU) scaffolds. *In vitro* and *in vivo* assays were performed to evaluate PCLU efficiency for bone repair.

METHODS: Porous cross-linked poly(ε-caprolactone urethane) (PLCU) scaffolds were prepared through a polyHIPE method. Scaffolds were sterilized by autoclave and bio-activated. For *in vitro* assays, human mesenchymal stem cells (hMSCs) were grown up to confluence. Thereafter, scaffolds were set down onto the cell culture to achieve cell infiltration. Environmental Scanning Electron Microscopy (ESEM) was used to visualise hMSCs within scaffolds. For *in vivo* assays, scaffolds were implanted into 3mm-cavitary defects drilled in Lewis rat's femoral bone. Bone reconstruction was monitored with micro-computed tomography (μCT) and histology.

RESULTS: The *in vitro* study demonstrated that hMSCs were unable to migrate into PCLU scaffolds, whereas the bio-activation improved the recruitment of cells. The *in vivo* assay showed better regeneration with bio-activated scaffold compared to PCLU.

DISCUSSION & CONCLUSIONS: In addition to enhance cell recruitment within PCLU scaffold, the bio-activation will promote cell adhesion and differentiation. As observed with the *in vivo* assays, the bio-activation leads to the increase of new bone formation. Deeper *in vitro* and *in vivo* investigations are underway to assess the efficiency of bio-activated PCLU scaffolds as graft substitute for the repair of bone defects.

P187 Cross-linked collagen-based scaffolds colonized by mesenchymal stem cells as a potential tool for bone regeneration – in vitro evaluation

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Two types of composite scaffolds (both consisting of natural collagen, poly(DL-lactide) electrospun nanofibers, calcium phosphate nanoparticles and sodium hyaluronate at different ratios) cross-linked by EDC/NHS were developed as a potential tool for bone surgery and bone regeneration based on mesenchymal stem cells (MSC) participation. Scaffolds were tested under *in vitro* conditions for their fundamental characterization and evaluation. Biological qualities of scaffolds in role of “artificial extracellular bone matrix” were determined by application of porcine MSC (pMSC). pMSC were cultivated on scaffolds for 48 h or 168 h under static or dynamic cultivation conditions. Cell adhesion, proliferation, morphology, penetration or cell differentiation were analysed. Obtained results were used for scaffold comparison, evaluation of different cultivation conditions and for study of „scaffold-pMSC“ potential for the future clinical application. Fluorescence visualization (wide-field and confocal microscopy) supported by other methods determined the scaffold with higher hydroxyapatite and lower poly(DL-lactide) and collagen content as the most suitable one for cell interaction and thus for subsequent *in vivo* analysis. Moreover, our observations noticed positive/harmless impact of used dynamic cultivation system on cell colonization in comparison to static conditions. Dynamic environment mostly caused less cells on seeding surface of scaffold and at the same time more cells with more homogenous distribution in scaffold depth in comparison to static environment. Because, in principle, the dynamic cultivation should better simulate *in vivo* conditions, this system provides more natural *in vitro* conditions in general. Taken together, our results helped us to choose the scaffold with higher potential for successful *in vivo* testing and possible future clinical application when colonized with pMSC. In addition, we noticed differences between dynamic and static cell cultivation which can finally mean an immoderate effect on *in vitro*results.

P188 Encapsulation and osteogenic differentation of human periodontal ligament fibroblasts in PRP/hydroxyapatite microbeads

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The use of the autologous products in personalized regenerative medicine and tissue engineering has become more attractive recently due to eliminating adverse inflammatory, immune rejection complications. Platelet-rich plasma (PRP) is a blood-derived autologous product obtained from whole blood, which contains high amounts of growth factors and cytokines responsible for regulating proliferation, differentiation, chemotaxis, wound healing and tissue regeneration processes. These growth factors are transforming growth factor-beta (TGF-β), platelet-derived growth factor (PDGF), epithelial growth factor (EGF), insulin-like growth factor (IGF-I) and vascular endothelial growth factor (VEGF). PRP is obtained from a sample of patients' blood through an easy and inexpensive method. For many years, autologous PRP in the form of solution or gel has been used safely and effectively in oral and maxillofacial surgery for the treatment of soft and hard tissue injuries; and is applied through direct injection method or by incorporating to the graft material. The aim of this study was to investigate the osteogenic effect of PRP/hydroxyapatite (PRP/HA) microbead encapsulation on human periodontal ligament fibroblasts (hPDLFs). The characterization of surface properties of PRP/HA microbeads was performed using SEM analysis. At regular intervals, the viability of encapsulated hPDLFs in extended culture was determined by using the Alamar blue assay. The osteogenic differentiation of hPDLFs encapsulated in PRP/HA microbeads was confirmed by the Von Kossa and Alizarin Red stainings. The results indicated that PRP/HA microbeads showed cellular compatibility and enhanced cell proliferation. ALP activity and histochemical stainings indicated that PRP/HA microbeads significantly promoted osteogenic differentiation of the hPDLFs compared to control groups.

P189 Studying the role of sensory innervation on osteogenesis in a microfluidic coculture system comprising sensory neurons and mesenchymal stem cells

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The number of musculoskeletal defects, including those resulting from trauma, infection and tumors, has dramatically increased over the last years, posing a serious public healthcare issue. Although angiogenesis is recognized as crucial in order to achieve successful bone repair, several studies have recently shown that innervation by the sensory nervous system plays a key role in skeletal development and in the orchestration of bone turnover and regeneration. However, the intricate molecular determinants and mechanisms on the interplay between sensory neurons and bone cells in a regeneration scenario remain unknown.

In the present study, we aim to establish a microfluidic coculture system between Dorsal Root Ganglion (DRG) neurons and Mesenchymal Stem Cells (MSCs) in order to further understand the impact of sensory innervation on the orchestration of osteogenesis and bone repair.

A two-stage photolithography negative, using standard techniques, was performed to allow the creation of compartmentalized microfluidic devices, consisting on a two-chamber system connected by microgrooves.

Bone marrow MSCs were collected from tibias and femurs of male Wistar rats and cultured for 1-2 weeks under standard conditions. Then, MSCs were cocultured with DRG neurons, isolated from adult male Wistar rats, in the microfluidic devices. Expression levels of osteoblast marker genes were analyzed by RT-qPCR to assess the influence of sensory neurons in the differentiation of MSCs into the osteoblast lineage. Additionally, Alkaline Phosphatase (ALP) assay was performed, and the impact of the coculture on metabolic activity of MSCs over time was verified by the AlamarBlue assay.

We show that the microfluidic coculture systems indeed enable to mimic the *in vivo* scenarioand the innervation process of bone tissue, where the projected neurons come into contact with the MSCs, without affecting their metabolic activity. An upregulation of osteoblast marker genes was observed in MSCs cocultured with sensory neurons, when compared with MSCs in monoculture. In addition, under the same conditions, we verify an increase of intracellular ALP levels in MSCs cocultured with sensory neurons.

These results suggest that sensory neurons regulate the commitment of MSCs to the osteoblast lineage.

P190 The influence of ordered 3D printed PLA scaffold microstructurization on rat dental pulp stem cells

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INTRODUCTION. Tissue engineering is an interdisciplinary science that seeks to create artificial organs and tissues, which could be a potential alternative for real ones, due to their limitless supply and non-immunogenicity. Today, bone is one of the most frequently transplanted tissues, as a consequence artificially engineered bone tissue grafts are in high demand. Appropriate cell source and scaffold selection are the basic elements required for successful creation of the artificial bone. This work is focused on the impact of orderly organised biodegradable polylactic acid (PLA) scaffolds surface microstructurisation to rat dental pulp stem cell (DPSC) adhesion, proliferation and osteogenic differentiation.

METHODS. Two different scaffolds were manufactured using fused filament fabrication 3D printer: microporous with pore size of ~300 µm and grooved topography [1]. DPSC were isolated from rat incisor dental pulp by combining two methods: outgrowth from intact tissue and direct isolation with magnetic beads. These cells were characterized by flow cytometry and by their differentiation ability into myocytes, adipocytes and osteocytes. Differentiation was induced and evaluated according to Kalvelytė et al.,2013 [2]. DPSC proliferation rates, adhesion and expression of bone morphogenesis-related genes were examined.

RESULTS. Isolated DPSCs expressed mesenchymal stem cells markers and showed the ability to differentiate into myocytes, adipocytes and osteocytes. PLA microstructurisation had effect on DPSCs attachment and proliferation. Cells attached equally well on both surfaces, but a greater cell number was observed on grooved scaffolds. However, cells proliferated faster on microporous PLA surfaces. Cells grown on microporous scaffolds likewise demonstrated higher osteogenic potential compared to grooved counterpart.

DISCUSSION & CONCLUSIONS. PLA surface topography influenced cell osteogenic differentiation capability, proliferation and adhesion. Ordered 3D microstructured scaffolds can be successfully applied as templates for cell proliferation and used as implants for tissue engineering applications.

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P191 Improved bioactivity and antibacterial properties by reduced graphene oxide coating

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Application of a two-dimensional (2D) nanomaterial coating improves biocompatibility and overcomes the drawbacks of low cellular activity on conventional bioactive substrates. Graphene oxide (GO) is 2D-nanomaterial with widely promising applications, e.g., drug or gene delivery, tissue engineering, and in-situ sensors. In the present study, to investigate the potential of GO to facilitate the bioactivity of titanium for use in orthopaedic applications, GO was deposited anodically-electrophoretically onto either titanium (Ti) or nanotubular anodized titanium (ATi). Physiochemical characterization studies using a scanning electron microscope, X-ray diffractrometer and X-ray photoelectron spectroscope suggested that GO was reduced onto Ti or ATi as a thin film. We next examined the biologic activity (adhesion, proliferation, and differentiation) of pre-osteoblasts (bone-forming cells) on the reduced graphene oxide (rGO) on Ti and ATi. The rGO coatings promoted local concentration in the bone extracellular matrix, including alkaline phosphatase activity, total protein content, and calcium deposition. The bacterial viability of *Staphylococcus aureus* and *Escherichia coli* was significantly decreased on the electrodeposited GO samples after 18 h of culture. The results suggest that the biocompatibility and antibacterial properties of Ti and ATi were significantly improved by an electrodeposited graphene oxide (GO) coating.

P192 Dentine matrix loaded silk-fribroin/gelatin scaffolds direct osteogenesis of bone marrow mesenchymal stem cells

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Silk has widely been investigated as a natural polymer for use in biomedical applications and blending of additional constituents such as gelatin confers excellent cell attachment. Gelatin is highly soluble and undergoes degradation, however this can be harnessed to permit the release of bioactive factors for enhancement of tissue repair. **Aim:** Analyse the potential for silk- fibroin/ gelatin based matrices to support the culture of bone marrow mesenchymal stem cells (BMMSCs) and to test the potential for osteogenic induction by incorporation of demineralized dentine matrix (DDM) with the intention of enhancing bioactivity of silk-fibroin biomaterials **Methods:** Silk-fibroin and gelatin (SF/G) films were cast by blending aqueous solutions of SF (5% w/v) and gelatin (5% w/v) in ratios of SF:G at 75:25, 50:50 and 25:75. Solutions were applied to 96-well and 12-well cell culture plates at 105µL/cm^2, and were allowed to fully evaporate under ambient conditions. For DDM inclusion into films, lyophilised DDM was reconstituted in the SF component of the solution and then blended with additional SF and gelatin to produce concentrations of DDM at 20, 10 and 5µg/mL. Dried films were treated with methanol to induce conformational changes to insoluble -sheet structure and air-dried. BMMSCs were seeded onto SF/G and SF/G/DDM films at 4x10^{3}cells/cm^2 and cultured for 48 hours and expansion determined through MTT assays. Osteogenic potential of SF/G/DDM on BMMSCs was assessed after 5 days culture through RunX2 gene expression (qPCR with GAPDH as an internal reference) and after 28 days of culture by measurement of mineral deposition through alizarin red staining. **Results:** BMMSCs seeded onto substrate blends of SF/G demonstrated cellular attachment to all blend ratios tested with those seeded onto 75SF/25G and 50SF/50G blends exhibiting spindle-like morphology whilst those on the 25SF/75G blend appeared smaller with of associated globular structures. SF/G blends provided a surface that facilitated cell expansion with the higher ratios appearing to support the greatest expansion. Cells cultured on DDM loaded SF/G films demonstrated cellular attachment and subsequent expansion with cells cultured on films loaded with 20µg/mL demonstrating highest expansion after 72 hours in culture compared with cells cultured on unloaded films. RunX2 expression of BMMSCs cultured on SF/G films was unchanged after 5 days in culture and no differences in expression levels between cells cultured on SF/G films loaded with varying DDM concentrations were observed, however positive alizarin red staining was observed in cells cultured on SF/G films loaded with DDM when compared with controls. **Conclusion:** This preliminary data suggests the SF/G blended films support expansion of BMMSCs, which is enhanced by additional loading with DDM. Further the osteogenic potential of DDM-loaded SF/G films suggests their potential as a substrate carrier for DDM and for loading of SF/G based matrices with bioactive factors for use in tissue engineering. Supported by Rosetrees Foundation, UK

P193 Cigarette smoke exposure impairs differentiation of human mesenchymal stem cells to osteoblasts and down regulates BMP signalling in vitro – Possible role in delayed fracture healing?

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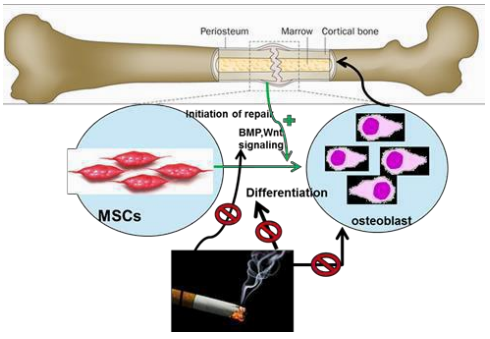
In the 21st century, cigarette smoking is continuously evolving as a massive socio-economic burden causing nearly 5 million deaths globally. Similar to many other systems of the body, the negative effect of cigarette smoke on bone health is undisputable. Several clinical studies including ours have shown the positive link between cigarette smoking and delayed fracture healing. However, the mechanisms that contribute to this delayed fracture healing are complex and not fully understood.

The process of bone regeneration is quite complex and involves the participation of bone marrow stem cells, chondrocytes and bones cells alongside the changes in the expression of several genes. The migration and differentiation of mesenchymal stem cells to osteoblast is a crucial step during the initiative phase of fracture repair. Our study shows that exposure of physiological concentrations of cigarette smoke extract not only significantly lowered the viability and proliferation of human mesenchymal stem cells (SCP-1) but also impaired its differentiation to mature osteoblasts. In order to understand the molecular mechanism underlying the impaired differentiation, the gene expression of BMP, Wnt signalling was investigated, since the cross-talks between these pathways have been implicated to play a central role in the differentiation of osteoblast, bone turnover and integrity. Our study revealed that CSE exposure caused a significant decrease in the gene expression of *BMP2*, *BMP7*, BMP receptors, transcription factors *Smads* as well as its final gene product *OPG* and *RUNX2* (markers of mature osteoblast). Furthermore, the expression of *SOST*, the inhibitor of Wnt signalling was significantly upregulated. The alterations in the expression of these genes could ultimately affect the bone remodelling events.

Thus an early intervention using growth factors to promote the differentiation of MScs to osteoblast could be crucial in promoting fracture healing in smokers. Our study broadens the current knowledge on cigarette smoke induced insult on bone remodelling and could promote developing alternative therapy to enhance fracture healing in smokers.



P194 Direct electrical stimulation may promote mesenchymal stem cell proliferation through a pathway that involves reactive oxygen species (ROS)

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Direct electrical stimulation has been capable of controlling the mesenchymal stem cell behaviours, e.g. cell alignment, cell proliferation and differentiation. It is suggested that reactive oxygen species (ROS) are also being produced during the stimulation as faradic by-products beside the electric field. This study hypothesises that ROS by-products could be involved in the signalling pathway from electrical stimulation towards the cellular responses.

To preliminarily investigate this hypothesis, human bone marrow-derived mesenchymal stem cells were cultured in 6-well plates, and treated with growth medium (GM) and osteogenic medium (OM). Direct electrical stimulation was delivered for 1 hour daily through L-shaped platinum electrodes to generate direct current electric field of 100mV/mm across the culture area. After 7 days of electrical stimulation, cell proliferation was characterised by alamarBlue® assay. Intracellular H_2O_2 was also measured from the cell lysates using fluorometric assay.

It is found that this regime of direct electrical stimulation could promote cell proliferation after 7 days. The intracellular H_2O_2 after the stimulation is also found to be higher than the control group. On the other hand, the H_2O_2 level in OM-treated group was relatively lower than the GM-treated group. This is expected to be the result of the catalase secreted from the cells after being exposed to OM; however, it needs further characterisation for validation. The results from this study have shown an accumulation of H_2O_2 inside the cells after direct electrical stimulation, which associates with an increase in cell proliferation rate. This finding has led to several future plans to understand the role of H_2O_2 in the signalling pathway between direct electrical stimulation and cell proliferation as well as the other cellular activities of mesenchymal stem cells.

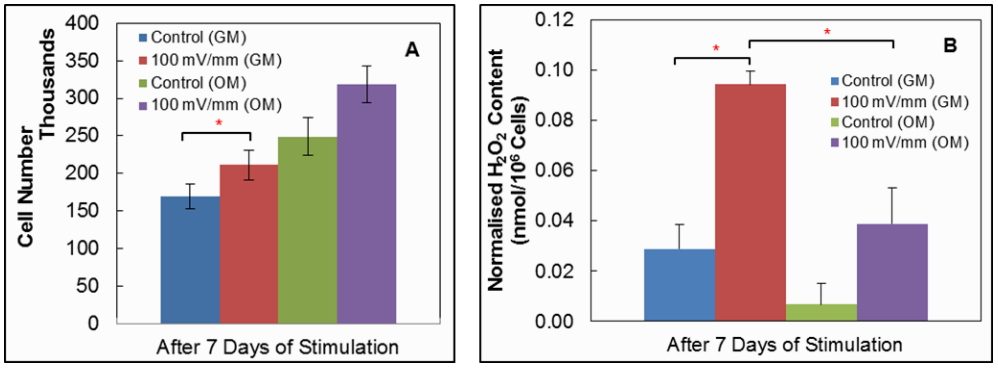

Figure 1 Cell number measured by alamarBlue® assay (A), and normalised intracellular H_2O_2 content (B) (Error bar represents standard deviation (n=3) and * represents $p<0.05$)

P195 Nanodiamonds enhance biocompatibility of copolymer scaffolds for bone regeneration

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Poly(L-lactide)-co-(ε-caprolactone) scaffolds modified with nanodiamond particles (nDP) for bone regeneration were investigated for their biocompatibility by evaluating for up to 27 weeks *in vivo* for their degradation and host tissue response and investigated *in vitro* for their tumorigenic potential. Modified scaffolds degraded faster than unmodified scaffolds. Gene expression of pro-inflammatory and angiogenic markers were upregulated from the nDP scaffolds 1 week post implantation *in vivo*. Inflammatory cells and foreign body giant cells' infiltration reduced significantly around the modified scaffolds after 8 weeks *in vivo*. A fibrous capsule was detectable by week 8 *in vivo*, thinner around nDP scaffolds and at week 27 thickest around unmodified copolymer scaffolds. Tissue healing markers were highly expressed in the modified groups. Interestingly, agglomerated nanodiamond particles were found in the implantation site after 27 weeks when 90% of the scaffolds had degraded without observed adverse effects. Culturing early neoplastic dysplastic keratinocytes (DOK^{Luc}) on nDP modified scaffolds reduced significantly their subsequent sphere formation ability and decreased significantly the cells' proliferation in the supra-basal layers of **in vitro** 3D oral neoplastic mucosa (3D-OT) when compared to DOK^{Luc} previously cultured on unmodified copolymer scaffolds. In conclusion, these **in vitro** and **in vivo** results demonstrate that nDP modified copolymer scaffolds do not aggravate tissue response and they are able to decrease the tumorigenic potential of DOK^{Luc}.

P196 Development of a novel RGD-coated porous polyurethane scaffold for bone tissue engineering purposes

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Introduction

The development of safe and efficient bone grafting techniques is dictated by the increasing need for bone grafts for orthopaedic procedures and the disadvantages associated with the use of autografts, allografts and xenografts. RGD is an extracellular matrix peptide which has been shown to serve as a cell adhesion motif with an effect on stromal cell proliferation and differentiation. Immobilization of RGD has been achieved on 2D substrates but remains a challenge for significant-thickness 3D scaffolds. For this reason, we created a novel RGD-surface modified polyurethane scaffold seeded with umbilical cord blood mesenchymal stem cells for bone tissue engineering purposes.

Materials & Methods

Polyurethane porous scaffolds, with 65-95% porosity and 70-120μm pore diameter, were fabricated by thermal phase separation and cut into 5X5X5mm cubes. RGD ($4.5X10^{-4}$ M) was then covalently immobilized by cross-linking to an aminated scaffold's surface. Mesenchymal stem cells isolated from umbilical cord blood (UCB MSCs) were cultured on RGD-functionalized scaffolds (PU-RGD) and compared to collagen coated PU (PU-Coll) and plain polyurethane (PU). Cells were expanded for 7 days in maintenance medium and cell numbers were assessed by DNA quantification (PicoGreen assay). Distribution of cells and cell spreading throughout the scaffolds was evaluated with confocal microscopy and scanning electron microscopy. Mineral production after 21 days of osteogenic induction of UCB MSCs was assessed by means of Alizarin Red S (ARS) quantification.

Results

PU-RGD scaffolds supported MSCs attachment and spreading, while achieving a 25% higher expansion than uncoated after 7 days of culture in maintenance medium ($P<0.05$). MSCs on PU and PU-Coll scaffolds maintained a spherical morphology (Fig.1A,B), whereas cells managed to adhere and spread inside the pores of PU-RGD (Fig.1C). In contrast to PU and PU-Coll scaffolds (Fig.1D,E), MSCs were homogenously distributed from the surface to the core of PU-RGD scaffolds (Fig.1F). Finally, at day 21 calcium deposition was also significantly higher in RGD-coated scaffolds ($P<0.05$).

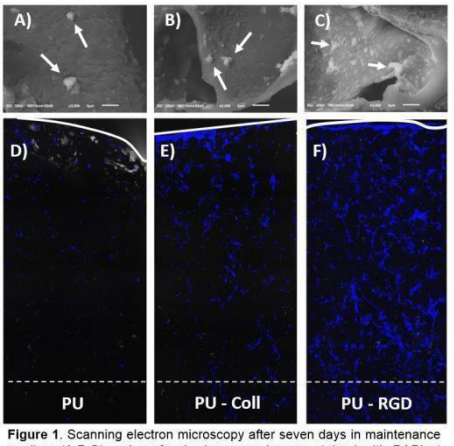

Figure 1. Scanning electron microscopy after seven days in maintenance medium (A,B,C) and confocal microscopy images stained with DAPI at day 21 of differentiation (D,E,F) comparing the ability of PU (A,D), PU-Coll (B,E) and PU-RGD (C,F) scaffolds to support cell adhesion and spreading

Conclusion

RGD-functionalised PU scaffolds supported the adhesion, expansion and culture mineralization of umbilical cord blood MSCs. Future research is going to further investigate the effect of RGD on osteogenic differentiation of UCBMSCs in terms of osteogenic gene and protein expression.

P197 Designating a 3D scaffold by platelet rich plasma/heparin sulfate/hydroxyapatite/zirconia for bone tissue engineering applications

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Hydroxyapatite (HA) was successfully coated on ZrO_2 to construct a porous scaffold with higher mechanical strength but there are still big challenges regarding to delivery of osteogenic growth factors, local reserve and sustain release of them. In this study, we fabricated a porous interconnected HA/ZrO_2 scaffold by slurry method with 71.6 + 0.5 % porosity and 310 + 150 µm pore size. Platelet rich plasma (PRP) used as an autologous source of growth factors and heparin sulfate (HS) sequestrated the PRP growth factors. After activating PRP with 2.5% $CaCl_2$, 5 µg/mL, HS was added and mixed well, followed by impregnating into the porous scaffold. The PRP/HS impregnating scaffolds showed a significant higher mechanical strength. Bradford assay showed HS led to a significant reduction in protein release at any point of time. After culturing MG63 cell line, cell adhesion test showed that 100 present of cells attached to the PRP containing compared with HA/ZrO_2 scaffolds. The cells also showed a significant higher alkaline phosphatase activity and Ca mineralization in scaffolds treated by PRP/HS. MTT viability test also demonstrated a significant higher proliferation rate on scaffold containing PRP/HS compared to those without PRP/ HS. The scaffolds with PRP/HS seem to provide a superior microenvironment for osteoblast activities and it can be suggested as a good vehicle for growth factor and cell delivery in bone tissue engineering applications.

P198 Osteogenic programming of human mesenchymal stem cells with highly efficient intracellular delivery of RUNX2

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Mesenchymal stem cells (MSCs) have tremendous scope in regenerative therapies due to their tri-lineage differentiation and immunomodulation potential. Currently there are two main challenges in directed differentiation of MSCs for therapeutic applications. Firstly, MSCs are difficult to transfect with existing approaches, either clinically unattractive (viral transfection systems) or have low efficacy (lipid-mediated transfection systems). Secondly, chemical strategies to direct osteogenesis *in vivo* lack specificity for targeted delivery with desired effects often having off-target side-effects. We overcame these challenges by delivering transcription factors (TFs) using a GAG-binding enhanced transduction (GET) delivery system (P21, 8R). We chose the osteogenic master regulator RUNX2 as a programming factor due to its stage-specific role in osteochondral differentiation pathways. We engineered GET-fusion proteins and compared sequential osteogenic changes in MSCs which had been induced by GET fusion proteins or conventional stimulation methods (Dexamethasone and BMP2). By assessing loss of stem cell surface markers, upregulation of osteogenic genes and matrix mineralization, herein we demonstrate that GET-RUNX2 successfully transduces MSCs and triggers osteogenesis directly by enhancing expression of target genes. Most importantly, GET-RUNX2 can prevent the effect of competing cues and retain osteogenesis in chondrogenesis-promoting conditions. The high transduction efficiency of GET-systems holds great promise for stem cell therapies by allowing a higher degree of control over directing stem cell differentiation *in vivo*, bypassing problems observed with high-concentration growth-factor therapies.

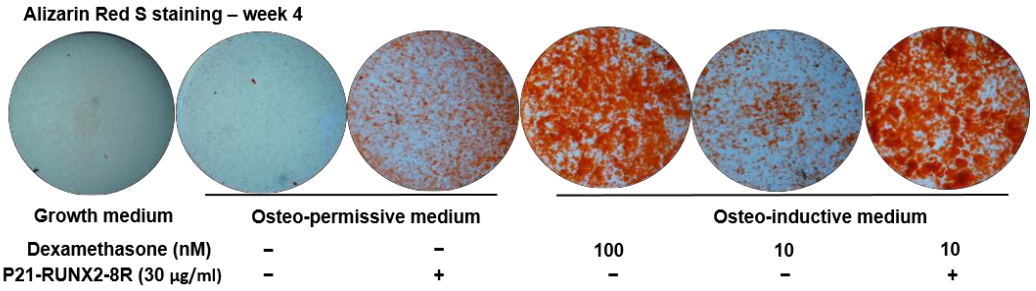

Figure 1: Matrix mineralization in hMSCs, 4 weeks after P21-RUNX2-8R (30µg/ml – twice during the first week) delivery, visualised using Alizarin Red S. The GET peptides significantly increased the delivery of RUNX2. The delivered RUNX2 retained their transcriptional activity and significantly increased the expression of osteogenesis related genes throughout differentiation resulting in enhanced matrix mineralization.

P199 Longitudinal monitoring of VEGFR2 and NFκB activity during the fracture healing after implantation of novel ß tricalcium phosphate scaffoldsin critical size bone defects in transgenic mice

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Large bone defects are still a challenge to the orthopaedic surgeon. The quality of the bone graft and soft tissue envelope are crucia. The presence of adequate blood supply is required to allow a high standard of care. VEGF and VEGFRs are important for generating a microenvironment that facilitates bone growth.

Aim of the present study is the investigation of the fracture healing process in VEGFR2-luc and NFκB- luc mice after implantation of novel ß-tricalcium phosphate (ß-TCP) scaffolds (with and without strontium (Sr)) in critical size defects of femora.

A critical size fracture was performed and stabilized using external fixation (AO). The fracture was bridged with a synthetic scaffold manufactured by slip casting in 3D printed wax molds, with a defined porosity to promote the regeneration. *In vivo* longitudinal measurements on VEGFR2-luc and NFκB-luc mice allow real-time monitoring of ongoing angiogenesis and inflammation process in the fracture area. After 2 months animals were eutanized and histological examination was performed on the fracture sites.

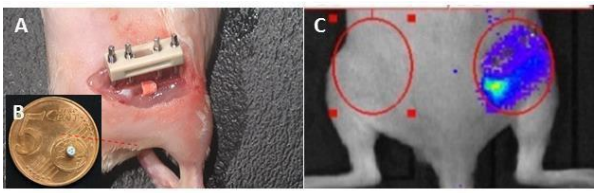

*Fig.1 (A) Implantation of n*ovel ß-tricalcium phosphate *scaffold (B) in critical size fracture. (B) Longitudinal monitoring of ongoing angiogenesis and inflammation process using detection of bioluminescence signal in vivo.*

We observed the first peaks of luciferase activity in the early (10th day) angiogenesis periods in all groups. While the level of VEGFR2 activity increases in the Sr doped ß-TCP (ß-TCP + Sr) group at the 15th day, the luciferase activity starts to decrease in this group and show significantly less activity compared to other groups in second half.

Additionally, Sr reduced inflammation, by means of NFκB activity, in the early phase of healing (15th days), but it was increased again in the late healing stage.

In both ß-TCP and ß-TCP + Sr groups the connection of newly formed tissue within the scaffold area to the fracture ends was clearly visible.

The histological analysis reveales that in case of a ß-TCP scaffold, much more osseous tissue has been formed when compared to the ß-TCP+ Sr scaffold.

This study for the first time gives an overview of VEGFR2 expression profiles and NfκB activity during fracture healing.

The addition of strontium in scaffolds influences inflammation in various stages of the

healing and leads to an increase of tissue formation.

This effect might influence the healing process and may prove to be advantageous for osteoporosis fracture healing.

P201 3D longitudinal characterization of swelling in Alginate/Hydroxyapatite scaffolds

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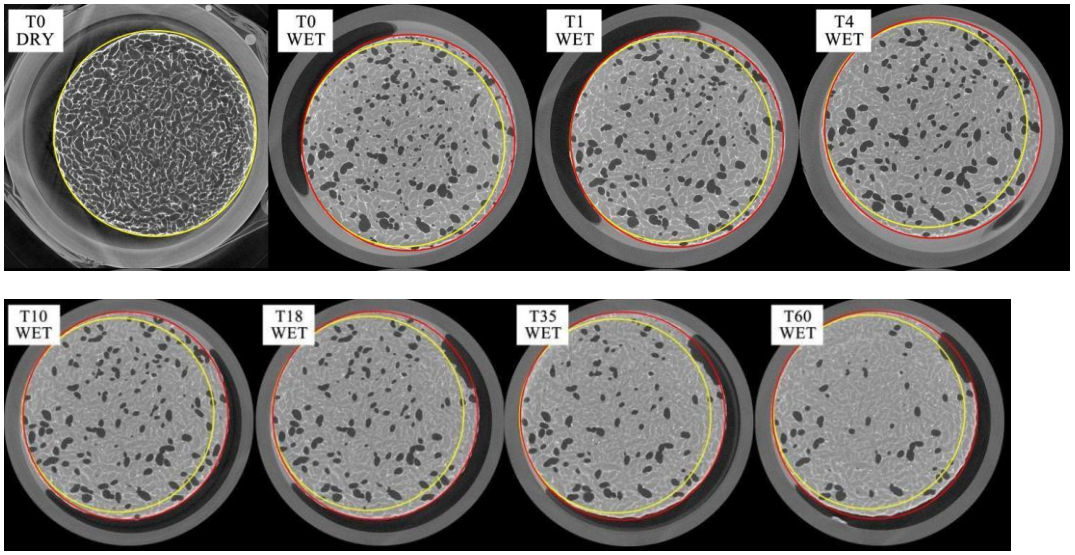
It is of paramount importance for a scaffold to preserve its shape and mechanical stability for a prolonged time. While an initial swelling is desirable because the resulting increase in pore- and throat-size facilitates cell attachment and growth, an uncontrolled swelling would lead to a reduced mechanical integrity and to a compressive stress toward the surrounding tissue. Thanks to non-destructive and three-dimensional (3D) computed microtomography (μ-CT), it is possible to characterize and monitor the wettability and the swelling processes through time. With additional image analysis, it is also possible to quantitatively assess the alterations induced by the liquid in the whole 3D domain. This allows for a better understanding of the structural and functional properties of the considered tissue engineering scaffolds. This approach has been here applied to nanocomposite Alginate/Hydroxyapatite scaffolds for bone tissue engineering aged in simulated body fluid (SBF). The same sample has been scanned at several time points (up to 60 days) with a conventional X-ray micro-focus benchtop system. The resulting voxel size is 8.33
µm. The reconstructed volumes have been then co-registered in 3D with respect to the baseline (first volume of the time series) in order to measure the swelling through time. A total volume dilatation of about 10% was recorded and the maximum swelling was observed after 10 days of embedding. After 18 days an erosion process started to corrode the walls leading to a slight contraction of the construct.



P202 Generation and finite element simulation of periodic bone scaffolds

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BACKGROUND: The design of scaffolds to support the restoration of bone defects must be optimised to support cell proliferation (factors such as porosity and pore size) and to meet the mechanical stresses the bone is physiologically subject to [1]. One starting point for the design of biomimetic extracellular support structures are triply-periodic-minimal surfaces (TPMS), because their periodicity makes them easier to generate, manipulate, additively manufacture, and model with the finite element method (FEM) [2]. METHODS: The models for the scaffolds were generated with a C++ program using the open-source computational geometry library (CGAL) [3]. The input is an implicit function that describes an approximation of a triply periodic minimal with a thickness added to it to make it printable, for example, to generate the gyroid, the equation $-\alpha \leq \cos x \sin y + \cos y \sin z + \cos z \sin x \leq \alpha$ is used, where α determines the “thickness” of the surface. After generating the objects, the open source software TetGen was used to tetrahedralize the volumes for use in FEM compression simulation. The simulation is done with a simple first-order polynomial linear elasticity code written in C++. As future 3D-printing and mechanical testing of the objects with the bioresorbable polymer polycaprolactone is planned, the material model is an isotropic linearly elastic solid with Young’s modulus of 80 MPa [4] and assumed Poisson’s ratio of 0.3. The applied force used was 0.1 MPa. The output of the compression simulation is the displacement of the objects in the z direction, from which the strain and effective Young’s Modulus can be calculated. Several different TPMS-based volumes were run through the simulation, as well as a solid cube of material to test the validity of the simulation. RESULTS: The simulation of the solid cube returned the same Young's modulus as assumed for the bulk material. Of the shapes tested, the gyroid performed the best (fig. 1), with an effective Young's modulus of 39.9 MPa at 80% porosity. CONCLUSIONS: Preliminary results of the FEM simulations suggest a reasonable result. Of the objects tested, the gyroid seems to perform quite well for a structure with 80% porosity. Future simulations are planned for torsion and shear, as well as non-linear analysis.

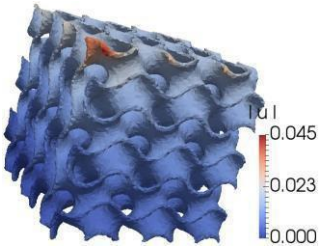

Fig. 1 Gyroid simulation result. Red indicates areas of maximum displacement

P203 The role of molecular mechanisms of the cell attachment and actin cytoskeleton in human adipose stem cell differentiation

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The cell adhesion mediated through integrins and focal adhesions (FA), is known to regulate differentiation of MSCs. Focal adhesion kinase (FAK) is a central mediator of integrin-activated signalling and interconnected with various cellular signalling proteins including extracellular signal-regulated kinase (ERK) and cytoskeletal regulator, Rho- (Rho-associated protein kinase) ROCK. However, the role and interplay of these molecular mechanisms in regulation of ***human adipose stem cell*** (hASC) differentiation process is unclear. ***The aim of the present study was to assess the significance of FAK, ERK and ROCK signalling on the morphology, proliferation and differentiation of hASCs*****.**

Small molecule ***inhibitors targeted to these signalling proteins were used to diminish the function of the kinase activity of these proteins in hASCs. The inhibitory effect of these molecules were analysed by Western Blotting (WB) and morphological impact by fluorescent phalloidin staining of actin cytoskeleton.*** The impact of kinase inhibition to osteogenic differentiation was evaluated by alkaline phosphatase (ALP) activity and Alizarin Red matrix mineralization assays. Adipogenic differentiation and cellular lipid formation was analysed with Oil red O staining by quantitative Cell Profiler analysis software, and the expression of adipogenic markers LEPTIN and aP2 by RT-PCR.

Our results indicated that the inhibition of FAK, ERK and ROCK function suppresses both cell proliferation and osteogenic differentiation, dose-dependently. Adipogenic differentiation was enhanced as a result of ROCK and FAK inhibition and this inhibition also modulated cell morphology leading to reduced tension of the actin cytoskeleton. Inhibition of ERK signalling decreased both courses of studied differentiation routes indicating also to its relevance in hASC fate decision. These results suggest that the cell adhesion, morphology and cytoskeletal arrangements modulated by FAK, ERK and ROCK signalling are relevant regulators of the hASC differentiation. The functionality of all these studied proteins is essential for the full differentiation potential of hASCs and our results suggests that FAK and ROCK functions in a switch-like manner, as definer of the fate decision of hASCs.

P204 Screening of layered nanosilicates for the osteogenic differentiation of dental follicle stem cells (hDFSCs) for the development of bone regenerative functional biomaterials

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Engineered bone tissue using synergistic combination of biomaterials, cells, and cues has been considered as most promising alternative to the conventional use of bone grafts, due to their limitless supply and no disease transmission. However, development of suitable functional biomaterials with inherent capacity to boost the proliferation and differentiation of stem cells to promote the bone tissue formation is challenging and yet to be developed. Nanoparticles formulation with essential trace elements (e.g., Si, Li, Na, F) is known to play important role in stem cell proliferation and differentiation. Nanosilicate is one of the promising candidates for the bone regeneration applications due to the presence of these trace elements and negligible toxicity. The objective of this studies is the systematic evaluation of cytotoxicity and comparative evaluation of the three commercially available layered nanosilicates compositions (laponite XL-21, XLG, RXL) on the osteogenic differentiation capabilities of human dental follicle stem cells (hDFSCs) under different culture condition (normal;NM, oteoconduction: OC and osteoinduction:OI). To investigate the effect cytotoxicity of nanosilicates to the hDFSCs, different concentration of silicate (0-5 mg/mL) was used. Different assays such as MTS assay, reactive oxygen species (ROS), and internalization of nanosilicates into hDFSCs were performed to understand the mechanism of toxicity. For the evaluation of differentiation potential of different nanosilicates, hDFSCs were incubated with different nontoxic concentrations (0ug-100 ug/ml) of nanosilicates for 72 hrs followed by culture using of different conditioned medium without nanoparticles. Alamar blue assay and live dead assay were performed for cell proliferation and viability determination. *In-vitro* osteogenic differentiation potential of nanosilicates was evaluated by monitoring the marker proteins expression (alkaline phosphatase; osteopontin) and quantifying the mineral deposition (alizarin red staining). MTS assay showed that all the three groups of nanosilicates were biocompatible, with very minimal cytotoxicity. Likewise silicate concentration (up to 1 mg) did not show significant ROS generation. Among the three nanosilicates particles RXL showed low ALP activity as compared to the other two particles system. Conversely, nanosilicates (XLG, XL-21) in normal culture medium showed a consistent increase in ALP activity and had a maximum peak on day 21. Whereas, in presence of OC and OI media nanosilicates showed a peak by 14^{th} day itself, which indicated that of all nanosilicates XL-21, XLG supported in the enhancement of ALP activity even in the absence of osteogenic factors. Alizarin red staining showed that nanosilicates concentration aided in the elevation of mineralized matrix in NM, OC, and OI media on 14th and 21th day respectively for XLG and XL21. But, RXL showed no significant staining. The above study highlights that XLG and XL-21 nanosilicates trigger in the osteogenic differentiation of hDFSCs. These layered nanosilicates are nontoxic, degradable and cost effective. It shows excellent osteoinducing properties due to their internalization/association with the stem cells and can be a promising candidate for development of functional biomaterials (scaffold, filler,injectable hydrogel) for effective in vivo bone tissue regeneration.

P205 Mechanisms of bone remodelling induced by tissue engineering products composed of mesenchymal stromal cells and natural bone matrices

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Background

Currently, the potential use of mesenchymal stromal cells (MSCs) in bone regeneration is being extensively investigated. To that end, distinct tissue engineering (TE) approaches aim to recovering bone healing deficiencies by implantation of functional combinations of cells with regenerative ability, natural or synthetic scaffolds and mechanical stabilization systems. In this context, our laboratory has developed therapeutic products based in bone particles from tissue bank dynamically repopulated by MSCs, from conception up to phase I/IIa clinical trials. Following some preliminary studies, several key questions, including cell source suitability and cell fate tracking after treatments, arise and were addressed in the presented work.

Materials and Methods

The suitability and bioequivalence of Umbilical Cord (UC)-MSCs and Bone Marrow (BM)-MSCs in TE strategies was assessed *in vitro* by means of flow cytometry analysis, differentiation assays, gene expression profiling and proteomic analyses of their secreted extracellular vesicles (EVs) isolated by size exclusion chromatography (SEC). Fate tracking of MSCs was evaluated *in vivo* in a large animal model of avascular necrosis of the femoral head by developing a method for isolation, expansion, characterization and genetic labelling of ovine MSC.

Results and Conclusions

Despite equivalence of basic properties (phenotype and multipotency) of both UC-MSCs and BM-MSCs, *in vitro* differentiation was significantly delayed regarding osteogenic commitment in the case of UC-MSCs with respect to BM-MSC. SEC-EVs derived from both sources of MSCs contained specific EV (CD9 and CD63) and MSC (CD90 and CD73) markers. Moreover, subsequent comparative analysis by proteomics indicated a high overlap between the two types of EVs, highlighting the common presence of a variety of regulatory proteins related to immune regulation, cell differentiation and migration, blood coagulation and stress response. Functionally, combination of MSCs and natural bone matrices supported osteogenesis both *in vitro* and *in vivo*. In particular, in the preclinical setting, the persistence of grafted cells in the host tissue and their lineage commitment into osteoblasts and osteocytes were demonstrated by tracking enhanced green fluorescent protein-labelled cells three months after implantation. In sum, our results strongly support the clinical use of MSCs for bone remodelling regardless of their source, being BM-MSCs highly committed to osteogenic differentiation whereas UC-MSCs take longertimes.

P206 Osteogenic differentiation of human adipose stem cells in 3D hydrogels

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Growing number of musculoskeletal defects increases the demand for functional engineered bone grafts. Human adipose stem cells (hASCs) are an abundant and readily available stem cell source that have been used for the development of engineered bone constructs. Hydrogels simulate better the natural 3D microenvironment compared to traditional biomaterials. This study proposes an *in vitro* model to differentiate hASCs towards bone-like cells embedded in 3D hydrogels.

The gellan gum (GG) and collagen type I (COL1) (rat tail) hydrogels were tested for their ability to support hASC viability and adhesion in 3D culture. Cell number and proliferation based on DNA content was quantified. Early osteogenic differentiation of hASCs was determined based on quantitative alkaline phosphatase activity (qALP) and the gene expression of bone marker genes. Late osteogenic differentiation of hASCs was analyzed based on the accumulation of hydroxyapatite mineralization residues and by immunocytochemical staining of late osteogenic marker osteocalcin. Raman spectroscopy was used to analyze different types of mineral residues in different hydrogels and Optical projection tomography (OPT) was used to image the hASCs in 3D hydrogel culture.

The results showed that the hydrogel encapsulated hASCs remained well viable, however, GG hydrogel promoted a tight and round cell morphology, whereas in COL1 hydrogel the hASCs were more spread out. The qALP results were low in all hydrogels whereas the gene expression of osteogenic markers was significantly elevated in COL1 hydrogel. Hydrogels supported hASC mineralization, seen in the formation of hydroxyapatite nodules, however, strong staining of osteocalcin was visible only in COL1 hydrogel.

These results demonstrated the potential of novel 3D hydrogel culture methods for the osteogenic differentiation of hASCs for the development of efficient and feasible bone tissue engineering applications.

P207 Influence of bone and tooth matrix on the ability of dental pulp and bone barrow mesenchymal stromal cells to orchestrate mineralised tissue repair

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The extracellular tissues of bone and tooth both contain common and autonomous protein compositions, specific to the respective tissue. Many of these proteins present have a major influence on the behaviour of MSCs by providing a scaffold structure and bioactive cell signals for promoting proliferation and differentiation, guiding mineralised tissue repair. **Aim:** To investigate the influence of bone and tooth matrix on the cellular viability and behaviour of clonal MSC populations derived from dental pulp (DPSCs) and bone marrow (BMSCs). **Methods:** Clonal DPSC and BMSC populations were obtained from the pulpal tissue and bone marrow of Wistar rats and characterised as previously described [1]. Teeth and bones were also obtained from rats, cleared of soft tissue, powdered and treated with EDTA, to produce either demineralised tooth matrix (DTM) or demineralised bone matrix (DBM). DPSCs and BMSCs were cultured in the presence of 0-2µg/ml of DTM or DBM and the influence of the matrix on cell viability was assessed by cell morphology and MTT assays. Studies also assessed the effects of 0-0.5µg/ml of DTM and DBM (concentrations determined above not to affect cell viability) on long term population doubling (PD rates) and osteogenic differentiation by measuring mRNA levels for bone sialoprotein and osteocalcin and deposition of mineralised matrix stained with Alizarin red. In addition, mineralised tissue surfaces of tooth and bone, cleared of soft adherent tissue, were prepared for the culture of DPSC and BMSC clones on the inner surface that had previously adjoined to the pulpal and marrow tissues respectively *in situ*. Surfaces were examined by SEM to assess cell attachment and viability. Immuno-localisation for PCNA provided a qualitative assessment of cell proliferation. **Results:** DBM at 0.5µg/ml had minimal effects in reducing PDs rates of BMSC and DPSC clonal populations. DTM demonstrated a concentration dependent decrease on PDs of clonal BMSCs and concentration independent decrease on PDs of DPSCs. Correlating with these results, DBM did not promote osteogenesis of BMSC, but did promote deposition of mineralised matrix by DPSCs. DTM demonstrated a greater osteogenic potential compared to DBM, promoting osteogenesis in both DPSC and BMSCs. Visualisation of the clonal MSC populations on the mineralised surfaces indicated that bone surfaces supported the attachment and the apparent proliferation of both BMSCs and DPSCs, leading to the synthesis of a collagen matrix. Tooth-dentine surfaces supported the attachment and apparent proliferation of DPSCs only. **Conclusion:** DTM demonstrates a greater osteogenic potential in stimulating mineralised tissue repair by both DPSCs and BMSCs. Further, DPSCs and BMSCs respond differently to the matrix micro-environments and the mineralised bone and tooth dentine surfaces. Differences may partially be due to inherent diversities in the differentiation capacity of DPSCs and BMSC [1], and also their respective cell signalling responses to the extracellular signalling environment and surface geometry. These results are significant as demineralised bone matrix has been proposed as a bioactive graft material for stimulating bone repair in clinical practice with mixed success.

P208 Fabrication of hybrid composite scaffolds using 3D bio plotting system for tissue engineering

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The scaffold plays an important role in tissue engineering, since they act as substrates for cell adhesion, proliferation and regeneration of new tissue. Notably, choosing appropriate materials to design a well-functioned composite scaffold that is biocompatible with human tissue is highly critical. Considering that magnesium is one of the most excellent biomaterials for orthopaedic applications. In this study, new hybrid composite scaffolds were composed of polycaprolactone (PCL) and $MgCl_2$, and the scaffolds were fabricated using 3D bio plotting system. The hybrid composite scaffold was characterized by compressive testing, cell culture and a rabbits implant test. Subsequently, a bone regeneration test was performed using 18 male rabbits (New Zealand white rabbits) older than 4 months (>3.5kg). The bone defect was made on radius of both front feet (defect length=10mm) and fixed with K-wire. The defect on the left radius was used as controls, the right radius was filled with Pure PCL and $MgCl_2$/PCL composite scaffolds (each n=6).The results shows that the composite scaffold's mechanical strength, cell proliferation and differentiation were enhanced those of the pure PCL scaffold due to the magnesium-ion compound. Additionally, the in-vivo test shows that the bone regeneration rates of the $MgCl_2$/PCL composite scaffolds are faster than those of negative and positive controls.

This research was supported by the KRIBB Research Initiative Program.

P209 Real-time dynamic fluorescent imaging of cell response to fluid shear stress

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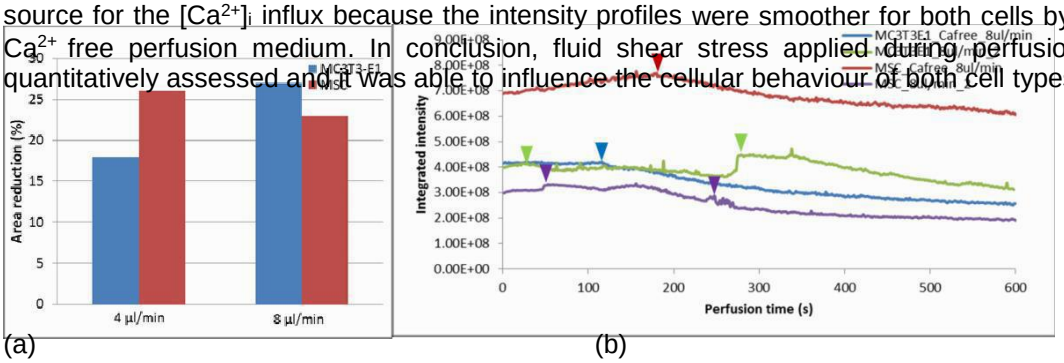
Introduction: Fluid shear stress plays a crucial role in regulating cellular behaviour in bone tissue engineering. Mechanotransduction studies how cells convert mechanical stimuli (e.g. fluid shear stress) into biochemical activity. Parallel-plate flow chambers (PFC) can introduce fluid shear stress to cells in culture and are useful tools for in vitro mechano-transduction studies. Although bone mechanotransduction has been widely studied, fluid shear stress experienced by the cell is evaluated through mathematical approximation and little is known about the local stress around the cell at the subcellular length scale. Multi-focal microscope (MuM) is a novel technique combining particle tracking velocimetry with advanced microscope set-up. It is able to map fluid flow around a single cell. Here we investigated the relationship between sub-cellular scale fluid shear stress and cellular behaviour of human mesenchymal stem cells (hMSC) and mouse osteoblasts (MC3T3-E1) in real-time up to 1 h upon application of fluid shear stress. Materials and Methods: MuM was employed in combination with PFC in our experiment to monitor the fluid field at sub-cellular level during perfusion. Live cell fluorescence label of cell membrane and intra-cellular calcium ($[Ca^{2+}]_i$) indicator were employed to examine cell morphology change and $[Ca^{2+}]_i$ flux. Cell morphology can indicate phenotype change whilst $[Ca^{2+}]_i$ flux can mediate numerous cell signalling cascades. Results and Discussion: The average fluid shear stress on the cell was calculated and the result was compared to mathematical approximation. The fluid shear stress distribution around the cell was visualised. In terms of cell morphology change, area of both cell types decreased by ~ 23% after 1 hour perfusion, and it was not flow rate dependent (fig 1a). Two $[Ca^{2+}]_i$ influx were visible for both cell types and the secondary flux in MC3T3-E1 was more significant than that in hMSC (fig 1b). Extracellular calcium was determined as the primary calcium source for the $[Ca^{2+}]_i$ influx because the intensity profiles were smoother for both cells by using Ca^{2+} free perfusion medium. In conclusion, fluid shear stress applied during perfusion was quantitatively assessed and it was able to influence the cellular behaviour of both cell types.



(a) (b)

Figure 1 a. Cellular area change of cells under 1 h perfusion at different perfusion rate.

b. $[Ca^{2+}]_i$ flux change with time in the normal or calcium free perfusion medium.

P210 The topographically defined biodegradable nanopatterned patch for acceleration of bone regeneration

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Naturally, bone-healing process could take several weeks, months or even years depending on the injury size. In terms of bone healing speed, many researchers have been used to deliver various growth factors with implantable biomaterials to shorten the date for bone regeneration. However, there may have occurrence with side effects such as nerve pain, infection and ectopic bone formation. As an alternative method, we focused on the biophysical guidance, which was provided similar topographical cues to cellular environment, to recruit host cells for bone defect healing. In this study, we hypothesized that biomimetic nanotopographical features have enhanced cell recruitment, migration and differentiation from intact cells without additional stimulation. We designed a biodegradable and transplantable poly (lactic-co-glycolic acid) nanopatterned patch using simple solvent casting and capillary force lithography. We confirmed that biodegradable nanopatterned patch (BNP) accelerated migration of osteoblast according to orientation of patterned direction. These highly aligned osteoblasts might contribute to in vitro osteogenic differentiation such as alkaline phosphate activity, mineralization and calcium diposion compared to the biodegradable flat patch (BFP). To demonstrate bone defect healing by guidance of BNP in vivo, we implanted whole and bridge BNP on critical size defect of mouse calvarial (ø 4 mm) and analyzed to use microcomputed tomography and histology. Only BNP treated group had faster new bone formation and compact bone regeneration at defect area compared to BFP at 4 and 8 weeks. Especially, the bridge BNP guided to be regenerated new bone formation along with the parallel direction of nanopatterned substrates. The BNP with biophysical guidance should be suitable utilized for tissue regeneration by accelerated intact cell migration.

P212 Development of a label-free cell separator for autologous stem cell enrichment for skeletal tissue repair

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INTRODUCTION: Autologous mesenchymal stem cell (MSC) therapies in regenerative medicine offer potential solutions to complex clinical challenges, such as fracture non-union and repair of critical bone defects. Current techniques used to isolate MSCs rely on centrifugation or antibody labelling. These have limitations including high costs, lack of specificity and unknown cellular effects in solid tissue. This project aims to develop a device which is able to separate MSCs with osteogenic potential from a mixed population derived from bone marrow or surgical discard in intra-operative time. An end product of an enriched population of MSCs, produced label-free and with minimal cell manipulation will be delivered for autologous cell therapy in combination with a suitable scaffold that will promote bone healing.

METHODS: A prototype label-free cell separator has been developed to deliver an enriched population of human dental pulp stromal cells (HDPSCs). Cells are captured utilising Leeds' non-antibody protein-binding protein technology [2]. Specific binders with affinity for TNAP (Tissue Specific Alkaline Phosphatase, a recognized cell surface marker for pro-mineralising HDPSCs [1]) have been identified which are extremely stable with high thermostability and melting temperatures when tethered to a surface. These binders were tethered to a substrate coated with a thin layer of gold to which a self-assembling monolayer is attached, within a prototype microfluidic device. Cells are injected into the microfluidic channel and incubated for 10 minutes allowing cells to be captured. Any non- bound cells are then eluted from the device by introducing a flow of cell culture medium. The binding protein is switchable, reducing the affinity for TNAP by a pH-induced change in conformation of the protein scaffold. This allows the release of the bound HDPSCs from the device's surface.

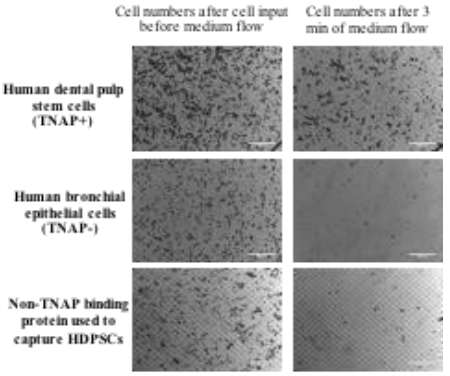

Fig 1. Specific capture of TNAP+HDPSCs by TNAP binding proteins after injection into the label-free cell separator, compared with capture of HBE cells and HDPSCs capture with non-TNAP binding proteins

RESULTS: Specific cell capture was seen when $TNAP^+$ HDPSCs were injected and incubated on the bio-functionalised surface for 10 minutes (Figure 1) and compared with $TNAP^-$ human bronchial epithelial cells. Reduced binding was also seen when HDPSCs were injected across a functionalised surface to which a non-TNAP protein binder was attached.

DISCUSSION & CONCLUSIONS: Specific $TNAP^+$ HDPSCs capture has been demonstrated when using TNAP binding proteins. Future work will focus on the increase of cell numbers from cell release before further characterisation of the captured cell population.

P213 A novel Mg2+ eluting degradable polymer shows increase in bone cell proliferation and adhesion

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Tissue substitutes require a three-dimensional, porous, biocompatible, and preferably biodegradable scaffold. For orthopaedic tissue engineering, Mg^{2+} enhances formation of new bone tissue and adherent of osteoblastic cells (Rude et al, 2004). In-situ polymerization was used to produce various novel 3D Mg^{2+} - containing polylactone acid ionomer network structures using Mg/Al layered-double-hydroxide (CO_3^{2-}) as the initiator (McCarthy et al, 2012). Importantly, the network materials can be easily manipulated and integrated with other base materials as particle-reinforced composite, including polyesters and hydrogels. In this study, we show the value of this technique for stimulation of bone tissue regeneration. The initial hybrid product was prepared by ring opening, melt polymerisation of 95% L,D-lactide with ε-caprolactone or δ-valerolactone in different mass ratios with 5% LDH by mass. To vary the metal ion content, the LDH initiator can be ion exchanged prior to use as an initiator in this reaction (Newman et al, 1998). SEM (Fig.1A) revealed a porous network structure indicating that polymerizing lactone monomer with carbonate-intercalated hydrotalcite is a credible route to the chemical synthesis of a bio-scaffold. Pico Green data (Fig.1B) shows a significant cell number increase at 14 days of in-direct culture of Saos-2 cells with the novel polymer granule culture medium extract. In particular, a doubling of cell number was seen with L:C 1:1 which is synthesized by initial lactide and caprolactone monomer at mass ratio 1:1. When these granules were combined in a composite with PCL, a significant increase in cell attachment to the PCL based scaffold was seen with the increasing mass ratio when network-PCL composite film samples were fabricated as seen inFig.1C.

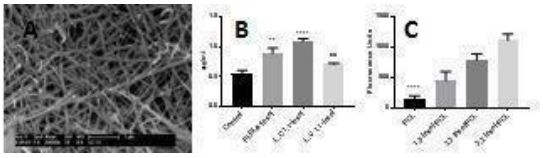

Fig.1 A. SEM of porous structure in polymer granule, B. Saos-2 proliferation measured by PicoGreen Assay, C. Cell attachment measured by Alamar Blue assay after 24 hours.

Different morphologies of our new Mg^{2+} eluting polymer can be tailored to the desired application. Bone cells are known to respond to surface roughness and surface topography and this can play a role in osteogenic activity. In addition to this topography, controlled Mg^{2+} elution has significantly increased cell proliferation. Cell adhesion is also significantly increased when these novel scaffolds are used in a PCL composite. We are currently completing further analysis including differentiation markers such as osteogenic gene and protein expression. The key advantage of this polymerization synthesis is the flexibility of using various metallic ions (e.g. Ca^{2+} and Sr^{2+}) potentially and polymeric moieties providing different mechanical and degradation properties in tissue engineering that are tailored for different applications.

P215 Evaluation of silymarin/duck's feet-derived collagen/hydroxyapatite scaffolds for bone tissu regeneration

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INTRODUCTION: Silymarin is a constitutive natural compound composed of an isomeric mixture of flavonolignans of silychristin, iso-silychristin, silydianin, silibinin and isosilibin. Silymarin is abundant in milk thisle (silybum marianum) and has been clinically implicated in various liver diseases such as alcohol or drug intoxication and viral hepatitis, whose pathogenesis involves an inflammatory response. Collagen is mainly used for cartilage, bone and skin regeneration owing to its well-known biocompatibility, biodegradability. And they are known for low antigenicity, hemostatic effect, and cell adhesion. The aim of this study was to fabricate three dimensional Smn/DC/HAp scaffolds and access its availability for bone grafts through in vitro and in vivo test.

METHODS: Smn/DC/hydroxyapatite (HAp) scaffolds 25μM, 50μM, 100μM were produced by freeze dried methods. Smn/DC/HAp scaffolds were studied mechanical properties and through SEM (Fig.1), FTIR, compressive strength, porosity, MTT, ALP, RT-PCR, histological staining.

RESULTS: Chemical bonding and with increasing Smn content the scaffolds. Also, compressive strength was increased. In vitro studies showed that cell proliferation and osteogenic differentiation were increased with rising Smn content. Especially, cell proliferation and osteogenic differentiation are highest in 100μM Smn/DC/HAp scaffold (Fig. 2).

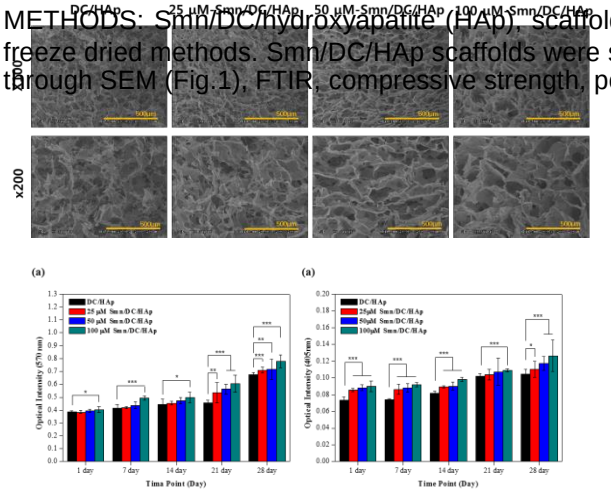

Fig. 1: SEM images of Smn/DC/HAp scaffolds

Fig. 2: (a) Proliferation of BMSCs on Smn/DC/HAp scaffolds after 1, 7, 14, 21, and 28 day studied by MTT assay. (b) Osteogenic differentiation of BMSCs on Smn/DC/HAp scaffolds after 1, 7, 14, 21, and 28 day studied by ALP assay.

DISCUSSION & CONCLUSIONS: The results showed that the 100μM Smn/DC/HAp scaffold efficiently increases the rBMSCs proliferation and osteogenesis.

P216 Biofunctionalisation of decellularised porcine mitral valve scaffolds

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INTRODUCTION

Decellularised heart valves represent a promising alternative to current heart valve prostheses. The implantation of tissue engineered heart valves in both aortic[1] and pulmonary[2] position has showed encouraging clinical midterm results. Implantation of *in-vitro*-endothelialised decellularised pulmonary scaffolds in the ovine model has shown that endothelialisation can improve scaffold biocompatibility[3], whereas the surface coating of biological scaffolds with proteins has been shown to accelerate the adhesion of recipient cells *in vivo*[4]. The aim of this work was to compare 4 different coating agents in 3 different concentrations in terms of coating efficiency, cell adhesion and proliferation as well as mechanical behavior.

MATERIALS & METHODS

Mitral valves (MVs) from 6-month old pigs were disinfected, treated with 0.5% (v/v) Triton X-100 and 0.5% (w/v) SDS for 24h each, followed by extensive washing cycles, nucleic acid digestion and sterilisation with peracetic acid. Decellularised MV leaflets were coated with fibronectin (FN), vitronectin (VN), Collagen IV or Chondroitin sulfate sodium (CS) in 3 different concentrations (5, 10 & 20 μg/ml). The efficiency of the coatings was examined immunohistochemically. Moreover, the amount of the HYP was quantified in scaffolds coated with Collagen IV whereas the amount of sGAG was quantified in the scaffolds treated with CS. The effect of the coatings on the adhesion and viability of ovine endothelial outgrowth cells (EOCs) was examined by scanning electron microscopy and live/dead staining, respectively. The effect of the coatings on the mechanical integrity of the scaffolds was assessed under uniaxial tensile loading to failure.

RESULTS

The coating procedure was effective for all 3 coating agents investigated, as observed under immunohistochemical staining (Fig.1), and HYP and sGAG quantification. Ovine EOCs appeared to attach and proliferate more on the decellularised leaflets coated with either FN or VN (Fig.2). None of treatments altered the mechanical behaviour of the scaffolds significantly.

DISCUSSION

The coating procedure was successful for all 3 agents tested, while the mechanical integrity of the tissue was maintained and seemed to improve compared to the decellularised-only control. Tissue coated with either fibronectin or vitronectin favored the attachment and proliferation of ovine OECs. Coating with either Collagen IV or CS did not show any beneficial effect on OEC adhesion. Future work will focus on the combination of the coating treatments and the biological and biomechanical assessment of the treated scaffolds.

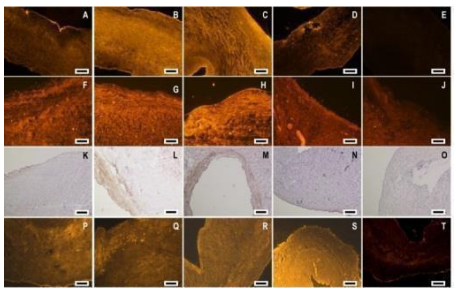

Figure 1: Immunohistochemical staining of the native (D,I,N,S), decellularised leaflet (E,J,O,T) and coated with FN (A-C/ 5,10,20 μg/ml respectively), VN (F-H/ 5,10,20 μg/ml respectively), Collagen IV (K-M/ 5,10,20 μg/ml respectively) & CS (P-R/ 5,10,20 μg/ml respectively). (Scale bar: 100 μm)

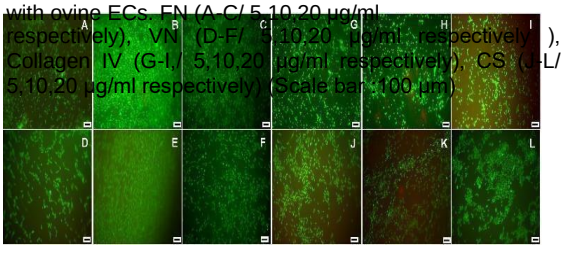

Figure 2: Live/dead staining of the coated MV leaflets, seeded with ovine ECs. FN (A-C/ 5,10,20 μg/ml respectively), VN (D-F/ 5,10,20 μg/ml respectively), Collagen IV (G-I/ 5,10,20 μg/ml respectively), CS (J-L/ 5,10,20 μg/ml respectively) (Scale bar: 100 μm)

P217 The regenerative potential of extracellular vesicles (EVs) from human cardiac-derived adherent proliferating (CardAP) cells

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There is a compelling need for new therapeutic strategies to treat cardiovascular diseases (CVDs) due to a steadily increasing morbidity rate and one of the highest mortality rates worldwide. A recent approach in cell therapy is the application of extracellular vesicles (EVs) as paracrine mediators derived from regenerative cells. One suitable candidate is cells from endomyocardial biopsies, the human cardiac-derived adherent proliferating cells (CardAP), which have been shown in previous studies to be anti-apoptotic, pro-angiogeneic, immune modulating and to significantly improve the heart functions of mice suffering from an acute Coxsackievirus B3-induced myocarditis.

The aim of this study is to unravel the possible contribution of CardAP-derived EVs for protective and regenerative effects. Therefore, we characterized the phenotype of EVs in comparison to their originating CardAP cells and analysed the *in vitro* capabilities of EVs to mediate beneficial effects.

In general, EVs were isolated by differential ultracentrifugation of the conditioned medium from CardAP cells (n = 6 donors), which were kept for 20 hours under serumfree conditions. Particles released into the medium showed a modal diameter of 95 nm as determined by Nanoparticle Tracking Analysis with no changes between different passage numbers. Similar surface proteins, e.g. tetraspanins and major histocompatibility complex I (MHC class I), could be observed on EVs as well as on CardAP cells, whereas other markers were exclusively expressed on cells, e.g. intercellular adhesion molecule 1 (ICAM-1). Co-cultures of peripheral blood mononuclear cells with EVs from CardAP cells for 5 or 7 days did not induce any T cell proliferation. However, immune modulating effects by CardAP EVs were detected, when co-cultures were additionally stimulated with phytohemagglutin. This lectin-induced T cell proliferation was diminished by the treatment with CardAP EVs as compared to the PBS treated control. In addition, tubal formation (total tube length, number of branching points) of human umbilical venous endothelial cells on matrigel was significantly increased by a 24h pre-treatment with CardAP EVs from different donors compared to the PBS treated control.

Our results indicate that CardAP EVs reveal a low immunogenicity, the potential to modulate immune reactions and pro-angiogenic effects, of which all would be very advantageous features for cardiac therapy. However, further functional *in vitro* and *in vivo* studies are necessary to evaluate if CardAP EVs comprise the regenerative potential to treat CVDs while being immunologically compatible.

P218 Biomimetic multilayer membrane for new generation blood propulsion systems

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Introduction: Commonly available blood propulsion systems present several issues such as blood coagulation, formation of thrombus and enhanced inflammatory response. In order to overcome these issues, a new approach is proposed here. A hyperelastic hybrid membrane (hhM) able to elicit an anti-thrombogenic action and to bear large elastic deformations is designed, aiming to mimic the architecture and functionality of blood vessels. For this purpose, polydimethylsiloxane (PDMS) is used as substrate for the integration of the hhM into novel blood propulsion device. By tailoring the surface chemistry of PDMS, nanofibers of poly vinylidene fluoride-co-hexafluoropropylene (PVDFhfp) are anchored to the PDMS to provide an environment allowing proliferation of cells in the system. In this work, we first present different approaches to achieve irreversible and stable bonding between the two layers (PDMS +PVDFhfp).

Methods: The first approach consists in directly electrospinning fibers on top of uncured PDMS; the second approach consists in spin coating an interlayer of acrylates (PDMS/ACR) at different velocities of 1000, 2000 and 3000 rpm on top of PDMS treated with 30W plasma for 60s at 0.4 mbar. PVDFhfp is subsequently deposited and the acrylates undergo UV curing after the electrospinning process. In this approach, plasma improves the hydrophilicity of PDMS and enhances bonding with the top layer while acrylates ensure stability of the bonding between the layers over time due to their stability, chemical inertia and resistance to biological corrosion. The third approach consists in applying a prolonged plasma treatment to achieve etching of the PDMS surface and subsequently deposit nanofibers inducing a velcro-like bridging between PDMS and fibers. The chemical modifications of the surface are thoroughly investigated by contact angle, X-ray photoelectron spectroscopy (XPS), energy-dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM). Then, mechanical tests are performed in order to evaluate how the different approaches influenced the mechanics of the hhM while pull-off tests are used to evaluate the strength of the bonding.

Results and discussion: Results showed the successful integration of a homogeneous interlayer of acrylates on PDMS. Elemental mapping by EDX revealed uniform carbon coverage on coated samples and the contact angle decreased from 115±5° to 57±6° on the PDMS and PDMS/ACR, respectively. Decreasing the spin coating velocity, XPS showed progressive decrease of the siloxane content and increase of carboxyl groups, further confirming the deposition of an acrylate layer. The third approach was investigated by SEM revealing increased etching of the PDMS surface with higher power plasma. Despite this, more extreme etching conditions can have a negative impact on the mechanical properties of PDMS leading to a brittlestructure.

Conclusion: The methods presented here give an overview on possible approaches to be considered for applications requiring a stable bonding between surfaces and are a promising solution in the development of an hhM for integration in new generation blood propulsion pumps.

P219 Novel cantilever-based approach for force measurement of 3D engineered cardiac microtissue within a microfluidic platform

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Introduction: Cardiovascular disease ranks as the leading cause of morbidity and mortality worldwide. However, despite the great effort in the field of tissue engineering to develop new therapeutic strategies targeting heart disease, recreating *in vitro* a phenotypically stable cardiac cell population resembling the host myocardium remains challenging [1]. Microfluidic platforms have shown unprecedented capabilities as high-throughput models to mimic *in vitro* the complexity of the native cardiac cell microenvironment, characterized by an orchestrated multiplicity of electromechanical stimuli and biochemical factors [2].

Methods: We developed a microfluidic bioreactor to enhance the maturation of 3D cell constructs into cardiac ventricular microtissues. The device was designed to provide cyclic uniaxial mechanical strain and medium perfusion to the 3D cardiac construct, allowing direct force measurement. Uniaxial cyclic strain mimics the systolic and diastolic phases that cardiomyocytes experience during heartbeat, while microfluidic perfusion enables predictable delivery of oxygen, nutrients and drugs. Our strategy for the measurement of force-frequency relationships exerted by cardiac microtissues was based on the use of specific PDMS pillars, designed to bend in response to the contraction force of the microtissues. Biological validation was performed on cardiomyocytes isolated from neonatal rat. Cells were embedded in fibrin gel, injected into the microfluidic platform and cultured for 7 days under medium perfusion and cyclic mechanical stimulation (10% uniaxial strain, 3 Hz frequency). Immunoflurescence microscopy was used to assess cardiac microtissue maturation by the identification of cell-cell and gap junctions. Deflection of vertical cantilevers was tracked to estimate the contractile force.

Results: Cardiomyocytes cultured up to seven days showed spontaneous synchronized beating, enhanced maturation and a dose-dependent inotropic response to isoproterenol. Preliminary experimental validation showed enhanced maturation of cardiac constructs under culture medium perfusion, as well as mechanical stimulation, as compared to static conditions. Additionally, force measurement showed results in agreement with computational modeling.

Conclusions: We developed a novel cantilever-based approach for contractility assessment of 3D cardiac microtissue, allowing a tight control of environmental parameters. Synchronized beating and positive force-frequency relationships were achieved, making the device an enabling platform to assess the correct recapitulation of myocardial physiology *in vitro*, and potentially an *in vitro* screening tool of chronotropic and inotropic drugs.

Aknowledgements: This work was supported by the European Commission within the Horizon 2020 Framework through the MSCA-ITN-ETN European Training Networks (project number 642458).

P220 Epicardial implantation of cardiac progenitor cell sheets is more promising method for myocardial regeneration than conventional cell injections

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The adult heart contains small populations of multipotent cardiac progenitor cells (CPC), which are participate in postnatal pathological cardiac repair and can be successfully isolated, expanded in culture. Several studies employing the direct injection of different types of cells have been already clinically performed, but injections were associated with a low engraftment rate, which limited the benefits of the procedure. Cell sheet (CS) technology has been investigated as alternative delivery methods providing better cell viability.

The aim of the present study is to compare the efficacy of transplantation of cardiac progenitor cells into the infarcted myocardium by conventional needle injection and CS epicardial deposition.

After ligation-induced myocardial infarction, rats were randomly allocated to receive intramyocardial injections of c-kit(+)CD45(-) CPC marked with CM-DIL or control medium or infarcted area covered with scaffold–free CS. Morphometry analysis of left ventricular remodelling, quantification of cell engraftment, assessment of CPC survival, apoptosis, differentiation, myocardium fibrosis and angiogenesis were analyzed by immunostaining.

Quantitative analysis showed that at 2 weeks after transplantation the number of engrafted CPC in injection group was significantly lower compare with CS group. No significant differences in the number of cells expressing the proliferation and apoptosis markers have been identified. CPC transplanted in CS had a more pronounced migration ability compared to injected cells The area of infarction contained labeled CPC was 2.5 times greater at epicardial delivery method than injection. Histological analyses of heart frozen sections revealed that on day 14 the CPC CS grafts had produced thick tissues, with a high-cell density, and promoted pronounced vascularization, compared with injected cells. Part of transplanted CPC in both groups showed signs of differentiation to cardiomyocytes and endothelial cells.

Compare with injections, delivery of CPC based CS in the infarcted area is associated with better graft functionality, structural integration and neovascularization and structural integration of CS in myocardium. These data strongly support the potential of CPC sheet transplantation for the treatment of damaged heart.

P223 Micro-scale engineered tissues as *in vitro* cardiac scar-like model

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Introduction. Research on cardiac biology and clinical translation might benefit from the generation of engineered three-dimensional (3D) micro-scale cardiac tissues suited to reproduce controlled key native biophysical conditions [1]. In particular, after myocardial infarction, tissue necrosis occurs and a wound healing process takes place to form a dense collagenous scar, which impairs the normal cardiac function. Our aim is to generate a micro-scale engineered tissue as in vitro scar-like model recapitulating the fibroblast activation, proliferation and switch into myofibroblasts, and their ultimate role in extracelluar matrix (ECM) deposition. Transforming growth factor - β1 (TGF-β1) supplementation is known to support the switch of cardiac fibroblasts towards myofibroblasts at the macro-scale [2]. We hypothesize that the combined exposure to TGF-β1 supplementation and physiological mechanical stimulation (as uniaxial strain) regulates fibroblast proliferation and fibroblast-myofibroblast phenotype transition.

Materials and Methods. For this purpose, we used a recently developed microfluidic bioreactor capable to apply controlled uniaxial strain to 3D cell-based constructs [3]. Rat cardiac fibroblasts were isolated from 3-day-old neonatal pups expanded in high glucose culture medium supplemented by 10% fetal bovine serum. Cardiac fibroblasts were embedded at the density of $1{,}45 \cdot 10^4$ cells/µl in a fibrin gel (composed by 20 mg/ml of fibrinogen and 5 U/ml of thrombin) and cultured for 7 days into microfluidic devices. In particular, fibroblasts were statically and dynamically (10% of strain and 1 Hz of frequency) cultured, with or without (control) the addition of TGF-β1. The effect of different culture conditions on ECM stiffness, cell phenotype, and proliferation were investigated through atomic force microscopy (AFM)-based nano-indentation and immunofluorescence, respectively.

Results. TGF-β1 supplementation yielded higher values of Young's modulus in both static and mechanically stimulated constructs compared to the controls without the addition of the growth factor. The mechanical cyclic strain significantly increased both the cell proliferation and the percentage of α-smooth muscle actin positive (α-SMA+) cells (myofibroblasts), compared to the static condition without TGF-β1 supplementation. In particular, cell proliferation was statistically lower in the static condition without TGF-β1 supplementation (0,95±0,60%) compared to both the static (3,35 ± 1,19%) and dynamic (3,75 ± 1,33%) conditions supplemented with TGF-β1. A significant increase in α-SMA expression was also found between the static condition without TGF-β1 supplementation (23,92 ± 15,30%), and both the dynamic conditions without and with TGF-β1 supplementation (58,55 ± 8,97% and 56 ± 21,03%, respectively), confirming the key role of the mechanical stimulation.

Conclusions. The here generated micro-scale scar-like engineered tissue represents a reliable model to investigate the effects of different molecules/drugs in the proliferation and maturation phases of the wound healing process not only for cardiac-related research but also for other types of tissues (e.g. skin, liver).

P224 Diffraction enhanced imaging computed tomography for monitoring implanted hydrogel cardiac patches by using a synchrotron light source

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Patch-based cardiac tissue engineering aims to stabilize and restore the myocardium for myocardial infarction repair [1]. Three-dimensional (3D) quantitative imaging of hydrogel cardiac patches is a strategic priority for longitudinal animal study of cardiac regenerative medicine [2]. Due to weak X-ray absorption of hydrogels, conventional radiography is unable to visualize microstructural features of cardiac patches [3]. This study aims to assess the performance of a state-of-the-art synchrotron-based diffraction enhanced imaging computed tomography (DEI-CT) compared to MRI and phase-contrast imaging tomography (PCI-CT) for 3D quantitative assessment of implanted 3D-printed hydrogel cardiac patches.

Alginate was 3D-plotted into a cardiac patch with a well defined microstructure. Left anterior descending coronary artery of rats was surgically occluded in order to induce heart attack and then the cardiac patch was implanted proximal to the ligation. Seven days after implantation, the heart including the patch was excised, embedded in a soft tissue mimicking gel and imaged using DEI-CT and PCI-CT at the Canadian synchrotron facility. Experiments were performed at four X- ray photon energies, three phase propagation distances (PPDs), four CT-scan times and two effective pixel sizes in order to identify optimum imaging parameters. DEI-CT and PCI-CT images were processed, reconstructed and then compared to the corresponding 3T MRI images.

DEI-CT and PCI-CT showed their superiority over MRI with respect to 3D qualitative and quantitative visualization of anatomical and microstructural features of myocardium and cardiac patch. Optimum quantitative/qualitative low-dose imaging was obtained at PPD of 147 cm and X-ray energy of ~30 keV at the detector effective pixel size of 25 μm. Both DEI-CT and PCI-CT were able to clearly distinguish the patch microstructure (e.g. alginate strands) and the myocardium anatomical features. While phase retrieval of PCI-CT images provided a relatively better quantitative visualization of the patch, DEI-CT was superior in terms of edge illumination of anatomical features.

Based on the findings of the present study, DEI-CT and PCI-CT have shown promises for quantitative/qualitative assessments of the implanted hydrogel cardiac patches, which were poorly detectable by MRI. As a result, it is concluded that DEI-CT and PCI-CT offer great potentials for monitoring implanted hydrogel patches for cardiac tissue engineering.

P225 Tissue-engineered cardiac microfibers for the treatment of conduction impairment

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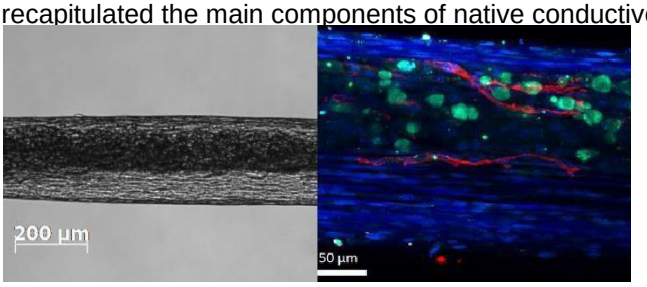
Introduction: Atrioventricular (AV)-block is a pathological condition affecting the conduction pathway of the heart, resulting in impaired heart beating. Current treatment using artificial pacemakers has many drawbacks especially for the paediatric patients, making a permanent tissue-engineered solution desirable.

Method: Fibrin micro-fibers encapsulating human induced pluripotent stem cell (iPS) derived cardiomyocyte (CM) cells were produced using a newly developed micro-moulding technique imparting a longitudinal topography on the gel and increasing cell density. Human umbilical vein endothelial cells (ECs) were added to the same fibers to provide pre-vascularization. Human dermal fibroblasts were encapsulated in a separate fiber that was placed parallel and touching the CM/EC fiber. These assemblies of fibers were cultivated for two weeks, after which electrophysiological (EP) recordings were made and IHC was performed.

Results: Brightfield images taken during cultivation showed synchronous beating of the CMs over the whole length of the fiber (~ 1.5 centimeter) starting at day 5 and continuing for the whole duration of the experiment. The presence of the fibroblasts notably affected the mechanical properties of the constructs as movement as a result of CM beating was markedly dampened compared to control constructs lacking fibroblasts. Visualization with two- photon laser scanning microscopy revealed that the two fibers had fused into one unit surrounded by a thick layer of collagen as demonstrated by second harmonic generation imaging. CD31 staining revealed the formation of capillary-like structures mainly located within the original CM/EC fiber. Staining of sarcomeric alpha actinin clearly showed a striated contractile apparatus in the cells, indicating cellular maturity. Connexin 43 was found on the surface of the cells. Preliminary EP recordings showed the conduction velocity in the fiber to be
~10cm/s, close to that of the native human AV- node.

Discussion and conclusion: The presented constructs formed a conductive network covering the length of the micro-fibers and featured vascular-like structures in between the CM network. This together with the mechanical strength provided by the fibroblasts means that the constructs recapitulated the main components of native conductive cardiac tissue.



Left: brightfield image after one week of cultivation, top part is the CM/EC fiber, bottom fibroblast fiber, the two fibers visibly merged into one. Right: Construct stained for CD31 (red), vascular-like structures can be seen formed in the CM/EC fiber. Collagen (blue) covering the construct.

P226 In vitro fabrication of 3D cardiac tissues with perfusable blood vessels by cell sheets engineering and measurement of the contractile force

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[Background and aim of this study]

Thick three-dimensional tissue engineered structures have a great future potential in various areas of regenerative medicine. However, the diffusion limit of nutrients, oxygen and metabolites represents a major challenge. The purpose of this study is to create thick myocardial implantable and functional tissues with pre-existing vascular networks. The constructs consist of stacked human cardiac cell sheets which are vascularized through tissue engineered vascular beds. Conventionally, stacking cell sheets are limited due to cell necrosis caused by lack of oxygen or nutrition. Therefore, in order to create thick tissues, it is necessary to create vascular networks in the stacked cell sheets and perfuse them with cell culture media.

[Method]

We created vascular beds from porcine small intestinal tissue by trimming it into a flat surface, removing mucosa and finally sterilizing it with peracetic acid. Triple-layers of human cardiac cell sheets were then stacked using stamp method. We performed mechanical analyses using a newly developed, 3D-printed device capable of real time measurement of contraction force during cardiac tissue culture (Fig 1). Cell sheet morphology was assessed using optical coherence tomography. In addition, cardiac tissue was stained with H.E. for histological analysis.

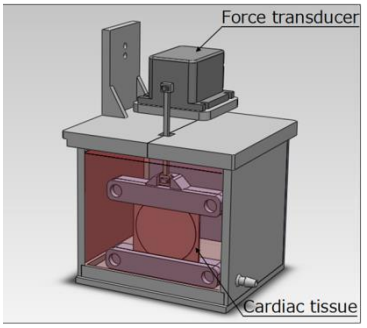

Fig 1. 3D-CAD image of the contractile force measuring device for cardiac tissue.

[Result]

Real time contractile force could successfully be recorded using the newly developed device. Optical coherence tomography recording revealed synchronously beating cardiac tissue. H.E. staining showed triple-layered cardiac cell sheets and vascular structure of porcine small intestine origin (Fig 2). [Future plan]

We aim to create thick tissues by multistep stacking of triple-layered cell sheets with neovascularization. We believe that this can be achieved by using vascular bed with porcine small intestine which have closed loop arteriovenous vascular network and that our newly developed device can be used to measure differences in contractile force.

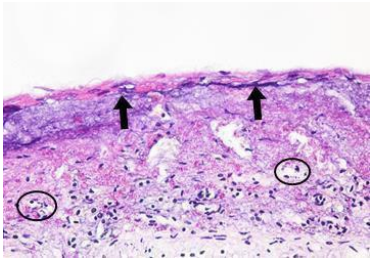

Fig 2. Triple-layers human cardiac cell sheets (arrows) and porcine small intestine tissue in which contain vascular structure (circles).

P227 A high-tech bioreactor system for cardiovascular tissue engineering - increasing quality, possibility and efficiency

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OBJECTIVES

The demands on bioreactors for cardiovascular tissue engineering are constantly increasing. More and more environmental conditions have to be controlled, measured and documented. Consequently the devices are increasingly complex and expensive. The aim of this work was to develop a flexible bioreactor system for heart-valve tissue engineering, that fulfils these demands and grants easy and failsafe handling.

METHODS

The combination of a precision pump with an adjustable compliance and resistance provides the means to create physiologic and pathologic flow patterns through the circuit. Furthermore, the bioreactor is equipped with a variety of sensors to monitor key parameters such as the pressure gradient, temperature, flow rate, pH, pO_2, etc. To improve the usability, all data is collected and processed in a programmable logic control and displayed in a clear way on a custom made user interface. To reduce downtime and workload, the bioreactor is equipped with an integrated sterilization procedure. Thus, the complete device can be sterilized without time-consuming disassembly and reassembly. The risk of contamination during assembly is eliminated as well. Consequently, the safety of the sterilization is increased while downtime is significantly reduced if compared to other systems.

To allow an autarkic use of the device, the temperature and CO_2 control is handled by the bioreactor as well. Thus, there remain no dependencies on any further equipment and the device can be used anywhere.

The modular design of the bioreactor allows adaptions at minimal effort and cost. Individual components can be rearranged or exchanged to meet specific requirements. Since all connections are standardized, the required engineering and consequently the financial effort, is kept at a minimum.

RESULTS

The bioreactor allows the simulation of all necessary environmental conditions for cardiovascular tissue engineering. Furthermore, the modular design provides the necessary flexibility to adapt to increasing and changing requirements. The simple way to monitor and control all important data via a clear user interface improves the usability and safety of the device. The possibility to log and export all data automatically significantly increases the efficiency and reproducibility of research.

CONCLUSION

The new system is a completely autarkic bioreactor which allows realistic in-vitro experimentation and research. Its modular design combined with key features such as the self-sterilization process, it is a powerful tool for cardiovascular tissueengineering.

P228 Increasing quality and safety of biological grafts - a comprehensive evaluation and mapping of the mechanical, structural and biological characteristics of the bovine pericardium

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OBJECTIVE:

Bovine pericardium is well-known and the most used biologic material in the field of cardiovascular surgery. To increase biocompatibility of this xenogenic material, the tissue has to be fixated or decellularized prior to application. Consistent material properties are required, to achieve reproducible graft quality. However, pericardium has significant differences in composition and microstructure, depending on the selected region. Surprisingly, the material characteristics of the pericardium have not been mapped so far.

METHODS:

Bovine pericardium (n=12) was gained from the local slaughterhouse. Preparation and evaluation was performed within 2h. A special backlighting-bench was designed to evaluate the fiber orientation. In regard to mechanical properties, the thickness, and Young's modulus was evaluated. The elastic modulus was determined by tensile testing of standardized S3a-specimen punched from the pericardium. Specimens in, as well as perpendicular to the fiber direction were examined, to evaluate the anisotropy.

The microstructure was further evaluated by Pentachrome and Picrosirius Red staining which offer a more detailed insight in Collagen and Elastin composition. The cell distribution on the pericardium was mapped by DAPI and HE staining. The cell density was automatically calculated using ImageJ. Finally, the surface topology of the pericardium was evaluated by SEM analysis.

RESULTS:

We proved that the composition of pericardium is highly dependent on the region where the probes are taken. The tissue not only varies in thickness and surface topology, but more importantly in cell density as well. Moreover, the fiber network varies significantly in composition as well as in regards to orientation: Some areas display a unidirectional fiber orientation, while others show an inhomogeneous network.

CONCLUSIONS:

This is the first work to comprehensively map material characteristics of the bovine pericardium. It highlights the significance of proper source selection, to achieve constant and repeatable results with pericardial tissue. This is of utmost importance, especially in regard to applications such as fixated or decellularized heart-valve leaflets where the tissue is immensely stressed and where long-term stability is crucial. Furthermore, for decellularization procedures it is of utmost importance, that the primary cell density can be estimated, to achieve stability and repeatability. The performed mapping of all these characteristics not only allows estimating the tissue properties, but can also act as a guideline to identify suitable regions and specific orientation to create biological grafts from pericardium of constantly high-quality.

P229 Engineering the microenvironment niche of human BM-derived MSC spheroids for enhanced cardiomyogenesis

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Introduction: The endogenous cardiac regenerative mechanism is incapable of recovering the lost cells and tissue following myocardial infarction. Current surgical and pharmaceutical interventions have lowered the mortality rates; however there is an unmet clinical need to replace the lost cardiomyocytes and restore the structure-function-property relationship of the injured myocardium. Here, we investigate the benefits of a 3D microenvironment, constituting collagenous hydrogels or electrospun nanofibrous scaffolds, and supplemented with 5-azacytidine (Aza), in inducing cardiomyogenesis of the human bone marrow (HBM)-derived mesenchymal stem cells (MSCs).

Materials and Methods: Spheroids of HBM-MSCs were formed from loose cells using a custom hanging-drop protocol. After 24 h, spheroids were cultured within collagen hydrogels (type-I; 1-3 mg/mL) or within aligned electrospun collagen nanofiber scaffolds in the presence of Aza (0-50 μM) for 4 weeks. Co-expression of the cell markers pertaining to their stemness (CD90), early (GATA) and late cardiac markers (cTNT, cTNI), and survival (LIVE/DEAD®) were quantified using IF labeling and imaging at various time-points, and gene expression quantified using RT-PCR. Roles of Wnt (GSK-3β and β-catenin) and Notch (DLL4 and Notch 1) signaling pathways on the cardiomyogenesis of BM-MSCs were investigated using fluorescence labeling and Western blots.

Results and Discussion: Within 2 mg/ml collagen hydrogels, ~50% of the cells expressed CD90 in controls on day 1, while 37% of them also co-expressed GATA. By day 12, CD90 expression dropped to ~30% in controls while that of GATA significantly increased, with the highest expression in 2 mg/ml (10 μM-Aza) compared to other gels. No significant increase in the standalone and co-expression of cTnT was observed in day 12 cultures. Standalone expressions of cTnI were significantly higher on day 28 cultures (10 μM Aza) compared to day 1 cultures. Similar trends in the standalone and co-expression of the markers were observed at various intermediate time points in 1 mg/ml hydrogels and nanofibers. However, 3 mg/ml hydrogels showed the highest standalone expression of cTnT, compared to other scaffolds, and was significantly higher on day 12 (10 μM Aza) compared to other hydrogels. PCR analysis from day 12 cultures showed detectable expression of both cTnT and cTnI, only within 3 mg/ml hydrogels, while cultures from 2 mg/ml showed detectable Ct values for cTnI.

Conclusions: Results showed the significant role of microenvironment (matrix stiffness, topography and Aza dosage) on cardiomyogenesis of human BM-MSCs. Increasing trends in the standalone expression of early and late cardiac markers were observed within collagenous scaffolds, with stiffer hydrogels exhibiting higher expression of mature cardiac markers, while higher cellular alignment were observed on nanofibers, inferring to the synergistic role of signaling molecules, matrix stiffness and topography on phenotypic, genotypic and morphometric traits of BM-MSCs differentiating towards cardiomyogenic lineage. Such differentiated stem cells can have potential scope in cell transplantation or tissue engineering applications for cardiac repair.

Acknowledgements: NSF (CBET), Cleveland State University Startup funds, Cellular and Molecular Medicine Fellowship.

P230 Semi-automatic quality control of decellularized human pulmonary roots

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Introduction

The current EU regulatory guidelines for the quality control of tissue engineering products have been reported to be time/resource consuming and subjective. The aim of this study was the development of a semi-automatic method for a faster, cost effective and objective assessment of the extracellular matrix (ECM) quality of decellularised pulmonary valve roots.

Materials and methods

The bag-of-features algorithm was used for the automatic classification of histological images. Histological slides of decellularized human pulmonary arteries were stained with Elastica van Gieson and assessed by expert histopathologists. The diagnosis of the slides from the histopathologists was either “in order”, “intima fibrosis” or “disturbed fiber network”. The slides were photographed and images were obtained at two different magnifications (×10, ×20). Before the analysis, stain normalization was applied on all the images in order to have a uniform appearance The image set, comprised of 735 fields of view, was divided in training and validation sets (70%) and a test set (30%). Different feature extraction and detection techniques (SURF, MSER, SIFT), classifiers (SVM, k-nn) and codebook sizes (100,500,1000,1500,3000,6000) were used for different models. The out-of-sample generalization error from 10-fold cross validation was used as the model selection parameter.

Results

The 10-fold cross validation results showed that the model that used the SIFT feature detection and extraction, the 5-nearest neighbor classifier and 1000 word codebook had the least generalization error. This model was subsequently applied to the test set. The performance on the test set demonstrated high precision, recall, f-measure and accuracy (Figure 1).

Discussion

The results indicated that the bag-of-features image classification algorithm is a potent tool for histological image representation and that it could be used for the assessment of the ECM integrity in a semi-automatic quality control scheme with performance that is comparable to the current gold standard, which is the histopathologist’s expertise.

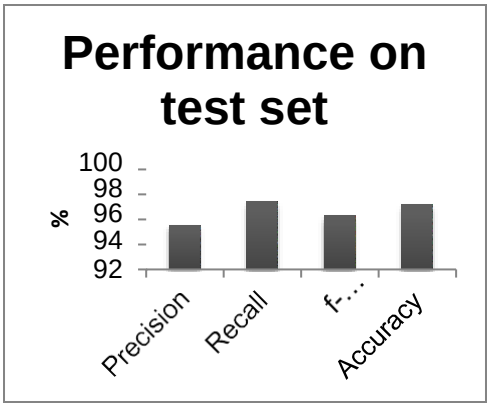

Figure 2: Performance of the optimal model on the test set.

P231 hPSC differentiation into cardiomyocytes cultured on biomaterials immobilized nanosegments (ECMs and oligopeptides)

Cheng Hui Liu

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Human pluripotent stem cells (hPSC) of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) have the potential ability to differentiate into many kind of cell types originated from three germ layers: endoderm, mesoderm and ectoderm cells such as dopamine-secreting cells for Alzheimer disease treatment and insulin-secreting cells for diabetes treatment. However, it is a challenging issue to guide hPSCs to differentiate into our desired lineages of cells due to their variety of differentiation ability. The fate of differentiation of stem cells is determined by different factors existed in the microenvironment of hPSCs: bioactive molecules, cell-cell interactions, physical factors and cell-biomaterial interaction. It is a reasonable strategy to mimic the stem cell microenvironment for the differentiation of hPSCs into specific lineages of cells using optimal biomaterials for hPSC culture. We investigated cardiomyocytes differentiation of hPSCs and evaluated the efficiency of differentiation using three differentiation methods. Currently, it has not yet investigated which extracellular matrices (ECMs) or nanosegments derived from ECMs promote hPSCs differentiation into cardiomyocytes. We developed nanosegment-grafted biomaterials having different elasticity for hPSCs differentiation into cardiomyocytes.

We developed nanosegment-grafted biomaterials having different elasticity for hPSCs differentiation into cardiomyocytes. We prepared (1) ECM-coated dishes where ECMs are fibronectin (CellStart), laminin, and recombinant vitronectin, (2) PVA-IA (polyvinylalcohol-co-itaconic acid) hydrogel dishes having different elasticity that are grafted with several ECMs where ECMs are fibronectin, laminin, and recombinant vitronectin, and PVA-IA hydrogel dishes having different elasticity that are grafted with cell-adhesion oligopeptide (oligovitronectin: KGGPQVTRGDVFTMP (O-VN1)). On day 0, we replaced the expansion medium into cardiomyocytes differentiation medium containing the GSK3B inhibitor. On days 1-2, we observed that 30%~40% of the cells were died and detached from the surface. However, the center of the colony of living cells were getting thicker and became compact (Fig. 1). The cells were differentiated into mesoderm stem cells between days 3-4. On day 8, we successively observed the contracting colonies on the surface.

We evaluated the optimal elasticity of biomaterials and preferable nanosegments immobilized on the biomaterials for hPSCs differentiation into cardiomyocytes. The results for cardiomyocyte induction from hPSCs will be used in clinical application and in the investigation of molecular mechanism of specification and maturation of cardiomyocytes.

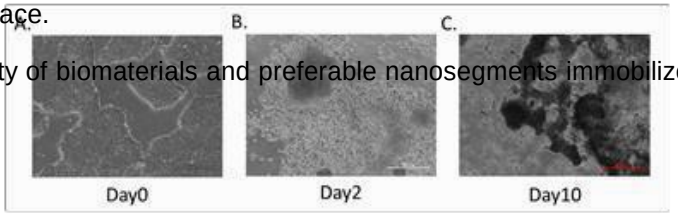

Figure 1. Cardiomyocytes differentiation from hESCs using a direct differentiation method on Matrigel-coating dish. (A) Human pluripotent stem cells. (B) Mesoderm stem cells. (C) Contractile cardiomyocytes.

P232 Elimination of residual iPS cells in bioengineered cardiac cell sheets using Dinaciclib

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Although bioengineering cardiac tissues derived human iPS cells is a promising method for regenerative medicine, the development of strategy to eliminate remaining undifferentiated iPS cells in bioengineered cardiac tissues without affecting the viabilities of some kinds of iPS cell-derived somatic cells is indispensable for clinical application. In the present study, we show that Dinaciclib, a multi cyclin dependent kinase (CDK) inhibitor, induces the apoptosis of human iPS cells without affecting the viability of iPS cell-derived cardiac tissues. The treatment with low dose (6nM) Dinaciclib induced the apoptosis of human iPS cells within 24 hours through CDK-1 inhibition-mediated p53 dependent MCL-1 degradation. On the other hand, the treatment with middle (8nM) and high dose (20-50nM) Dinaciclib induced the apoptosis of human iPS cells through CDK9 inhibition-mediated p53 independent suppression of MCL-1 transcription. These findings suggest that Dinaciclib might promote iPS elimination through multidisciplinary mechanisms. When human iPS cells were cultured with human cardiac fibroblasts, one of the essential components of bioengineered cardiac tissues, and cultivated with several doses of Dinaciclib for 6 hours, the significant reduction of remaining iPS cells was observed, suggesting that Dinaciclib might be effective to eliminate iPS cells even in the co-culture environment with other cell types. Finally we elucidated the influence of Dinaciclib for human iPS cell-derived cardiomyocytes. Even after the treatment with high dose Dinaciclib (50nM), most of cardiomyocytes were still kept in viable. Furthermore when iPS cell-derived cardiomyocytes were cultured on temperature responsive culture dishes with Dinaciclib, cell sheets were fabricated after the lowering the culture temperature and the transplanted cardiac cell sheets onto the subcutaneous tissues of nude rats showed the spontaneous beating at 7 weeks after the transplantation. Although Dinaciclib induced the expression of p53 in cardiomyocytes, MCL-1 transcription and protein were maintained, indicating that Dinaciclib might not be toxic for human iPS cell-derived cardiomyocytes. These findings suggest that the difference of MCL-1 expression regulatory machinery through Dinaciclib treatment between iPS cells and iPS cell-derived cardiac cells could be exploited to eliminate remaining iPS cells in bioengineered cell sheet tissues, which will further reduce the risk of tumour formation.

P233 Cardiac progenitor cell mechanoresponse in 3D fibrin hydrogels

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The interaction of cardiac progenitor cells with the stimuli provided by the surrounding microenvironment is crucial for the success of stem cell-based therapies for cardiac regeneration. However, very little is known about this complex interplay. In particular, mechanical stimuli are of great importance in the mechanically active heart. We have recently shown that, in 2D, human cardiomyocyte progenitor cells (CMPCs) only develop stress fibers upon early cardiac differentiation, and as of that moment become sensitive to cyclic strain, as was evidenced from their re-orientation perpendicular to the applied strain direction (strain avoidance).[1] Here, we investigated the effects of cyclic strain on undifferentiated CMPCs in 3D environments, and the role of actomyosin stress fibers in 3D CMPC mechanoresponse. Uniaxial cyclic straining of CMPCs, embedded in biaxially constrained 3D fibrin mini-tissues, resulted in re-orientation parallel to the direction of the cyclic stretching (strain alignment). Furthermore, when the cytoskeleton was perturbed by inhibiting the mechanotransduction pathways Rho-A kinase (ROCK) and myosin light chain kinase (MLCK), CMPCs displayed distinct responses to the applied strain. Addition of the ROCK inhibitor Y27632 resulted in strain alignment, similarly to control samples where no inhibitor was added. On the contrary, when using the MLCK inhibitor ML-7 CMPCs displayed a strain avoidance behavior. The presented findings suggest that multiple mechanisms regulate the mechanoresponse of CMPCs in 3D, and highlight the importance of investigating these phenomena, particularly in 3D environments. A deeper knowledge of the interplay between CMPCs and their mechanical niche can contribute to the improvement of cardiac regeneration therapies.

P234 Differentiation of mesenchymal stem cells on fibrin assemblies with immobilized growth factors

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Bioartificial heart valves and vessels prepared from decellularized tissues could be recellularized with bone marrow-derived mesenchymal stem cells (MSCs). MSCs differentiation into endothelial cells, smooth muscle cells or valve interstitial cells will be facilitated by sustained release of growth factors.

In this study, fibrin, fibrin with heparin, fibrin with adsorbed or covalently-immobilized vascular endothelial growth factor (VEGF) or/and basic fibroblast growth factor (FGF-2) via binding to heparin attached to fibrin have been prepared and evaluated for their stimulation of MSCs differentiation. MSCs were seeded onto fibrin assemblies and cultured for 21 days. The mRNA expression of genes, such as ACTA2 and DES (specific for smooth muscle cells), ALP, Col1A1, and BGLAP (osteoblasts), VWF and CD31 (endothelial cells) were estimated on days 1, 3, 7, 14, and 21 after seeding. The MSCs prepared for seeding were used as a control. The presence of some of these markers was proved by immunofluorescence staining.

We have found that VEGF either adsorbed or covalently bound increased more than 600 times the expression of ACTA2 on day 14. The maximum expression of osteogenic markers ALP, Col1A1, and BGLAP was about 200 times, 50 times, and 20 times higher respectively compared to the control, on fibrin with heparin alone and fibrin/heparin/VEGF on day 21. The differentiation into endothelium was very poor.

We can conclude that MSCs differentiate mainly into the smooth muscle cells on fibrin containing VEGF. Thus this assemblies should be more convenient for the bioartificial heart valve replacements.

Supported by the Technology Agency of the Czech Republic (grant No. TA04011345) and Ministry of Health of the Czech Republic (grant No.15-29153A).

P235 Contribution of heart extracellular matrix bioscaffolds to disclose the mechanic dynamics behind cardiac pathologies

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The complex architecture of cardiac extracellular matrix (ECM) provides a unique 3D environment fundamental for cardiac development, homeostasis, and functionality. The derangement of ECM integrity occurring during the onset and progression of cardiac diseases drives a modification in the nanotopography and mechano-physical properties of the ECM itself, thus impairing cardiac cell contractility and proper organ function. Furthermore, our immunohistochemistry and gene expression analysis, on infarcted mouse hearts and human cardiac biopsies, confirms that the dramatic structural changes triggered by myocardial infarction and tissue remodelling, leading to heart failure, causes a switch in cardiac cell mechanosome.

Cardiac decellularized ECM (dECM) obtained from physiological and pathological specimens feature the three dimensional cues, mechanical properties, chemical complexity and the native organization of heart tissue in healthy and diseased condition. For this reason, they are here proposed as an *in vitro* model to investigate the mechanisms of cell-ECM interaction, especially consequent to cardiac pathologies.

In this study, we show that second harmonic generation imaging, scanning electron microscopy and atomic force microscopy can be implemented to obtain high-resolution 3D maps of cardiac dECM structure and mechanical properties, as an essential asset to the conventional immunostaining and protein analyses. Furthermore, recellularization of physiological and pathological myocardial scaffolds resulted in distinct cell-dECM interactions, thus bringing further hints to disclose a possible window of action for heart cell therapies. Altogether, our findings show that cardiac dECMs are powerful and reliable toolboxes to monitor cardiac nanostructural changes and to investigate cardiac system mechanobiology.

P236 Tissue adaptation in human saphenous veins cultured ex-vivo in a bioreactor enabling the full-mimicking of coronary-like by pass grafting hemodynamic conditions

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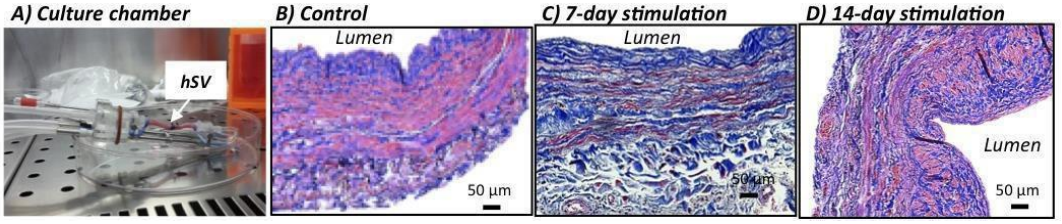
Introduction - The incidence of vein graft failure after coronary artery bypass grafting is well documented. However, the early events associated to the hemodynamic loading changes are poorly understood. We devise *ex-vivo* culture systems (EVCS) for the stimulation of human saphenous vein (hSV) segments[1,2,3] to investigate metabolic and mechanical cues leading to graft (mal)adaptation. Recently, we have developed a novel EVCS[3] that enables stimulating hSVs with coronary pressure/flow patterns, i.e., a sphygmoid pressure (80-120 mmHg) in counter-phase with a pulsatile flow rate (100/200 ml/min). Panel A shows hSV mounted in the ECVS culture chamber.

Methods - SV segments from patients undergoing bypass surgery (n=18) were subjected to stimulation in our EVCS for 7 and 14 days with dextran-enriched DMEM (3.5% w/v, 3.07±0.07 cP viscosity). Samples treated with steady flow regimen were used as control groups. After the stimulation, hSVs were fixed and stained with H&E, Masson's trichrome and Weigert van Gieson staining for morphometric evaluation. Cell proliferation (Ki67) and apoptosis (TUNEL) were performed together with immunofluorescence to identify smooth muscle cells (SMCs) and endothelial cells (ECs).

Results – While 7-day stimulation (panel C *vs.* B) did not affect vessel wall and lumen perimeter integrity, morphometric analysis revealed a thinning of the intima and media. The media consisted mainly of SMCs, aligned both circumferentially and longitudinally. On the luminal lining, most cells expressed CD31/vWF. Apoptosis was significantly increased in both stimulated and control hSVs, while increased cell proliferation was observed only in stimulated hSVs. After 14-day stimulation (panel D *vs.* B), the media was consistently thinner compared to control group, with a trend towards increased intima thickness. Analysis of proliferation, apoptosis and SMCs phenotype are currently ongoing.

Conclusion - Our novel platform enables prolonged *ex vivo* stimulation of hSVs mimicking the complex hemodynamic environment of the coronary circulation. Our results are consistent with the early vessel remodelling events observed in vein grafts in patients shortly after implantation. We are now extending the timeframe of the experiments up to 21 days to obtain data on the pathological evolution of vein graft disease.



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P238 A medium-throughput microfluidic platform for the generation and functional assessment of engineered cardiac microtissues

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Introduction. The generation of functional cardiac tissues in vitro remains an open issue. In vivo, cardiac cells undergo multiple cues deriving from the local heart tissue "niche", which instructs cells through dynamic biochemical, electrical and mechanical signalling, eventually guiding tissue development and maturation. Cardiomyocytes (CMs) are subjected to periodic contraction/relaxation sequences deriving from the beating of the heart. A robust and predictive in vitro model of mature cardiac tissues should thus enable the recapitulation of (i) the three-dimensional (3D) architecture of complex cell-cell and cell- extracellular matrix (ECM) interactions and (ii) electro-mechanical stimuli resembling the native myocardial environment, (iii) in the presence of controlled biochemical signals [1].

Materials and Methods. Here, we report a new medium-throughput platform to generate mature and highly functional cardiac microtissues within a perfused microfluidic device. The platform features 6 independent culture chambers, and it builds upon a previously developed micro-bioreactor for the culture of 3D cell constructs, designed to recapitulate the physiological strains experienced by cells in the native myocardium (about 10%) [2]. Briefly, arrays of hanging posts limit cell-laden gels during injection, and a pneumatic actuation system induces uniaxial cyclic strains to the 3D constructs (generated by neonatal rat or human pluripotent derived cardiomyocytes). The presence of auxiliary channels allows to perfuse medium during culture, while providing biochemical stimulation and transporting pacing signals during the functional evaluation of microtissues.

Results. Stimulated cardiac constructs express higher levels of connexin-43 (Cx43) as compared to control, suggesting superior electrical connection among neighbouring cells and cardiac maturation. Moreover, the cyclic mechanical stimulation promoted spontaneous synchronous beating of the constructs, all over their volume. Electric pacing experiments further demonstrated the superior maturation of the stimulated constructs.

Conclusions. We introduced a medium-throughput heart-on-a-chip platform able generate functional cardiac microtissues from both neonatal rat and human pluripotent derived cardiomyocytes. This result was achieved through the application of a highly controlled cyclic uniaxial strain to 3D microtissues in culture. The compatibility with electrical pacing makes the platform suitable for physio/pathological states investigations. Furthermore, current experiments aim at assessing the ability of the platform to predict in early stages drug benefit and safety.

P239 Oxygen-controlled double-compartment culture system for investigating the tissue response induced by different adventitial hypoxic conditions in saphenous vein grafts

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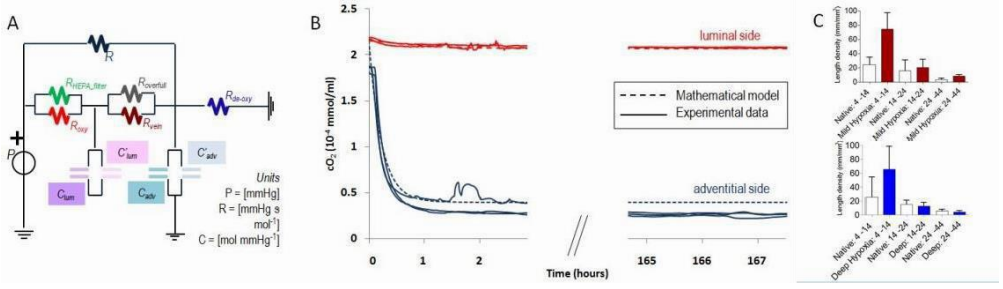
Introduction: After Coronary-Artery-Bypass-Graft surgery (CABG) with autologous human saphenous vein (hSV), the graft is exposed to an altered biochemical environment. In particular, oxygen levels affect the cell and tissue adaptation and induce inflammatory response. Aim of this work is to develop an *ex vivo* culture system to study the effects produced on the tissue by different oxygen conditions.

Methods: Here we developed a culture system [1] composed by a double-compartimental culture chamber to keep separated the intra-luminal and the extra-advential vessel areas. A lumped parameter model was developed to predict the oxygen trend in the two compartments (Panel A). Based on the model predictions, a de-oxygenator module was designed to impose different oxygen levels in the extra-adventitial compartment (in a range between 21% and 2.5%). The system was used in experiments with hSVs, imposing different oxygen concentrations in 3 experimental groups: two different arterial conditions (mild hypoxia: 21% intra-luminal, 5% extra-adventitial; deep hypoxia: 21% intra-luminal, 2.5% extra-adventitial) were mutually compared and compared with the standard condition (21% in both compartments). After 7 days conditioning, hSV central portion was processed for the histological and immunofluorescence analyses.

Results: The system was suitable to impose controlled oxygen conditions during human SV experiments (Panel B). In comparison with the standard condition where no differences with the native samples were found, hSV experiments showed an increased density of adventitial small caliber *vasa vasorum* both in the two arterial oxygen conditions (Panel C). Moreover, deep hypoxia provokes a decrease in the number of smooth muscle cells in the tunica media. The quantification and the evaluation of change in cell density (Immunoistochemistry, Tunel assay) is ongoing.

Discussion: The results proved that the culture system is versatile and easy-to-control device to study the effects of different oxygen levels on hSV grafts. Preliminary results from hSV experiments suggest that the advential hypoxia is responsible for neovascolarization. Moreover, deep advential hypoxia is incompatible with the cell viability. All these events contribute to the vein remodelling that can increase the risk of graft failure.



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P240 Engineering of vascularized tubular myocardial tissues in vitro by using cell technology

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[Background and aim of this study]

The purpose of this study is to create a thick tubular myocardial tissue *in vitro* by cell sheet engineering (CSE), with the potential for independent cardiac assistance. The roadblock, is the limitation in the number of cell sheet layers due to the limitation of oxygen diffusion. Therefore, to create a thick tissue, the introduction of a vascular network is essential for providing oxygen and nutrient. Multi-step layering of 3-layer rat cardiac cell sheets on a resected tissue with a connectable artery and vein realized vascularized thick cardiac tissues. In this study, we attempted to construct a human tubular cardiac tissue with perfusable blood vessels using an original bioreactor system.

[Method]

Small intestine of a rat was harvested and connected to a bioreactor. Mucosa of the small intestine was eliminated by osmotic shock after ultrapure water perfusion. Three-layer human cardiac cell sheets co-cultured with endothelial cells were constructed and transplanted inside the small intestine repeatedly to create the tube. This tubular cardiac tissue was cultured using a bioreactor system and perfused with perfusion medium. After 6-day perfusion culture, function was analysed by inner pressure, tissue morphology was analysed by Azan staining and immunostaining.

[Result]

Artificial flow was created by connecting the system to a rat´s artery. Inner pressure following electric potential as well as contraction motion of tubular cardiac tissue was confirmed. Blood vessel presence and formation was clearly observed in the CSE engineered area. This suggests that blood vessels have been induced within the tubular cardiac tissue. Actually, Azan staining revealed that perfused red blood cells reach into cardiac tissue. In addition, immunostaining with CD31 revealed that neovascularization occurred within the tubular engineered cardiac tissue.

[Conclusion]

We have successfully fabricated *in vitro* tubular cardiac tissue accompanied by a vascular network using cell sheet engineering. Our results may open the possibility to overcome thickness limitation during the fabrication of thicker cardiac tissue and contribute to future treatments for patients with serious cardiac problems.

P241 Well-defined conjugated materials for the engineering of electroactive tissues

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Introduction: Conjugated polymers are promising materials for the engineering of electroactive tissues as they may provide a means to stimulate differentiating cells or facilitate electrical signalling upon implantation. However their utility is limited by the use of highly heterogeneous materials, ill-defined in terms of architecture and electroactivity, which remain difficult to integrate into bio-composites in a controlled manner. As such, there is a pressing need to create well-defined conjugated materials which can be assimilated into more complex 3D architectures. Here, we demonstrate the production of homogeneous oligomers of the commonly used conjugated polymer PEDOT with versatile end-functionality, for integration into bio-activestructures.

Methods: EDOT was treated with oxalyl chloride and a suitable amine to generate a diverse range of keto-acid end-caps. Oligomerisation was achieved through iterative C-H activation (90 ˚C, 1 hr, $Pd(OAc)_2$) and bromination (1 hr, NBS, THF, AcOH). Through rational choice of end-group, bis-capped oligomers could undergo further derivatization, such as the growth of polycaprolactone from amine capped oligomers (140 ˚C, 4 hrs).

Results: Previous attempts to access and utilise oligo-EDOTs in tissue engineering have been hindered by oligomer insolubility, instability, and a lack of functional handles. These problems are exacerbated by traditional synthetic routes to monomer functionalisation and oligomerisation. Here, we developed a novel route to install keto-acid end-groups bearing a diverse range of reactive handles in high yields for further derivatization (eg. bioorthogonal conjugation, polymerisation). Furthermore, we demonstrate that mild iterative C-H activation reactions can effectively furnish end-functionalised oligomers of a defined length in good yields, including hetero-bifunctional oligomers and structures with tunable physical and electrical properties through the controlled introduction of chain defects. Importantly, by combining these methods, we demonstrate the synthesis of homogenous oligomers which may undergo facile subsequent modification to produce functional block co-polymers. This key development will greatly facilitate the integration of conjugated materials in to tissue engineering systems and scaffold architectures.

Conclusion: We present a novel route to create homogenous conjugated oligomers structures bearing functionalities for further derivatisation. Such structures will greatly facilitate the production of electroactive bio-composites.

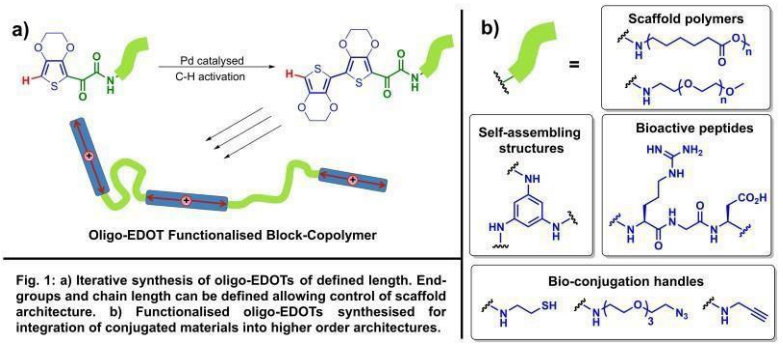

Fig. 1: a) Iterative synthesis of oligo-EDOTs of defined length. End-groups and chain length can be defined allowing control of scaffold architecture. b) Functionalised oligo-EDOTs synthesised for integration of conjugated materials into higher order architectures.

P242 Measurement of the contractile force of the stacked hiPSC-CMs cell sheets on the fibrin-gel

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[Background and Aims]

Fabricating 3-D tissues using the human induced pluripotent stem cell- derived cardiomyocytes (hiPSC-CMs) which is thick and beating robustly like a human heart, is essential for the therapy for heart failure. Our laboratory succeeded to create 3-D cardiac tissues by stacking cardiac cell sheets, however the contractile force of them is unknown. Thus, the purpose of this study is to measure the contractile force of the stacked hiPSC-CMs cell sheets on the fibrin-gel.

[Methods]

Using the hiPSC-CMs expressing the puromycin-resistant gene, we did puromycin processing to them after the differentiation into the cardiomyocytes. The hiPSC-CMs cell sheets incubated on the temperature-responsive culture dishes were transferred on the fibrin-gel sheet using the low temperature treatment. Repeating this process, we fabricated some samples by stacking the iPSC-CMs cell sheets up to 3-layers. Installing the sample in the strain gauge, we measured the contractile force with 60 BPM by providing electrical stimulation. The stacked cell sheets were analysed by H.E. and immunostaining, and measured their own thickness.

[Results]

The samples of 1-layer, 2-layers, and 3-layers revealed the contractile force of 0.58±0.75 mN, 1.28±1.32 mN, and 1.53±1.61 mN respectively(Fig. 1), and the thickness of the cell sheets of 7.5±2.6 µm, 13.9±8.6 µm, and 32.7±17.6 µm respectively(Fig.2) (Average±S.D., each n = 2). The contractile force of the stacked cell sheets was successfully quantified, and we elucidated the linear relationship between the number of the stacking cell sheets and the contractile force of them using the novel developed measuring system.

[Future plan]

The limit of the thickness of stacking cell sheets without necrosis is about 100 µm in vitro. By stacking further hiPSC-CMs cell sheets, we will reveal the number of the limit of layering them. Moreover, by fabricating the 3-D tissues beating with higher contractile force than ever, this measuring system would contribute to a high sensitive drug screening field.

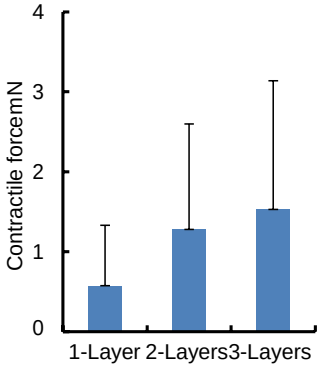

Fig. 1 Contractile force

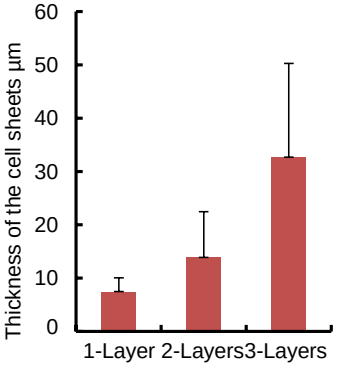

Fig. 2 Thickness of the cell sheets

P243 Where to go in cardiovascular research? A comprehensive evaluation of different tissue engineering and regenerative approaches

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Background: Over the last decade, cardiovascular tissue engineering (TE) became a major field in medical research. Common materials are synthetic, biologic or biohybrid. At the moment research groups using them in “classical” tissue engineering or regenerative strategies. The aim of this study was to provide a guideline for further cardiovascular research by investigation of different materials in a regenerative and a tissue engineering approach.

Methods: Fibroblasts (FB) and endothelial cells (EC) were obtained from human saphenous vein segments. Electrospun non-degradable polyurethane (heart valves and patches) and decellularized biologic materials (heart valves and pericardium) were used as scaffolds. In the first group (G1), polyurethane scaffolds were consecutively colonized with FB and EC. Seeded scaffolds were conditioned by exposure to increasing pulsatile flow for 5 days, representing a „classical“ TE approach. To simulate the implantation procedure transcatheter heart valves were crimped and reperfusioned. In the second group (G2) fibroblast-seeded polyurethane was cultivated statically for 10d to induce extracellular matrix (ECM) expression and to generate a biohybrid scaffold. Scaffolds of G2 and native biological materials (G3) were decellularized (0.5% desoxycholate, 0.5% dodecylsulfate) for 24h, washed for 6d and finally the DC efficiency was analyzed. For evaluation of regenerative approaches the re-seeding efficiency and biocompatibility were evaluated by cell seeding. Scanning electron microscopy, endoscopy, live-dead assays, immunofluorescence and standard histological staining were used for analysis.

Results: FB and EC enabled easy isolation and fast cultivation. Furthermore, both cell types demonstrated effective seeding characteristics. Pulsatile conditioning of G1 resulted in an intact cellular coverage of the scaffold and a strong ECM formation. Subsequent crimping and reperfusion inflicted severe cellular damage, cell layer delamination and upregulation of inflammatory factors. Cultivation of fibroblasts on scaffolds (G2) resulted in comprehensive ECM formation. After decellularization, acellularity and preservation of fibrous ECM was proved for G2 and biological scaffolds (G3). Long-term storage (12 months) of biologic materials revealed no degradation of the components in histological analysis. Re-seeding of regenerative scaffolds lead to good cell adherence, viability and proliferation.

Conclusions: Production of functional “classical” TE constructs as well as regenerative biohybrid and biologic scaffolds was successful. However, manipulation – for example transcatheter heart valve implantation – of living TE constructs inflicts massive cell death and can cause inflammation. Therefore, in cardiovascular research we recommend focusing on regenerativeapproaches.

P245 A novel bioreactor chamber combining interstitial perfusion and electrical stimulation for culturing 3D cardiac constructs allowing non-invasive maturation monitoring and testing

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Nowadays cardiac diseases still represent one of the leading causes of death worldwide. For this reason, in the field of tissue engineering (TE) great efforts have been made to generate functional cardiac constructs to be used both as an *in vitro* models or as functional tissue substitutes. Different TE bioreactors have thus been successfully designed to recreate *in vitro* a cardiac-specific biomimetic environment providing cells with different stimulations (i.e. perfusion, electrical). However, classical bioreactors used for macro-scale construct developing, often lack the possibility to monitor cells and evaluate tissue maturation directly within the system without interrupting culture.

In this perspective, we developed a new bioreactor culture chamber fitting in an oscillating perfusion bioreactor providing 3D cardiac constructs with a bidirectional interstitial perfusion and electrical stimulation, allowing direct cellular optical monitoring and contractility test. The chamber design was optimized through finite element models (fluid dynamics and electrical) to ensur e a uniform perfusion speed, a uniform electric field and an efficient current density pa ssing throughout the scaffold.

The chamber, mainly realized in PDMS, was equipped with AISI 3 16L stainless steel electrodes and with two glass windows for high definition imaging (Fi .1a-c). The presence of tworound arrays of pillars allows to hold the scaffold during interstitial perf usion (culture) and to rele ase it for contractile performance evaluation (electrode pacing) within the chamber. The complete bioreactor supports up to 18 constructs in parallel, cultured independently, optically assessed, and test for maturation without interrupting culture.

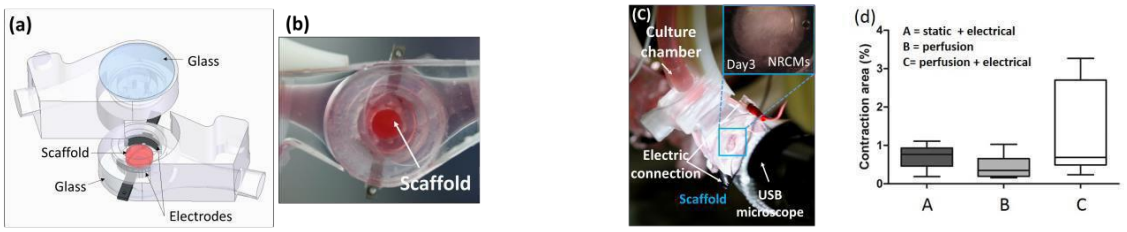

Figure 3: Bioreactor chamber sketch (a) and prototype (b) allowing optical inspection of collagen scaffold seeded with cardiomyocytes (c). Scaffold fraction area changes during pacing test.

Neonatal rat cardiac fibroblasts seeded on collagen scaffolds (8mm diameter; 2mm thickness) were subjected to a combination of bidirectional perfusion (speed 100µm/s) and electrical stimulation (5V/cm, 2ms, 1Hz) showing positive cell viability over time. Furthermore, the maturation of neonatal rat cardiomyocytes culture up to seven days in the bioreactor chambers was successfully assessed by evaluating construct beating performances (spontaneous beating\pacing tests) and key protein gene expression (i.e. cTnI-T, Cx43, α\β MHC). Perfusion together with electrical stimulation enhanced construct beating, as evaluated measuring scaffold border displacement during contraction (Fig.1d).

This new modular bioreactor, providing cells with a relevant biomimetic environment, offers the possibility to independently culture, real-time monitor and test each single constructs.

P246 New pu-based scaffold for 3D-mimicking model of engineered cardiac tissue

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Three-dimensional (3D) tissue models can be used in early stage research to study the efficacy, safety, and mode of action of therapeutic agents as also as novel cell-based therapeutic treatment for myocardial diseases. For engineering cardiac muscle, instead of trying to mimic the complexity of the whole organ, a realistic goal would be the design of a scaffold able to replicate the tissue-specific architecture and mechanical properties, so that it recapitulates the main physiological functions. This work is focused on design and biological test of innovative PUR scaffolds mimicking cardiac tissue properties.

A porous PUR-based scaffold, with a Young modulus comparable to native tissue, was synthesized starting from poly(e-caprolactone) diol (Mn=2000 g/mol), 1,4-butane diisocyanate and L-lysine ethyl ester and processed by TIPS technique. Scaffold functionalization was performed by plasma treatment with acrylic acid and the grafting of fibronectin. XPS and contact angle measures were performed scaffold characterization. Primary neonatal cardiomyocytes deriving from Sprague-Dawley rats were seeded on the biomimetic scaffold and their colonization, survival and beating activity were analysed for 14 days with histological analysis (haematoxylin and eosin staining), CellTiter Blue assay and time-lapse video, respectively. Signal transduction pathways (western blot of PI3K/AKT and MAPK) involved in cell survival as well as gene (RT- PCR of natriuretic peptide and endothelin system) involved during structural development of the heart have been analysed and compared to cardiomyocytes plated on culture plate.

Results of XPS analysis confirmed the surface immobilization of the fibronectin by changes in both chemical composition and C1s signal deconvolution. Fibronectin immobilization led to a reduction in the contact angle value, according to hydrophobic properties of fibronectin. Staining showed the presence of multiple cell layers on the scaffold surface. Cell viability showed a high and stable value as also cell beating until day 14. Results for signal transduction demonstrated that the phosphorylation of AKT and ERK1/2 was higher in cardiomyocytes cultured on the PUR scaffold compared to those cultured on plastic plates. No statistical differences were observed for RT-PCR analysis between scaffold and plastic surface at different culture time and the only significance was observed for BNP mRNA expression that resulted to be lower after 14 day on scaffold surface.

Analysis of results highlighted a porous-aligned structure in PUR scaffold, highly functionalized with fibronectin, resulting in a good adhesion of cardiomyocytes, with strong viability and long-time beating activity. Signal transduction, crucial for mechanical stretch sensing and cardiomyocytes survival, is increased while BNP show a physiological significant decrease.

Tissue engineering is an attractive approach to build in vitro models that can recapitulate various physiological functions and may serve as representation of tissue development, regeneration and disease progression. This work, focusing on the mimicking of tissue architecture and mechanical properties, could represent a promising approach for cardiac tissues modelling.

P247 Pathophysiological mimicking in-vitro model of young and aged pu-based scaffold for cardiac aging studies

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Heart is a dynamic organ subjected to various mechanical signals, which modulate transduction pathways, gene expression and protein synthesis. Aging leads to its decreased functionality undergoing progressive structural changes. In vitro cardiac tissue engineering, trough the use of scaffolds able to favour cell adhesion and survival, is a promising tool for identification of aging-related molecular mechanisms. Aim of this work is to show a new approach focused on tissue-specific architecture and mechanical properties mimicking of young and aged tissue (scaffold) integrated with mechanical stimuli (loading) to generate an in-vitro pathophysiological model of cardiac aging.

Young and aged artificial tissues were produced by polyurethane (PUR) and polyurethane-polycaprolactone blend, respectively. The polymer blends were studied to simulate the aged muscle, which is stiffer compare to the young one. Polymer scaffolds were produced by Thermal Induce Phase Separation to obtain oriented fibres texture like cardiac tissues. Scaffolds surface was functionalized with fibronectin, one of the main components of cardiac Extracellular Matrix.

Primary neonatal cardiomyocytes from Sprague-Dawley rats were seeded on young and aged scaffold and cultured for 7 days. For mechanical tests, scaffolds were placed in SQPR bioreactor and subjected to a cyclic loading stimulus (1Hz) for 24 hours. To mimic ischemic pathology, a hypoxia/reperfusion protocol (3 hours of hypoxia followed by 2 hours of media reperfusion) was applied. Cell viability with CellTiter Blue assay was evaluated. Natriuretic Peptides (NPs) and Endothelin (ET-1) system mRNA expression, to evaluate cardiac phenotype, and Connexin (CX)-43, to confirm cellular interaction by gap junction formation, were measured byRT-PCR.

Results showed a good viability in static and after mechanical loading stimulation in SQPR. An increased expression of ANP/BNP in parallel to a reduction of CNP mRNA levels in young scaffold with respect to old ones were observed in static condition. An activation of NPR-A and NPR-B were also found. After mechanical stimulation, ANP and BNP trend significantly decreased in old scaffold with respect to young ones ($p<0.0001$/$p=0.0008$, respectively) and, on the contrary, CNP was significantly higher ($p=0.011$) with a counter-regulation of NPR-B. At the end of hypoxia/reperfusion protocol, an acceptable reduction of 30% in cell viability was observed. During I/R, only CNP was up regulated in SQPR bioreactor scaffold. ET-1 mRNA was higher in old scaffold while CX43 mRNA decreased. During I/R CX43 mRNA levels resulted significantly higher in SQPR bioreactor scaffold with respect to static conditions ($p=0.0028$) and plastic surface ($p=0.014$).

Aging is associated with a progressive decline in numerous physiological processes, leading to an increased risk of health complications and disease: this process has a remarkable effect on the heart causing an increase in CVD including atherosclerosis, hypertension, myocardial infarction and stroke. The engineered model described in this work, thanks to integration of structural properties and mechanical stimuli, furnishes a new approach to study in-vitro cardiac aging.

P248 Development of xenogeneic, self-organizing small-caliber vascular grafts

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Objectives:

Small-caliber synthetic vascular grafts (< 6 mm) for the use of coronary bypass or peripheral vascular repair are unsatisfactory. We have developed *in vivo* tissue-engineered autologous small-caliber vascular grafts, named "Biotubes", which is constructed by a novel concept of regenerative medicine. We have reported Biotubes withstood systemic blood pressure and exhibited excellent performances as small caliber vascular prostheses in animal models. However, as it takes 4 weeks to fabricate Biotubes, they cannot respond to emergency surgery. Also, in order to flexibly respond to various types of surgery, it is ideal that grafts of various diameters and lengths are readily available for use in advance. The objectives of this study are to fabricate off-the-shelf small-caliber vascular grafts using xenogeneic animals.

Methods and Results:

Silicone rod molds (diameter: 2mm, length: 20 mm) were placed into subcutaneous pouches of beagle dogs, and after 4 weeks the implants with their surrounded connective tissues were harvested. Biotubes with internal diameter of 2 mm were obtained as tubular connective tissues from the implants after pulling out the impregnated molds.

Biotubes were perfused with 1% sodium dodecyl sulfate (SDS) for 12 h, deionized water for 15 min, 1% Triton-X for 30 min, and 500 U/ml of DNase in phosphate-buffered saline (PBS) for 24 h. After decellularization, total DNA content was less than 50 ng/mg dry tissue weight, which was considered as complete removal of genetic material. Decellularized biotubes were stored in PBS at 4 degree for 1 week. Deccellularized biotube grafts were transplanted to the abdominal aorta of the rats. After implantation, neither antiplatelet, anticoagulant nor immunosuppressive agents were administered. After implantation, the rats survived without any signs of abnormal inflammation or immunological problems due to the xenogeneic material. After 1 month, echocardiography revealed no stenosis of the grafts. Histological evaluation revealed that grafts formed neointima on the luminal surface and graft walls had cell infiltlation.

Conclusions:

The xenogeneic decellularized biotube functioned as a small caliber vascular graft as well as an autologous biotube. With this technology, grafts with any form could be fabricated using xenogeneic animals in advance and stored for a significant period, which satisfy the condition for off-the-shelf grafts.

P249 Si-HPMC/Si-Chitosan hybrid hydrogel for cartilage regenerative medicine: from in vitro to in vivo assessments in nude mice and canine model of osteochondral defects

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Articular cartilage may be affected by many injuries including traumatic lesions that greatly predispose to osteoarthritis (OA). Because of the increase in life expectancy, such OA- associated cartilaginous disorders are nowadays a serious public health issue. Currently there is no long-term clinically efficient cure for cartilage lesions and total joint replacement remains often the ultimate procedure to alleviate painful symptoms. In this context, new strategies for regenerating articular cartilage are contemplated with a growing interest. Among these innovative strategies, tissue engineering (TE) is propably one of the most promising. TE is based on the association of cells with a biomaterial capable of supporting cell growth and differentiation. To address the clinical issue of cartilage TE, we have recently developed an injectable, self-hardening and mechanically reinforced hydrogel (Si-HPCH) composed of silanised hydroxypropymethyl cellulose (Si-HPMC) mixed with silanised chitosan. The *in vitro* cytocompatibility of Si-HPCH was tested using human nasal chondrocytes (hNC) or human adipose stromal cells (hASC) with a live and dead assay kit. The *in vivo* biofunctionality of our hydrogel was then determined by implantation in nude mice subcutis. Six different conditions were implanted: Si-HPMC or Si-HPCH alone, Si-HPMC or Si-HPCH mixed with hNC and Si-HPMC or Si-HPCH mixed with hASC. Samples were collected 6 weeks after implantation and characterized by immunohistochemistry. Si-HPCH was then tested for the repair of calibrated osteochondral defects performed on the medial femoral condyle of twelve 4-year old beagles. 6 defects were filled with Si-HPCH alone or mixed autologous ASC. As negative control, 2 additional defects were left empty. Four months after implantation, histological and immunohistological analyses were performed. Our data demonstrated that Si-HPCH supports hNC and hASC viability in 3D culture. Si-HPMC or Si-HPCH also allowed the maintenance of hNC and hASC viability in the subcutis of nude mice. Subcutaneous explants of hNC showed the formation of cell clusters surrounded by a cartilage- like extracellular matrix (ECM). ECM was more abundant with Si-HPCH than with Si-HPMC. Interestingly, the explants containing hASC showed the presence of a low but significant number of cells expressing chondrogenic markers. In the canine osteochondral defect model, the two hydrid constructs tested provided with satisfactory clinical results. While the empty defects were only partially filled with a fibrous tissue, defects filled with Si-HPCH with or without ASC, revealed a significant osteochondral regeneration. In empty defects, the repair tissue was thus mainly composed of fibroblast-like cells expressing type I collagen and no chondrogenic marker had been detected. On the contrary, in the defects filled with Si-HPCH, whatever the presence of autologous ASC, the ECM of the repair tissue was positively stained for type II collagen and aggrecan. To conclude, Si-HPCH is an injectable, self-setting and cytocompatible hydrogel able to support the formation of a cartilage-like tissue in nude mice subcutis when implanted with chondrocytes. Interestingly, this hydrogel also supports the regeneration of osteochondral defects in dogs when implanted alone or with ASC. Taken together, these data make Si-HPCH a promising candidate for the cell-free regeneration of articular cartilage.

P250 The fate of meniscus-implants - is their clinical performance dependent on the grade of degradation?

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Introduction

Lesions of the meniscus are relevant risk-factors for the development of osteoarthritis. Therefore, the regeneration of meniscus would represent a potential preventive strategy for osteoarthritis. However, currently available implants do not fulfil all the high demands placed on them. An important requirement for the improvement of these implants would be a better understanding of the regenerative niche as well as that of the microenvironment of the implantation site. Since the meniscus represents a highly organised anatomical structure with an inner avascular "white zone", the intermediate "red-white-zone" and the vascularized outer "red zone" it remains unclear until today what impact pathological changes of the meniscus have on the potential performance of a meniscus-implant. In this context, neither a widely accepted histological scoring system nor a standardized preparation technique allowing the evaluation of the regeneration niche exists.

Materials and Methods

In a retrospective analysis we studied 17 meniscus resections. The specimens were subdivided in three groups (mildly altered, n=10, moderately altered, n=10, severely altered, n=10). For evaluation histological (H & E) and histochemical staining (safranin-O-, alcian-blue staining) were performed. The slides were analysed with a view to different parameters of available scoring systems (IKDC, Mankin, Fründ).

Results

The parameters known from the evaluation of hyaline cartilage, such as "number of cells", "irregularities of the surface", and "swelling" are also relevant for evaluation of menisci. In addition, parameters like fibrillations, mucoid-degeneration or the vitality of chondrocytes were analysed. The higher the grade of alterations, the higher was the amount of fibrillations, mucoid-degeneration and of avital chondrocytes .

Discussion

The combination of elements of the Mankin-score and the newly determined parameters result in an appropriate histological score for the evaluation of menisci with a proper description of the microenvironment even with respect to the different anatomical zones of the meniscus. From our findings it seems that a high grade of degradation results in an unfavourable environment for meniscus implants, a hypothesis which is being tested in an ongoing study.

P251 Infant chondrocytes from polydactyly patients as a new cell source for cartilage engineering

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Clinically juvenile and infant chondrocytes have a number of advantages compared to adult chondrocytes. First, they have great chondrogenic potential due to their ability to synthesize higher amount of cartilage-like extracellular matrix (Smeriglio et al. 2015). Second, juvenile chondrocytes produce all the autocrine morphogens which are necessary and sufficient for tissue regeneration (Adkisson et al., 2001). Lastly and importantly, juvenile chondrocytes are considered immune privileged as they do not elicit immunogenic responses in patients even in the absence of immune suppressive therapy. It was proposed that juvenile chondrocytes actively inhibit T cell proliferation (Adkisson et al. 2010).

This study evaluated the use of infant chondrocytes from polydactyl patients as a new source of cells for articular cartilage repair techniques. Infant chondrocytes were isolated from articular cartilage of young patients undergoing the surgical removal of supernumerary digits. The native tissue was assessd histologically revealing high levels of collagen 2, glycosaminoglycans (GAGs) and lubricin with no presence of collagen 1 and 10. The cells were isolated by enzymatic digestion and expanded up to passage 5. Infant chondrocytes had a stable expansion profile, with a proliferation doubling rate significantly higher than adult chondrocytes. Infant chondrocytes expressed high levels of chondromodulin 1, and no CD80 and CD86, confirming their immune protected status. In addition, the cells were highly chondrogenic in vitro both in centrifuged pellets culture and in a newly developed 3D hydrogel made from hyaluronic acid modified with transglutaminase peptide substrates (HA-TG). After 3 weeks of culture in HA-TG, the cells re-expressed high levels of collagen 2, without upregulating collagen 1 (Figure 1). Moreover, they produced a great amount of cartilage-like matrix, able to homogeneously fill the hydrogels (Figure 2).

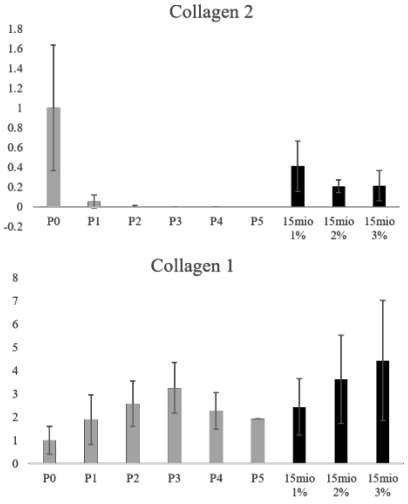

Figure 1: Collagen 2 and 1 expression of infant chondrocytes from passage 0 (native tissue) to 5 and after 3 weeks in vitro in HA-TG gels (at p3)

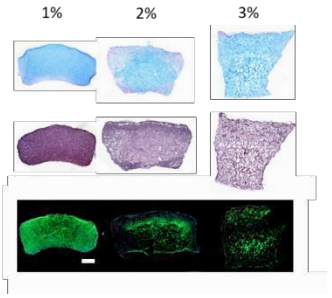

Figure 2: Alcian blue (first row), Safranin-O (second row) and collagen 2 staining of 1, 2 and 3% HA-TG gels after 3 weeks in vitro. Scale bar: 100µm.

In conclusion, infant chondrocytes represent a viable source of cells for cartilage engineering applications.

P252 GDF-5-augmented fibrin glue to improve osteochondral integration of tissue engineering constructs in cartilage defects

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Background: Long-term regeneration of cartilage defects often fails because of insufficient integration of tissue engineering constructs into the host tissue. We here investigated whether growth and differentiation factor-5 (GDF-5)-augmented fibrin glue is promising to enhance bonding of cartilage constructs to bone tissue and thus improve subchondral integration.

Methods: Expanded human bone marrow stromal cells (BMSCs) were embedded in fibrin glue and subjected to in vitro chondrogenesis with TGF-β with or without 150 ng/mL GDF-5 before constructs were implanted subcutaneously into SCID mice. Bonding of cartilage constructs to bone was tested in vivo with hydrogel and collagen constructs that were glued onto bone disks with fibrin glue augmented with 900 ng GDF-5.

Results: BMSCs treated with GDF-5 in chondrogenic medium in vitro expressed ACAN and COL2A1 and deposited proteoglycans and collagen II at similarly high levels as controls. Importantly, the mineralizing enzyme alkaline phosphatase was significantly increased by GDF-5 compared to controls, but collagen X production remained unchanged. Pellets pre-treated with GDF-5 mineralized faster in vivo and formed more bone. In our integration model, GDF-5 strongly supported formation of calcified cartilage by grafted BMSCs and increased tight bonding of this connecting zone with bone tissue (figure 1).

Conclusions: The pro-chondrogenic and hypertrophic activity makes GDF-5-augmented fibrin an attractive bioactive glue with a high potential to increase bonding of cartilage constructs to subchondral bone. Thus, GDF-5-augmented fibrin glue promises to overcome lacking integration of cartilage constructs into the host tissue and facilitate long-term regeneration of cartilage defects.

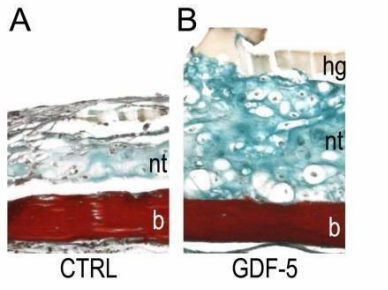

Figure 1: GDF-5 induced BMSCs to form in vivo calcified cartilage integrating with bone. Masson-Goldner staining of three-layered constructs consisting of a bone disk (b), fibrin glue with BMSC with carrier (CTRL, A) or 900 ng GDF-5 (B) forming novel tissue (nt), and a cell-free hydrogel (hg) after 7 weeks in vivo.

P253 Arthroscopic airbrushing for cell-based treatment of knee cartilage defects - a preclinical *in vitro* study

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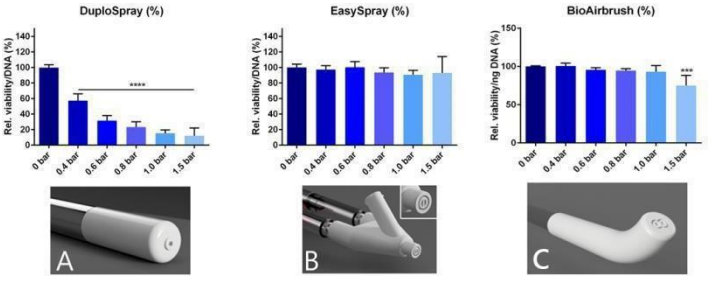
Introduction: Recent research has shown the feasibility of arthroscopic airbrush assisted filling of cartilage defects using fibrin glue [de Windt, 2015]. However, currently available fibrin spray device-nozzles need to be optimized for arthroscopic use. Current changes include internal to external mixing to prevent fibrin clogging and developing a 45-degree tip for increased arthroscopic maneuverability. Our work aims at (I) optimizing current technology with the design of custom-made spray nozzles and (II) at investigating cell viability after spraying at varying pressures and nozzle-substrate distances.

Methods: Custom-made spray nozzles were designed with 3D CAD software and produced by high-resolution 3D printing. Two commercially available fibrin spray devices using an internal mixing nozzle (Baxter® DuploSpray, endoscopic), and external mixing nozzle (Baxter® EasySpray, topical use) were used as a comparison. To study the influence of the spraying pressure on cell viability, human chondrocytes and human mesenchymal stromal cells (MSCs), were sprayed *in vitro* in culture medium under increasing pressure ranging from 0 to 1.5 bar, from a distance of 1.5 cm. This distance was varied to study the influence of nozzle-substrate distance. Cell viability was determined after 24 hrs (AlamarBlue) and normalized to the DNA content (PicoGreen).

Results: Spraying under increasing air pressure (0-1.5 bar) with an internal mixing nozzle (DuploSpray) had a highly destructive effect on cell viability (fig A), while an external mixing nozzle (EasySpray) had a limited effect on cell viability (fig B). Using custom-made external mixing nozzles for the endoscopic DuploSpray system, the effect of increasing air pressure on cell viability of hMSCs and chondrocytes was not statistically significant for pressures up to 1.0 bar (fig C). A short nozzle-substrate distance was shown to have only a minor effect at a distance of 5 mm. Additionally, it was shown that homogenous mixing of the fibrin glue components was achieved using the newly developed system.



Conclusion: The current commercially available spray nozzles are not applicable for arthroscopic cell spraying due to their design or influence on cell viability. However, modification of this technology with 3D-printed custom-made nozzles enabled cell spraying at a short distance without significantly affecting cell viability. This provides an easy-to-use technology for arthroscopic application of (cell-laden) hydrogels as well as a useful tool that can potentially be used in tissue engineering. Ongoing experiments focus on the ability of cells to produce cartilage matrix components after spraying.

P254 Evaluation of cartilage regeneration strategies in an osteochondral ex vivo cartilage defect model: Effect of cell type and oxygen concentration

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Introduction: Degeneration of articular cartilage is a major cause of chronic pain and current treatment options like microfracture or (matrix assisted) autologous chondrocyte implantation result in fibrocartilaginous repair tissue with insufficient mechanical properties at the defect site. Hence, new therapeutic strategies need to be developed for generation of functional repair tissue. Here we used a recently established ex vivo osteochondral model (Schwab A et al., 2016) to evaluate the effect of embedded cell type and oxygen concentration on the production of cartilaginous tissue. Due to its clinical relevance, collagen type I hydrogel was chosen as defect filling material for this comparative study.

Methods: Cylindrical osteochondral explants (8mm diameter; 5mm height) were isolated from porcine medial condyles. Full-thickness defects (4mm diameter) were created with a biopsy punch and filled with autologous porcine chondrocytes (pCHON) respectively a mixture of chondrocytes and mesenchymal stem cells (pMIX), embedded in collagen type I hydrogel. Culture was performed for 28 days under static conditions in normoxia (20% O_2) respectively hypoxia (2% O_2). Samples were analysed histologically for cell viability (live/dead staining) and presence of newly synthesized extracellular matrix proteins. Evaluation of immunohistochemical stainings (Safranin-O-staining, aggrecan, collagen type I, II & X) was done based on the Bern and ICRS II scoring systems with blinded slides. Glycosaminoglycan (GAG) content in hydrogels was also quantified by DMMB assay.

Results: In all approaches, embedded cells remained viable after 28 days culture in ex vivo osteochondral model. Averaged scoring values, obtained from two independent experiments and evaluated by three independent observers ranged from 8.3 (pMIX hypoxia) to 11.8 (pCHON normoxia) [max. possible score: 18]. Higher values were reached in pCHON approaches, compared to pMIX. This finding correlates with GAG content (µg/mg wet weight) analysis. Referring to our results, oxygen concentration has less influence on overall matrix production than cell type embedded in hydrogel.

Impact: The effect of cell type and oxygen concentration on production of cartilaginous tissue was evaluated using collagen type I hydrogel as clinically relevant defect filling material. Best culture conditions will be applied for testing new material-cell-compositions in the ex vivo osteochondral model.

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P255 Effect of spatial PTHrP signalling gradients on human mesenchymal stem cell chondrogenesis and hypertrophy

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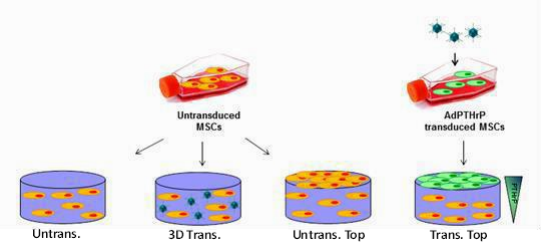
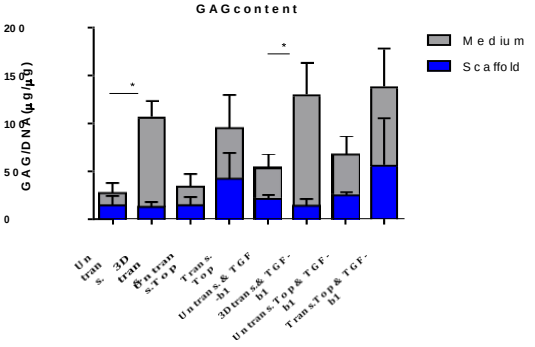
Introduction: Mesenchymal stem cells (MSCs) are considered a promising cell source for cartilage repair strategies due to their chondrogenic differentiation potential[1]. However, the tendency of chondrogenically differentiating MSCs to progress towards hypertrophy limits their clinical use. This unfavourable result may be due to the fact that MSCs used in tissue engineering approaches are all at the same developmental stage, and have lost crucial spatial, temporal and mechanical cues. Parathyroid hormone-related protein (PTHrP) is a factor known to regulate chondrocyte maturity within the developing growth plate[2]. The aim of this study was to examine the effect of PTHrP overexpression and spatial PTHrP signalling gradients on MSC chondrogenesis and hypertrophy.

Methods: $2x10^6$ human bone marrow-derived MSCs (n=4) were transduced with adenoviral vectors overexpressing PTHrP via 3D mediated transduction, and seeded into 8x2 mm fibrin- poly(ester-urethane) scaffolds. To investigate the effect of a spatial PTHrP signalling gradient, scaffolds were seeded with 400,000 pre-transduced MSCs on top with 1,600,000 untransduced MSCs within. Scaffolds were cultured with or without additional 2 ng/ml TGF-β1 for 28 days.





Results: PTHrP was overexpressed by all transduced MSC groups, with levels reaching a maximum at day 7 and reducing by day 28 of culture. PTHrP overexpression significantly increased GAG production and release in to the culture medium compared to untransduced MSCs, irrespective of TGF-β1 treatment (Figure 1, $p<0.05$). Furthermore, constructs containing transduced MSCs positioned on top on the scaffold had increased GAG retention compared to all other treatment groups. In addition to promoting GAG production, transduced MSCs were associated with an increase of endogenous TGF-β1 production and reduced total matrix metalloproteinase-13 secretion compared to untransduced controls.

Conclusion: PTHrP overexpression may support chondrogenic differentiation of MSCs and promote the formation of a stable cartilage phenotype. Additional investigation is required to evaluate the effect of temporal PTHrP signalling on functional tissue formation.

Figure 1. PTHrP overexpression promotes GAG production by MSCs.

P256 A model system to test tissue engineering approaches for intervertebral disc regeneration under cyclic compressive loading

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1McGill Scoliosis and Spine Group, Orthopedic Research Laboratory, Montreal, Canada, 2Department of Surgery, Faculty of Medicine, McGill University, Montreal, Canada Introduction: Intervertebral disc (IVD) degeneration is considered as one of the most common causes of chronic low back pain. Autologous stem cell based therapy is a promising concept to treat early stage degeneration. The ability to differentiate into multiple cell types make mesenchymal stem cells an attractive source to promote the repair processes of IVDs. However, delivery of cells to the affected tissue likely requires a suitable injectable scaffold to enhance viability, promote neo-matrix integration and reduce stress. The purpose of this study is to 1- Evaluate the potential of a (HA-PNIPAM) hydrogel for mesenchymal stem cell differentiation. 2- To asses the effect of mechanical compressive loading on cell differentiation, viability and matrix production.

Methods and materials: Human mesenchymal stem cells were obtained commercially. The potential of mesenchymal stem cells to differentiate into multi-linages was assessed histologically. To assess whether mesenchymal stem cells differentiate towards a disc-like phenotype and produce matrix, we have designed an in vitro, dynamic compressive culture system simulating the disc physiological environment. Mesenchymal stem cells were encapsulated within HA-pNIPAM injectable hydrogel and embedded within custom-made silicone/agarose constructs mimicking IVD structure. These were cultured either without load or under dynamic compression (10% of construct height) for three weeks. Cell viability was determined with LIVE/DEAD assay and DAPI staining, while histological manifestations were assessed using safranin-O staining, and DNA was quantified with HOECHST assay.

Results: The silicone/agarose model can withstand 10% cyclic compressive loading over the 3-week culture period. The bioreactor system proved a consistent around 10% throughout whole culture period. Hydrogels seeded with human mesenchymal stem cells showed over 80% cell viability after 3 weeks of culture. Histological analysis of constructs showed positive proteoglycan staining in both unloaded and loaded cultures. DNA quantification showed no significant difference in cell number between unloaded cells or loaded constructs.

Conclusion: Our results showed that Silicon/agarose constructs could withstand compression within the boundaries of physiological load, which around 10% of construct height. The HA-pNIPAM hydrogel is viable composition to be used as an injectable medium for delivery of cells into degenerate discs. Mesenchymal stem cells have the ability to differentiate and produce nucleus pulposus like matrix in HA-pNIPAM hydrogel. This system can be further used to evaluate various hydrogels, bioactive factors and cell types. It can also be used to study the effect of co-culture of mesenchymal stem cells and nucleus pulposus cells isolated from IVDs of different degrees of degeneration.

P257 Genetical modified equine mesenchymal stem cells for osteoarthritis treatment

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Among cell based therapies, the use of mesenchymal stem cells (MSCs) has great potential for regenerative medicine because of their unique paracrine, differentiation, and self-renewal abilities. Genetically manipulation under standard cell culture conditions can further reinforce their therapeutic potency, making them an attractive target for cellular gene therapy. Hence, the development of systems allowing transgene expression, particularly regulated by natural disease-induced substances is highly desirable and represents a promising field for further research.

Bone marrow-derived equine MSCs were transduced with a lentiviral vector expressing interleukin-1 receptor antagonist protein (IL-1RA) gene under control of an inducible NFkB-responsive promoter. The IL-1RA production was analysed in transduced MSCs upon cytokine stimulation with TNFα and IL-1β. An equine chondrocyte culture-based cytokine model of osteoarthritis (OA) was established using quantitative real-time PCR (RT-qPCR) analysis of disease-specific gene expression. The biological activity of the produced IL-1RA protein and, moreover, the therapeutic effect of IL-1RA-expressing MSCs was assessed in this in vitro model.

In transduced MSCs, dose-dependent increase in IL-1RA expression was found upon TNFα stimulation. This effect, however, was absent upon IL-1β stimulation. Repeated cycles of induction allowed on/off modulation of the transgene expression. Using different experimental settings, IL-1RA protein production from these genetically-modified MSCs changed the expression of OA-specific genes in equine OA chondrocytes and thereby clearly showed their potential for attenuating the OA process.

On demand expression of a therapeutic transgene was induced in genetically modified equine MSCs using naturally occurring inflammatory cytokines. The produced IL-1RA protein is fully active and influences the expression of disease-induced genes in an equine OA in vitro model.

P258 The effect of conditioned medium from human Adipose-derived Stem Cells on Chondrocytes *in vitro*

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Adipose-derived Stem Cells (ASCs) are multipotent progenitors able to participate in tissue regeneration and to modulate immune response. Their regenerative and protective action seems to be mainly mediated by the release of soluble factors and/or extracellular vesicles (EVs). In a recent work we demonstrated that ASC-derived conditioned medium (CM) counteracts neuropathic pain symptoms in different preclinical mouse models (Brini et al., under revision to Scientific Report 2017). Since ASC secretome represents a promising cell-free approach in the treatment of inflammatory pathologies affecting the musculoskeletal tissue, here we investigate its *in vitro* effects on primary human chondrocytes.

ASCs, derived from subcutaneous adipose tissue, were cultured for 72 hours in starving conditions to collect CM, which was then concentrated through Amicon Ultra-15 Centrifugal Filter Unit (Merck-Millipore). CM was concentrated 46±10-folds (n=26) and its trophic effect was tested on primary human chondrocytes (CHs). 9 days of treatment with CM slightly improved CH viability (increase of +10% respect to untreated CHs). In order to mimic articular inflammation *in vitro*, we treated CHs with 1 or 10ng/ml TNFα and then we evaluated CH proliferation up to day 9. Just the higher TNFα concentration determined a clear increase (+40% of control) in CH proliferation, suggesting the induction of a hypertrophic growth status. To determine a possible therapeutic effect of CM administration, we investigated cartilage-specific (ACAN and SOX9), inflammatory (COX2, MMP3 and MMP13) and hypertrophic (type X Collagen) markers. As expected, the inflammatory status induced by 10ng/ml TNFα was characterized by a strong increase in MMP3 and MMP13 gene expression at 24 hours (more than 50 and 10 folds the baseline values, respectively) that was considerably diminished by CM treatment (-50% and -30%, respectively). At later time points (48 and 72 hours), the effects of 10ng/ml TNFα±CM on gene expression became less straightforward. Furthermore, TNFα induced an increase in type X Collagen transcription at 24 hours that was partially counteracted by simultaneous CM administration. We did not observe major differences in the expression of cartilage-specific genes after treatment with TNFα in the presence or absence of CM.

Taken together, our *in vitro* data show the trophic action and the anti-inflammatory potential of ASCs-derived CM and we strongly believe that concentrated CM represents a powerful source of factors with promising therapeutic potential.

P259 Development of a one-stage cell therapy for meniscus regeneration using a combination of meniscus cells, mesenchymal stromal cells and the collagen meniscus implant®

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Introduction and Aim: Damage to the meniscus is a very common intra-articular knee injury in young active patients. Since meniscus tissue has a very limited healing capacity, surgical treatment still consists of partial or subtotal meniscectomy, which after decennia causes early osteoarthritis. To overcome this, meniscal replacement has found a place in clinical practice (i.e.: Collagen Meniscus Implant (CMI®)), providing an environment for ingrowth of cells and tissue formation while the biomaterial is slowly degraded. However, this is a slow process and could be accelerated and improved using a cell-based treatment. Therefore, the aim of this study was to investigate the feasibility of a one-stage cell-based treatment for meniscus regeneration with a combination of meniscus cells and mesenchymal stromal cells (MSCs) using CMI as carrier.

Methods: Human meniscus cells isolated from meniscus tissue retrieved after total knee replacement and bone marrow derived MSCs were cultured in different ratios in cell pellets and type I collagen hydrogels (n=9 donors). The same cell types (n= 5 donors) were seeded in a 10:90 ratio (based on previous results of chondrocytes and MSCs) with 500.000 cells per 150mm ^{3} of CMI in fibrin glue. To mimic clinical circumstances of *ex vivo* pre-seeding and *in vivo* seeding during arthroscopy, dry CMIs and CMIs immersed in culture medium were used and seeded either statically or by injection. All cultures were harvested after 28 days and analysed for glycosaminoglycan (GAG, by DMMB), DNA (by Picogreen) and total collagen (by HYP) content, histology (H&E and Safranin-O) and immunohistochemistry for type I and II collagen.

Results: There is a trend towards more GAG and collagen production in the ratios with a higher percentage of MSCs in both pellets and collagen hydrogels but the observed differences were not significant. A significant difference was found in DNA content between wet and dry seeded CMI's but not for GAG production. In addition, a more homogenous cell distribution was found in dry seeded CMI's and the produced tissue consisted mainly of type I collagen with minimal deposition of type II collagen and proteoglycans.

Impact of investigation: Seeding a CMI with a combination of meniscus cells and mesenchymal stromal cells leads to fibrocartilage-like tissue formation. Static seeding of a dry CMI results in significantly more cells and a homogenous distribution of cells and neotissue. Ratios of 10 : 90 and 20 : 80 meniscus cells : MSCs are clinically feasible for one-stage procedures and perform better than only meniscus cells. The next challenge is how to translates these findings to fit surgical reality since seeding the CMI before arthroscopic implantation seems less practical and creates a considerable loss of cells during implantation and fixation.

P260 Study of the effect of synovial fluid and synovial fibroblasts from patients with OA on the healthy knee joint cartilage and influence of human mesenchymal stem cells on the process of regeneration - in vitro studies

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Osteoarthritis (OA) is characterised by progressive changes and degeneration of joint tissues. Synovial fluid (SF) of healthy joints functions as a lubricant and as a medium for the distribution of nutrients and information molecules within the joint cavity. Injury and inflammation of the joint results in dramatic changes of the ratio of different molecules of the SF. Synovial fibroblasts (SFs) during OA play a significant role in the cartilage destruction process. Mesenchymal stem cells (MSCs) may play a significant role in cartilage regeneration and repair and modulate the joint OA environment by production of different factors with the large scale of biological effects. The aim of our study was to 1) investigate whether SFs and SF from OA patients can initiate and develop process of the degradation of healthy human cartilage (HC) *in vitro* and 2) observe the *in vitro* effects of hMSCs on the process of HC degradation caused by SFs and SF. HC was obtained from healthy tissue donors (Articular Engineering, LLC, USA), synovial membrane (SM) from OA patients (grade IV) during surgery and SF from OA patients (grade I - II) keeping conditions of patient informed consent and Ethical committee approval. MSCs were isolated from subcutaneous adipose tissue (ASCs) and SFs from SM also respecting and fulfilling the Ethical committee requirements. ASCs and SFs expanded in vitro were characterised by flow cytometry. SF (diluted 1:10) was added to SFs, culture media were collected for multiplex molecular analysis. RayBio® Quantibody Human Array was used to characterise media collected after 10 days of SF and SFs co-culture. HCs (5 mm diameter) were embedded in agarose gel and cultured in DMEM/F12 supplemented with 2% ITS-A and 1% ATB as previously described [1]. Degradation was performed for 2 weeks by co-cultivation of HC with: 1) SFs, 2) diluted SF and 3) combination of SFs and SF. After 2 weeks cultivation, ASCs were added on HCs for subsequent 2 weeks. Degradation of HCs and the effect of ASCs on cartilage were observed by scanning electron microscopy (SEM). OA synovial fluid demonstrated high levels of MMP-1 and MMP-3. Metalloproteinase inhibitors 1 and 2 (TIMP-1 and TIMP-2) as the protective mediators were also highly expressed. Activation of SFs by SF was confirmed, 10 days after co-cultivation, levels of IL-6, IL-8, MMP-1 and MMP-3 were increased compared to levels from culture medium of SFs without OA SF. SEM analysis has confirmed the most significant changes in upper layer of cartilage in case of co-cultivation of HC with SFs co-cultivated with OA SF. ASCs have formed smooth layer on the surface of degenerated HC. We conclude that ASCs had extremely positive effect - in the sense of regeneration on degenerated cartilage *in vitro.*

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P261 Chondrogenesis study in rabbit cartilage cells using gellan-gum/silk-microfiber hybrid scaffolds

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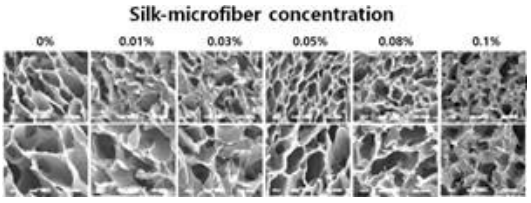
INTRODUCTION: Tissue engineering refers to regeneration of new tissues by transplanting cells into living tissues by culturing cells on a scaffold that is completely absorbed in vivo and has affinity for cells. Scaffolds play a very important role in tissue engineering because they provide tolerable environment for cell growth, proliferation & differentiation.

In this study, gellan gum, which is a natural polymer material and silk-microfibers was used to study the development of scaffolds that can induce similar regeneration of cartilage tissue as a study of scaffolds for cartilage regeneration.

METHODS: Gellan gum (GG)/Silk-microfibrers scaffolds 0, 0.01, 0.03, 0.05, 0.07, 0.1 at different wt% of were produced by boiling and cooling methods. All Samples characteristics were studied by FTIR, compressive strength, porosity and SEM (Fig. 1). Also, chondrogenic differentiation of rabbit cartilage cells cultured in scaffold was evaluated by MTT assay, RT-PCR, SEM.

RESULTS: We found that with increase in silk-microfibers content increases, the porosity in the scaffolds, and compressive strength was decrease. In vitro studies showed that cell proliferation and chondrogenic differentiation were increased with rising silk-microfiber content. Notably, cell proliferation and chondrogenic differentiation are highest in 0.1wt% gellan gum/silk-microfibers scaffolds (Fig. 2).



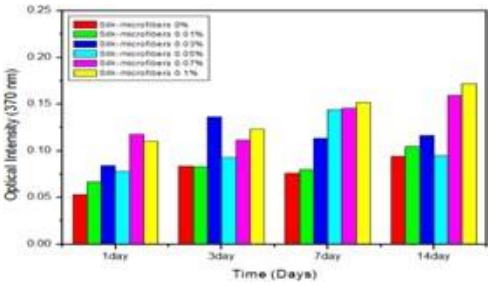


Fig. 1: SEM images of Gellan-gum/Silk-microfibers hybrid scaffolds

Fig. 2: Chondrogenic differentiation and cell proliferation of Rabbit cartilage cells in Gellan-gum/Silk-microfibers hybrid scaffolds was analysed by MTT assay after 1, 3, 7 and 14 days.

DISCUSSION & CONCLUSIONS: This study showed the highest cell proliferation and chondrogenesis in 0.1wt% GG/Silk-microfiber scaffold.

P262 BMP signaling in chondrocyte differentiation of embryonic stem cells

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Chondrocyte differentiation is regulated by various signaling pathways especially bone morphogenic protein (BMP). The balance between BMP levels/activity and its antagonists are likely to influence signaling and chondrocyte differentiation. Human embryonic stem cells (ESCs) can be induced to differentiate toward chondroprogenitors by sequential addition of factors including BMPs as previously described (Oldershaw et al., 2010). The aim of this study was to investigate the influence of BMP antagonists during the directed chondrogenic differentiation protocol. Transcription levels of key cartilage associated extracellular matrix molecules and molecules which play roles in regulating both extracellular and intracellular BMP signaling were investigated at different stages. The results showed that transcripts for extracellular antagonists especially noggin (*NOG*) and twisted gastrulation (*TSG*) were gradually upregulated, reaching their highest levels at day 14 of the protocol. *NOG* was barely detected at the pluripotent stage but started to increase around day 8 while *TSG* was stably expressed from day 0 to day 7 before being upregulated. Increase in BMP antagonists and their distinct patterns of gene expression during the protocol may affect BMP2 activity during chondrogenic differentiation. To further investigate the influence of BMP antagonists on ESC-chondrocyte differentiation, either 1-fold, 5- fold and 7.5-fold molar excess noggin or 10-fold molar excess TSG were added during the protocol from day 9-14. The results demonstrated that 7.5-fold molar excess noggin significantly downregulated *COL1A1* expression compared to control while transcripts of other chondrogenesis-associated genes including *SOX9*, *COL2A1*, and *ACAN* were not significantly different. TSG had no effects on the expression levels of *SOX9*, *COL2A1*, *ACAN or COL1A1* even at 10-fold molar excess, compared to control. Noggin and TSG effects on BMP induced phosphorylation of Smad1/5/9 were analyzed by western blotting. The result showed that noggin and TSG inhibited BMP2 activity. Our study indicates the regulation of BMP signaling by use of specific BMP antagonists at optimal concentration is necessary for generating chondrocyte from ESCs. Understanding the role of noggin and twisted gastrulation in ESC- chondrocyte differentiation and manipulating them may lead to the optimization of procedures for generating chondrocyte from ESCs for cell therapy or understanding disease.

Oldershaw, R. A., Baxter, M. A., Lowe, E. T., Bates, N., Grady, L. M., Soncin, F., . . . Kimber, S. J. (2010). Directed differentiation of human embryonic stem cells toward chondrocytes. *Nat Biotechnol, 28*(11), 1187-1194. doi:10.1038/nbt.1683

P263 Regenerative tissue after matrix-associated chondrocyte implantation has better quality using amplified chondrocytes compared to synovial derived stem cells in a rabbit model with short-term follow-up

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Background

The autologous chondrocyte implantation is an accepted method for the treatment of full-thickness cartilage defects. However, known problems regarding donor site morbidity and the 2-step design make the search for cell alternatives ongoing. Aim of the study was to test the potential of synovial derived stem cells (SMSC) to regenerate cartilage using a matrix-associated chondrocyte implantation.

Methods

Despite a differing phenotype, both cell types were able to form cartilage in-vitro. In an osteochondral defect model of the medial femoral condyle in rabbits, a collagen type I/III membrane was seeded with either amplified allogenic chondrocytes or SMSC and then transplanted into the lesion. A tailored piece synovial membrane served as a control. Besides macroscopic and histological evaluation, the regenerated tissue was examined biomechanically analyzing thickness, instant and shear modulus.

Results

The evaluation of the macroscopic degree of healing using the ICRS score and the area of healing did not show differences between the groups after 6 weeks. Moreover, the thickness of the regenerated tissue was higher in all intervention groups than in natural cartilage, but there was no difference between the groups. However, the instant and shear modulus, reflecting the biomechanical strength of the repair tissue, was superior in the implantation group using chondrocytes. This correlated with the histological analysis. The regenerated tissue after matrix-coupled chondrocyte implantation had less cells, a more chondrogenic structure and expressed more proteoglycans stained with Safranin-O.

Conclusion

Regenerated cartilage using undifferentiated synovial derived stem cells for matrix-associated implantation in a defect model in rabbits did not show a comparable or higher quality than the current standard utilizing amplified chondrocytes.

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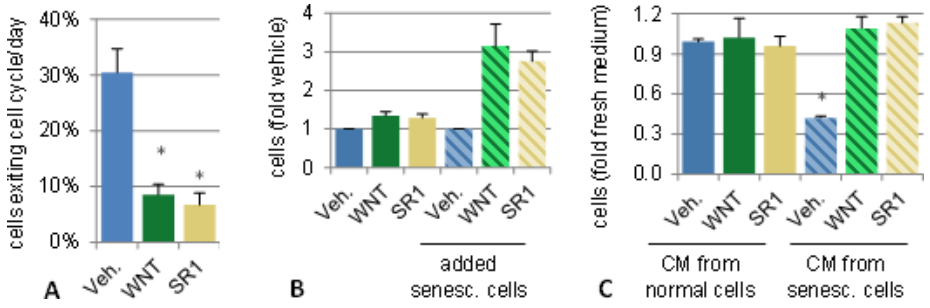
INTRODUCTION: Multipotent Stromal Cells (MSCs) are interesting for tissue engineering of bone, cartilage, muscle, tendon, fat and haematopoiesis-supporting stroma. They can be derived from bone marrow, however upon in vitro expansion MSCs rapidly cease proliferating and lose the ability to differentiate. Here we show that deterioration of MSCs in vitro is due to senescence spreading rapidly via paracrine signalling, but preventable by activating WNT signalling or inhibiting the aryl hydrocarbon receptor (AHR) pathway.

METHODS: Human bone marrow MSCs were expanded with WNT3A or AHR antagonist SR1, their vehicles or medium conditioned by senescent MSCs (CM). Over the culture period, MSCs were harvested for ClDU/EDU proliferation assays, senescence-associated beta-galactosidase staining or for differentiation into cartilage, fat and bone.

RESULTS: In vitro, MSCs rapidly became senescent, but WNT3A or SR1 treatment significantly slowed entry into senescence (A). In cultures with few senescent cells neither WNT3A nor SR1 affected proliferation rate, but when senescent MSCs were introduced, the effect of WNT3A and SR1 was restored (B). While proliferation was unaffected by the secretome of non-senescent MSCs, the secretome of senescent MSCs, unless they had been WNT3A or SR1 treated, abrogated proliferation. (C).Concurrent with reducing senescence, WNT3A and SR1 maintained proliferation and differentiation capacity.



FIGURES: **A** Cells proliferating (ClDU incorporation) at day one, but not (lack of EDU incorporation) at day two in cultures with WNT3A, SR1 or their vehicles. *n=3, SD, *p<0.005* **B** Expansion of low senescence MSCs cultured with vehicles, WNT3A or SR1 without (solid bars) or with addition of MSCs rendered senescent by irradiation (striped). *n= 2, SD* **C** Expansion of MSCs in medium conditioned (CM) by non-senescent MSCs (solid bars) or by senescent MSCs (striped) treated with vehicle, WNT3A or SR1. *n=3, SD, *p<0.005.*

CONCLUSIONS: We found that factors secreted by senescent MSCs induce senescence in surrounding cells, triggering a chain reaction that renders the entire culture senescent. We identify a previously unknown role for WNT and AHR signalling in modulating paracrine senescence and thus tools to facilitate MSC-based regenerative tissue engineering.

P265 Observe limb development to improve cartilage tissue engineering: selecting chondroprogenitor cells from adult MSCs by using *TWIST1* expression

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Introduction: Mesenchymal stem cells are clinically promising to repair and regenerate damaged cartilage. However, an incomplete understanding of the mechanisms that regulate chondrogenic differentiation impedes clinical progress. It is known that during limb development the expression of the transcription factor *TWIST1* is kept high in proliferative mesenchymal progenitors and then, following mesenchymal condensation, *TWIST1* expression decreases with the progression of the chondrogenic differentiation. By using classical differentiation assays and an innovative method for sorting living cells based on gene expression, we investigated the role of *TWIST1* in human bone marrow-derived MSC (BMSC). We aim to verify whether during their chondrogenic induction, the regulation of *TWIST1* was similar compared to the pattern described during limb development, and then to propose a new strategy for the use of BMSC for cartilage repair.

Methods & Results: Significant downregulation of *TWIST1* was observed in adult human BMSCs (N=7 donors) between day 1 and day 21 of chondrogenic induction in classical pellet cultures (~3-fold). However, silencing of *TWIST1* expression in BMSCs by specific small interference RNA (siRNA) prior chondrogenic induction did not improve chondrogenic differentiation potential, measured by thionin staining, glycosaminoglycan content and gene expression for the chondrogenic markers *COL2A1* and *SOX9*. Additional investigation of *TWIST1* expression over time during chondrogenic differentiation revealed that the observed downregulation of *TWIST1* in chondrogenic BMSCs started at day 3, but was preceded by initial upregulation (day 0 to day 1). Similar initial upregulation of *TWIST1*, associate with the pellet formation was also observed in non-chondrogenic BMSCs (N=5 donors). In these BMSCs, however, we did not observe subsequent *TWIST1* downregulation during chondrogenic stimulation, and they fail to differentiate.

Interestingly, combining the data from all BMSC donors (chondrogenic and non chondrogenic) we observed a significant correlation (p=0.016) between *TWIST1* expression in monolayer prior differentiation and their chondrogenic capacity (measured as *COL2A1* levels at day 21 of chondrogenic induction). To further confirm this observation, we used a novel live-sorting technique based on mRNA expression to separate high/bright *TWIST1*-expressing BMSCs from low/dim *TWIST1*-expressing BMSCs (N=3 donors) and then tested those populations for proliferation and chondrogenic capacity. High *TWIST1*-expressing BMSCs were more proliferative (0.39 vs 0.22 doubling/day), less (pre)senescent (~50% vs ~92% of beta- galactosidase positive cells detected after one further passage in expansion post-sorting) and more chondrogenic after differentiation in pellet cultures (collagen type-2 staining) than BMSCs expressing low *TWIST1*.

Conclusion: We demonstrated that a dynamic regulation of *TWIST1* expression is required for chondrogenic differentiation of BMSCs. A high expression of *TWIST1* in proliferating progenitor cells followed by a downregulation during chondrogenic induction is necessary for chondrogenic differentiation. Selecting high *TWIST1*-expressing BMSCs for clinical applications could significantly improve their efficacy for cartilage repair.

P266 An animal study evaluating the appropriate cell source for autologous meniscus treatment in an early osteoarthritis situation – a comparison between mesenchymal stem cells and meniscal cells

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The meniscus is a tissue located between the femoral condyle and tibial plateau of the knee. It aids in the force transmission, shock absorption, joint stability, lubrication and proprioception of the knee joint. The meniscus is composed of two compartments: an inner avascular region and a vascularized outer zone. Treatment of meniscus tears within the avascular region represents a significant challenge particularly in a situation of early osteoarthritis. Cell based Tissue Engineering approaches have shown promising in vitro results and in vivo animal studies have demonstrated positive outcomes. However, *in vivo* studies have not found a consensus on the appropriate autologous cell source in a clinical situation. The present study sought to evaluate the appropriate cell source for autologous meniscal repair within the avascular region in an early osteoarthritis situation.

Methods: New Zealand white rabbits were used as the animal model for testing autologous meniscal repair, whilst human cells were used to test the *in vitro* model. Rabbits were anaesthetized and bone marrow and medial meniscus was harvested, four weeks prior to surgery. Bone marrow Mesenchymal Stem Cells (MSCs) and meniscal cells were isolated and expanded in culture prior to surgery. Cells were seeded onto collagen-hyaluronan scaffolds and were implanted into the defect site without pre-culture. A punch defect model was performed on the lateral meniscus of the rabbit and then a cell-seeded scaffold was press-fit into the defect. Rabbits were sacrificed after 6 or 12 weeks, gross joint morphology was assessed and menisci were harvested for histological and immunohistochemical processing. Evaluation of macroscopic, histological and immunohistochemical results were evaluated using a validated meniscus scoring system. In an *in vitro* model, human meniscal cells isolated from non-repairable bucket handle tears and human MSCs were expanded and using the pellet culture model, assessed for their meniscus-like potential in a translational setting through collagen type I and II immunostaining, collagen type II ELISA and gene expression analysis.

Results: Following resections of the medial menisci, all knees showed early osteoarthritic changes. However, successful repair of meniscus punch defects was performed using either meniscal cells and MSCs in an animal model. Gross joint assessment demonstrated donor site morbidity for meniscal cell treated defects. Furthermore, MSCs had significantly increased collagen type II gene expression and production and collagen type I immunostaining compared to meniscal cells ($p < 0.05$).

Discussion: Regenerative potential of the meniscus by an autologous cell-based Tissue Engineering approach was shown in an early osteoarthritis situation. MSCs were found to have improved meniscal healing compared to meniscal cells in an animal model in an autologous setting, thus demonstrating its feasibility in a clinical setting. The reason for this intrinsic repair may be related to the differentiation of progenitor cells or the secretion of bioactive factors by MSCs in a cell therapeutic manner. Furthermore, this model may also be used to test other autologous cell sources to test their feasibility for cell-based meniscal repair.

P267 Preparation of in vitro model of osteoarthritis mediated by pro-infalammatory cytokines activated synovial fibroblasts for analysis of therapeutic potential of mesenchymal stem cells

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Osteoarthritis (OA), the most common form of arthritis, is characterized by cartilage breakdown and by synovial inflammation that is directly linked to clinical symptoms such as joint swelling, synovitis and pain. During OA, synoviocytes, mononuclear cells or chondrocytes may increase their expression of catabolic proteins following stimuli such as cytokine or chemokine exposure, including interleukin (IL)-1β and TNF-α, which are present in the joint following synovial inflammation. Pro-inflammatory cytokines are ideal candidates for the experimental in vitro induction of OA-like biological changes in cartilage and synovial cells or tissues, in which temporal and concentration effects can be explored. The objectives of the present study were to standardise *in vitro* model of OA using of cytokine activated synovial fibroblasts for the future possibility of OA treatment by using of adipose tissue derived mesenchymal stem cells (ASCs).

Synovial fibroblasts (SF) were isolated from human synovial membrane taken from patients with OA history during total knee joint replacement and ASCs were isolated from adipose tissue by enzymatic digestion and cultured *in vitro* in α-MEM medium supplemented with 10% foetal bovine serum and 1% of antibiotics/antimycotic solution. SF were activated with IL-1β and TNF- α. Activation was confirmed by fluorescence staining of actine cytoskeleton, by multiplex system - Quantibody Human Array and by flow cytometry. Cartilage discs (CDs) from healthy donor (Articular engineering, LLC, US) were inserted into pre-shaped agarose and SF were cultivated directly on the surface of cartilage discs and medium (with or without cytokines) was changed two time a week. There were 4 groups: CDs in culture medium without cytokines (1), CDs in medium containing IL-1β and TNF-α (2), synovial fibroblasts seeded on CDs in normal culture medium (3) and CDs with SF in medium containing IL-1β and TNF-α (4). After two weeks of cartilage degradation, ASCs were seeded on the surface of CDs and cultivated for another two weeks. Degradation and subsequent treatment of cartilage with ASCs were monitored by SEM analysis and histologicaly. After activation of SF with IL-1β and TNF-α - expression of CD55, CD54, CD106 and TLR3 were up-regulated, actin cytoskeleton was rearranged and levels of degradation enzymes (MMP1, MMP3, MMP13) were increased.

SEM analysis has clearly showed that in group 4 - CDs with SF in medium containing IL-1β and TNF-α - there was the most significant cartilage surface disturbance. Adherence and proliferation of ASCs on degraded cartilage surface were influenced depending on the grade of degradation.

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P268 Donor variability of ovine mesenchymal stem cell differentiation potential - clinical implications for cell therapies

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Introduction: Mesenchymal stem cells are the focus of cell-based repair strategies, but little is understood about the impact of donor variability on their clinical outcome. Due to similarities in size, weight, architecture and healing mechanism sheep animal models enable clinical translation of cell-based orthopaedic treatments. To date, ovine mesenchymal stem cells (oMSC) have not been fully characterized and their differentiation potential is not well understood. This study aims to investigate donor variability and chondrogenic potential of oMSCs *in vitro* for the translation of cell-based therapies for osteoarthritis and cartilage defects. **Methods:** Bone marrow oMSCs were isolated from 12 adult English mule ewes (age group 2-4 years) to assess the donor variation. To assess the differentiation potential of oMSCs for both adipogenesis and osteogenesis in 2D and for chondrogenesis in 3D cell organoids, cells were cultured over 20 days. Donor variation was assessed histologically using Oil-o-Red, Alizarin red and Alcian blue staining respectively and semi-quantitatively for both adipogenesis and osteogenesis, while chondrogenesis potential, was assessed by DMMB assay for GAG production. CD marker expression was studied by flow cytometry. MNP labelled STRO-4 positive oMSCs of five donors were seeded in 3D collagen I gels, cultured for 20 days and subjected to mechanical stimulation in a magnetic bioreactor (MICA) to assess the chondrogenic potential. Simultaneously, native sheep cartilage (6 donors, 8mm diameter samples, n=4) was harvested from femoral distal condyle. Native and engineered cartilage's tissue matrix composition (GAG, total Collagen, Aggrecan, COL II, DNA, total protein) were assessed by histology, and biochemical assays and mechanical testing. **Results:** Our data revealed donor variation in the tri-lineage differentiation and CD marker expression across the all donors. No clear correlation between donors was observed among the three lineages. For example, a donor that was highly responsive during osteogenic differentiation was not necessarily as responsive to chondrogenic or adipogenic differentiation. In addition, mechanical stimulation resulted in enhanced chondrogenesis compared with unstimulated controls indicated by increased GAG production (23.3-28.6 µg GAG/µg DNA) but fell short of native cartilage (73.2-119.4 µg GAG /µg DNA). Variations were observed across the donors. Similarly, there is clear variation in characterisation of native cartilage between different donors. **Conclusion:** oMSCs appear to share similarities in characteristics across individual donors, but at the same time, express differences regarding biological properties that may influence the number, phenotype and *in vitro* biological characteristics. This study investigated the use of 2D and 3D culture to assess differentiation capacity between different donors before successfully transmitted to preclinical animal studies. **Acknowledgements:** Al-Mutheffer was supported by Iraqi Ministry of Higher Education. Special thanks Dr J McLaren from the University of Nottingham for her assistance in collecting sheep bone marrow and cartilage and to Prof El Haj's group in particular Dr Markides.

P269 Influence of the addition of BMP2, BMP6, TGFβ1, TGFβ3 and PTHrP and their combinations on chondrogenesis of stem cells

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Cartilage regeneration is a challenging process. Stem cells may be used. To achieve stem cell chondrogenesis of them, diverse growth factors have been reported. However, during chondrogenic differentiation of stem cells hypertrophy frequently occurs. This study aims to evaluate different growth factor combinations and timing in order to identify superior chondrogenesis inducers and simultaneously suppress hypertrophy in human adipose-derived MSCs (hAMSCs) cultured in a 3D hydrogel matrix. hAMSCs were cultured in a 3D collagen hydrogel ($1.0x10^5$ AMSCs per gel construct). Next, cell seeded constructs were stimulated with different growth factor combinations added at different time points (Figure 1). A combination of TGFβ1/BMP2 or TGFβ3/BMP6 was used for a period of 8 weeks of culture. TGFβ1 and TGFβ3 were supplemented at 10 ng/ml. BMP2 and BMP6 were supplemented at 50 ng/ml. Two weeks after 3D culture with this stimulus, PTHrP (100 ng/ml) was added either immediately or 1 week after interruption of BMP2 or BMP6 stimulation.

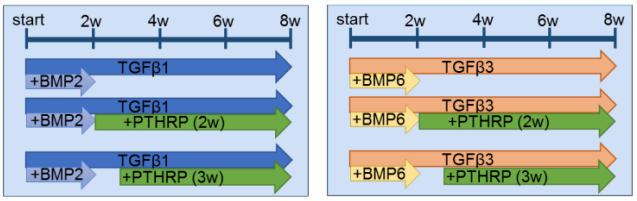

Figure 4 Schematic overview of the supplementation of the different growth factors

Proliferation with the different stimuli was evaluated by dsDNA quantification and GAG content was measured with the Blyscan Kit. Gene expression was evaluated by qPCR for Aggrecan, ALP, BMP-R1 and -R2, Coll I, II, III and X, PTHrP, RunX2, Sox9, TGFβ-R1 and Versican. Finally, the occurrence of chondrogenesis was also evaluated histologically (Alcian blue) and with IHC (e.g. Coll II, Col X). All combinations of BMPs and TGFβ resulted in initial increase of GAGs with a subsequent decrease at 8 weeks of culture. Interestingly, the addition of PTHrP resulted in a significant decrease of GAGs at all times of observation evaluated. Stimulation with TGFβ1/BMP2 resulted in upregulation of versican after 6 weeks with concomitant upregulation of Sox9 at earlier time of observation. This effect was enhanced by addition of PTHrP. Hypertrophy did not occur with all combinations of TGFβ1/BMP2 and PTHrP as indicated by Coll X expression. This effect was even more pronounced when the combinations of TGFβ3/BMP6 with PTHrP were used. Stimulation with TGFβ1/BMP2 resulted in upregulation of BMP-R1 and -R2. This lead also to upregulation of ALP. On the contrary, TGFβ3/BMP6 dowregulated BMP-R1 and resulted in an upregulated of BMP-R2 only after 8 weeks. At that time point, ALP also increased. Histological and IHC evaluations supported these findings.

The combination of TGFβ3/BMP6 is more beneficial for induction of chondrogenesis. Indeed, it even inhibits hypertrophy. The addition of PTHrP induced Sox9 expression. Osteogenesis related genes are not activated or only at a later time point.

P270 Novel markers for quality control of cell-therapeutic approaches to show the cell fate of annulus fibrosus and nucleus pulposus cells from human intervertebral disc

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Introduction: Cell-based therapies become increasingly important for the treatment of intervertebral disc (IVD) injuries. Strategies such as the autologous disc cell transplantation (ADCT) aim to regenerate the affected IVD and to structurally and functionally restore its properties with the patient's own cells. Nevertheless, the patient's safety and the quality of such advanced therapy medicinal products (ATMP) are of first priority. Therefore, the identity of the cells has to be shown before transplantation, to make sure the cells have retained their IVD cell character during the manufacturing process. Although much research was done to find molecules characteristic for IVD cells and to describe their diversity (1, 2), there is still no marker to exclusively distinguish the cells from the two distinct IVD compartments, annulus fibrosus (AF) and nucleus pulposus (NP) (3). The aim of this study was to identify markers to discriminate AF cells from NP cells using gene array analysis of adult human IVD tissue.

Materials & Methods: AF and NP tissue was obtained from 32 donors during cervical spine surgery and the tissues underwent a quality sorting by macroscopic and haptic evaluation as well as histological examination. Only samples with typical AF or NP characteristics, free from foreign tissues and with low degeneration were selected. A total of 11 AF and 9 NP samples of 13 donors (53±10 years, female/male 6/7) were used for gene array analysis (Affymetrix). Quantitative PCR (qPCR) was performed using the LC480 real-time PCR system with UPL Technology(Roche).

Results: The gene array analysis showed that 1.7% of the present probes was differently expressed in AF and NP tissue with a fold change larger than 2 in at least of 80% of the samples ($p<0.05$). There were 5x more probes higher expressed in AF than in NP tissue. We found five genes exclusively present in AF tissue and six genes with significantly higher expression signals in NP tissue. The gene expression profile was verified by qPCR analysis. These novel AF and NP markers will be presented.

Discussion & Conclusions: Although AF and NP tissue have different gene expression profiles, the observed gene expression differences in adult IVD tissue is lower than was expected. However, we identified 11 genes differently expressed in AF and NP. None of the genes was reported before in connection with either AF or NP tissue. These novel markers can be used to test cell identity and are of high value in quality control of ATMPs used for regenerative treatment of IVD diseases.

P271 The efficacy of intra-articular PRP injection following the microfracture technique or local adherent application of adipose-derived stem cells for the treatment of articular cartilage lesions in a rabbit model

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Therapy with platelet-rich plasma (PRP) and mesenchymal stem cells (MSCs) can enhance the process of healing of avascular tissue, so it can be particularly effective in the treatment of osteoarthritis (OA) which is one of the most prevalent diseases of today. However, microfracture is one of the most common techniques used to treat cartilage defects because of its low cost and simple method, it does not properly repair full-thickness cartilage defects. Application of ex vivo expanded MSCs directly into the defect using a minimally invasive method of transplantation by local adherent technique could enhance the adherence of MSCs to the cartilage defect and improve the healing process. PRP was used in combination with both techniques to increase and accelerate the regeneration capacity of MSCs in a rabbit model. Our work investigates the efficacy of repairing articular chondral defect in the rabbit knee joint using two different therapeutic methods prior to autologous PRP injections.

Full-thickness cylindrical defects (3-3.5 mm in diameter, 1.5-2 mm in depth) were created in the medial femoral condyle without traumatizing subchondral bony structures in nine, five month old New Zealand White rabbits (4.65 kg ± 0.20). This animal model was used to evaluate in vivo cartilage repair via microfracture technique (Group 1, n=3) or local adherent application of autologous adipose-derived stem cells (ADSCs) (Group 2, n=3). One week after postsurgery treatment application, autologous PRP (1 mL) was applied by direct intra-articular injection to the injured knee three times in weekly intervals in Group 1 and 2. Three rabbits were not subjected to treatment (Group 3, n=3) and were only used for examination. The defect in control group (Group 4, n=3) was no treated. The knee joints were harvested at 12 weeks after surgery. The outcome was assessed macroscopically, histologically and immunohistochemically. The repairs were scored according to semiquantitative macroscopic scoring system.

At 12 weeks, group 2 showed better defect filling compared with group 1. More hyaline cartilage-like tissue was found in the defects of group 2 at 12 weeks according to the results of histological and immunohistochemical evaluation. We also received significantly better outcome in the ***ADSCs***-treated knees (Group 2) than in the non-treated control knees (Group 4). Intra-articular injection of PRP in combination with transplantation of autologous ADSCs using the local adherent technique enhances the quality of full-thickness cartilage defects repair, increased hyaline-like cartilage formation with better defect filling in comparison with microfracture surgery in a rabbit model. Our in vivo study will advance and extend the clinical application of tested therapeutic option for cartilage injury.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0684-12 and by VEGA grant 1/0217/16.

(P272)

P272 Clinical efficacy of in vitro propagated adipose tissue-derived mesenchymal stem cells (AT-MSC) in the treatment of knee osteoarthritis

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Introduction: Various authors report successful utilization of adipose tissue-derived mesenchymal stem cells (AT-MSC) applied directly in the joint in order to treat degenerative joint disease. Stem cells are used as minimally modified (filtration, separation) or in vitro proliferated in order to achieve therapeutic dosage, and the exact threshold is still unclear.

We present a series of 11 cases diagnosed with knee osteoarthritis, treated with in vitro propagated AT-MSc injection using a single dose of 1-2 million of cells.

Inclusion and eclusion criteria: Patients older than 18 years with Outerbridge stage II-III knee osteoarthritis evaluated by clinical examination, radiographically and by MRI were included in the study. Exclusion criteria were chronic endocrine or neurological condition and history of malignant disease.

Method: A 5 ml sample of subcutaneous fat tissue was taken by small incision, followed by in vitro stem cell isolation and cell culture propagation until therapeutic dose is reached. After harvesting, a suspension of stem cells was then injected into the affected knee joint. Patients are evaluated by four clinical scores (Hospital for Special Surgery Knee Score, Knee Society Score, Tenger-Lysholm Score, VAS of pain), x-ray and MRI prior to injection, and in 6 months interval after the injection. During follow-up, all the patients were instructed not to refrain themselves from regular daily activities, but they were not allowed to take physical therapy or painmedications.

Results: A highly significant improvement of all scores after 6 months was observed:

HSS-KS from 59 ± 12.68 to 92.9 ± 5.26;

KSS from 42.1 ± 15.71 to 88.2 ± 2.23;

TLS from 46.7 ± 20.5 to 97 ± 2.4;

VAS of pain from 5.4 ± 1.65 na 0.9 ± 0.65.

These results are maintained improved throughout the 18 month follow-up period. Analysis of x-ray and MRI data documented a halt of further joint space narrowing.

Conclusions: Adipose tissue-derived mesenchymal stem cells, used in the dose greater than 1 million of cells, express significant clinical efficacy and safety for the treatment of knee osteoarthritis. Further follow-up is needed in order to determine the extent of their therapeutic effect.

Keywords: Stem cell therapy, Knee osteoarthritis, Adipose tissue derived mesenchymal stem cells

P273 MSC-Derived extracellular vesicles stimulate cartilage regeneration and modulate inflammatory responses

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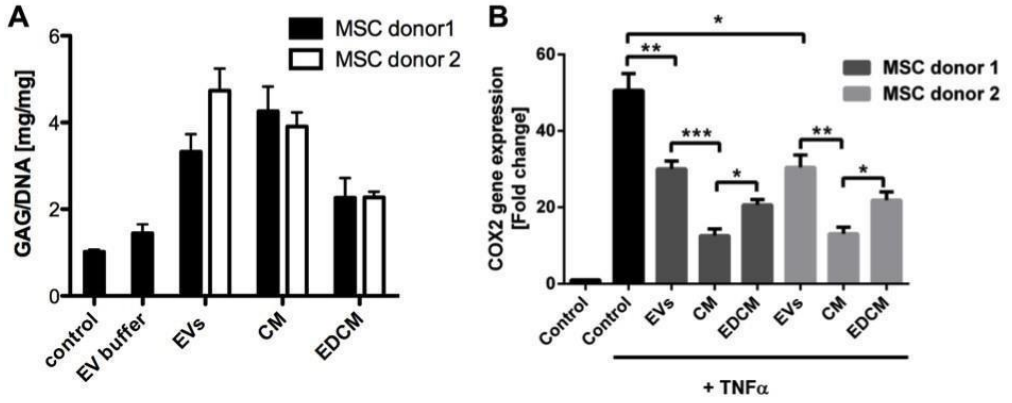
Introduction: Multipotent mesenchymal stromal cells (MSCs) stimulate tissue repair and modulate immune responses by paracrine signaling. This signaling might be mediated by extracellular vesicles (EVs), which are small membrane-enclosed particles released by cells. The aim of this study was to investigate the effects of MSC-derived EVs on cartilage formation by osteoarthritic (OA) chondrocytes and tumor necrosis factor alpha (TNF-α)-induced inflammation.

Methods: MSC-derived EVs were added to regeneration cultures of passage 2 OA chondrocytes in fibrin glue and to monolayers of OA chondrocytes treated with TNF-α. MSC-derived conditioned medium (CM) and EV-depleted CM were used as controls. COX2 gene expression and PGE2 protein levels were measured in the monolayer cultures and culture supernatant, respectively after 48 hours. The regeneration cultures were analyzed after 4 weeks for glycosaminoglycan (GAG) content by DMMB (normalized for DNA as measured by Picogreen assay) and paraffin sections of the regenerated tissue were stained for proteoglycans (safranin-O) and type II collagen (immunostaining).

Results: The matrix deposited by the MSC-EV and MSC-CM treated cultures contained more GAGs and type II collagen, while EV-depleted CM only showed minor effects (Fig. 1A). MSC- derived EVs decreased TNF-α -induced COX2 gene expression and PGE2 production. MSC- derived CM also decreased COX2 gene expression and PGE2 production and the effects were less prominent with EV-depleted CM (Fig. 1B).

Conclusion: MSC-derived extracellular vesicles showed positive effects on cartilage regeneration and demonstrated anti-inflammatory properties in TNF-α -induced inflammation in OA chondrocytes. This was supported by the differences observed between cultures treated with conditioned medium and EV-depleted conditioned medium from MSC. This suggests that great part of the MSC paracrine signaling in tissue repair and immunomodulation is mediated by extracellular vesicles and opens possibilities for the treatment of OA by intra-articular injections with MSC-derived extracellular vesicles.



P274 Development of a mesenchymal stem cell-based aggregate reinforced by fiberized collagen fibrils for soft tissue repair

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Introduction: Mesenchymal stem cells (MSCs)-based self-assembled tissue (scSAT) are a great potential as a repairing material for cartilage tissue. However, the collagen density and amounts of extracellular matrix elements contained in the scSAT are insufficient for clinical application. To solve the problem, we focused on the composition of the MSCs and self-assembled collagen. It is known that self-assembled collagen is obtained when collagen-dissolved acidic solution is neutralized at 37°C. In the present study, we have developed an aggregate consisting of mesenchymal stem cells and collagen fibers as a novel tissue-engineered material. The structural properties of the aggregate were analyzed.

Materials and Methods: Porcine dermal collagen-suspended acidic solution of 3 mg/mL was neutralized by a culture medium. The mixture was incubated at 37°C for 1 day, then the self-assembled collagen was obtained. Meanwhile, MSCs were obtained from the synovial membranes of human knee joints. After 4-time passage, self-assembled collagen and MSCs at the density of 4×10^5 cells/cm^2 were put on a Millicell® cell culture insert installed on a 6-well culture plate. Then, they were dehydrated by centrifugation of 1000 rpm. The aggregate of self- assembled collagen and MSCs (collagen/MSCs) was cultured in a culture medium for 7 days or 14 days. After cultivation, they were observed using an optical microscope after stained by hematoxilin and eosin (HE) and a scanning electron microscope(SEM).

Results and Discussion: Temporal change of gross appearance of collagen/MSCs aggregates were shown in Fig.1. Collagen/MSCs aggregate after 1 day culture attached to and spread on culture surface. At 4 day culture, the aggregates detached from culture surface with a morphological change of shrinking and thickening observed. After 4 day culture, the morphological change seemed to be more remarkable. It should be noted that obtained Collagen/MSCs aggregate was larger than conventional scSAT.

An optical microscopic image and a SEM image of the collagen/MSCs aggregate were shown in Fig.2. It was observed that many MSCs were uniformly dispersed in a well collagen matured fibrous collagen structure. These results suggest that MSCs in the aggregate generated extracellular matrix and self-assembled with circumjacent collagen fibers.

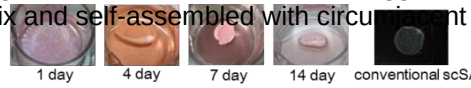

Fig.1 Temporal change of gross appearance of collagen/MSCs aggregate

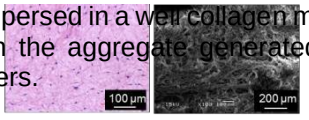

Fig.2. Optical microscopic image after HE-staining (a) and SEM image (b) of collagen/MSCs aggregate.

Conclusions: In this study, we have developed a collagen/MSCs through a MSC culture in collagen fiber-suspended neutralized medium. Due to the centrifugal process during cell culture, the aggregate has abundant MSCs of a high density with a well matured fibrous collagen structure.

P275 Fast screening method for cell recruitment into growth factor modified PEG-hydrogel

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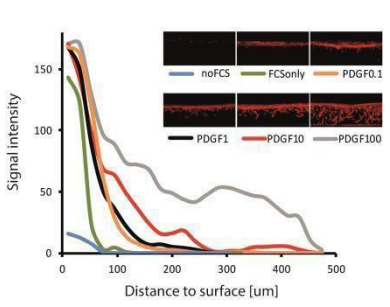
INTRODUCTION: The healing of tissues is a complex process in which recruitment of cells into the wound and matrix deposition play essential roles. In order to better understand the underlying biological processes and to test elements influencing regeneration, it is important to dissect the problem into separate approachable questions and develop in vitro assays for each of them. Here we present a poly(ethylene glycol) (PEG) hydrogel-based 3D-in vitro model that allows to test the recruitment of cells into a healing-inducing material in a relatively high-throughput manner. In this case, we evaluate the effects of the concentration of platelet-derived growth factor (PDGF), known to trigger cell migration.

METHODS: PEG-hydrogels were made in 96-well plates using electrochemistry in order to inhibit hydrogel polymerization on the top of the gel[1]. This inhibition creates a gradient of gel density permitting cells to be seeded on top and migrate into the gel if triggered by the right growth factors.

MSCs were seeded on top of the hydrogel and standard culture media was added with different concentration of PDGF. Each experimental group had five samples. The cells were kept in culture for 3 days without changing medium until fixation of the samples.

Cells were stained with Rhodamin-Phalloidin for actin cytoskeleton and DAPI for nuclear bodies. After that, confocal imaging of 500μm stacks was automated and done on each sample. A lateral view was generated using Fiji 3D-viewer software and signal intensity at each depth was averaged. This enabled to assess the presence MSCs into the gel and evaluate recruitment efficiency depending on the growth factor concentration.

RESULTS: We were able to reproducibly form PEG-hydrogels with density gradients by electro-chemical inhibition of polymerization in a 96-well plate. MSCs showed migration into the hydrogel in a dose dependent manner. The analysis showed that MSCs don't migrate at all into the gel if not activated by any growth factor. However, the penetration depth of the cells increased significantly depending on the concentration of PDGF present in the medium. Both the fabrication of the gel and the analysis were automated, enabling to significantly increase the throughput of the experimental procedure and its analysis.



DISCUSSION & CONCLUSIONS: The combination of advanced biomaterials with confocal imaging enabled us to advance one step towards a faster assessment of cell recruitment. In the more precise context of wound healing, this platform offers a very powerful way to screen for factors and help finding their best dose to optimise recruitment. This is absolutely necessary to understand part of the biological process of healing as well as to build the next complex material triggering wound healing.

P276 Hydrogel matrix stiffness regulates the phenotype of glioblastoma cells

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Stem cell biology provides new insights into cancer biology, thus leading us to the notion that tumors might contain functionally subsets of cells with stem-like properties named cancer stem cells (CSCs). These cells are characterized by chemotherapy resistance and considered one of the key determinants driving tumor initiation and relapse. Glioblastomas are the most common and lethal primary brain tumors of the central nervous system and derived from glial malignancies. Previous literature demonstrated that CSCs of Glioblastomas resides in particular tumor niches that are necessary to support their behavior. Herein, hydrogel materials composed of agarose and hydroxypropyl methyl cellulose (HMC) in different concentrations were prepared to mimic the microenvironments of brain tumor niche for CSCs selection and enrichment.

3D Hydrogel based in vitro system provide environment regarding the role of tissue dimensionality. In addition, hydrogel composed with different concentrations provide stiffness variation, nutrient gradient variety, and oxygen concentration difference to facilitate analysis of glioblastoma CSCs -niche interaction. Viscosities of HMC in different concentrations were measured. Colony formation on series of agarose-HMC hydrogel matrices was investigated. Cell cytotoxicity and cell viability were determined by MTT and LDH assay. CSCs marker expression of colonies culture on hydrogel system was examined by flow cytometry. Drug resistance and cell apoptosis after anticancer drug treatment were evaluated by Annexin V and PI double staining. Colony formation on hydrogel system was observed and the size and number of colonies were increased with time. After four days of culture, the percentage of CD133+ cells on agarose-HMC system show a significant difference in comparison with that on TCPS control group. Furthermore, cells on hydrogel system demonstrate highly drug resistance effect after anti-brain cancer drugs treatment. The successful demonstration of this study may provide a new insight of CSC biology and CSC-niche interaction.

P277 Tuning collagen hydrogel stiffness to enhance the regenerative phenotype of adipose stem cells for peripheral nerve repair

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Following a nerve injury, denervated Schwann cells in the distal part of the nerve adopt a regenerative phenotype and provide support to regenerating axons from the proximal stump. It is therefore potentially beneficial that therapeutic cells for peripheral nerve tissue engineering exhibit a similar regenerative phenotype. Adipose-derived stem cells (ASCs) are an accessible source of adult stem cells that are potential candidates for autologous cell transplantation. These cells have the ability to adopt a Schwann cell-like differentiated phenotype (dADSCs). Previous studies have demonstrated that engineered neural tissue (EngNT) made using dADSCs improved recovery when implanted into a preclinical model of peripheral nerve injury [1]. EngNT involves the self-alignment of cells within a tethered type-I collagen gel, followed by removal of some interstitial fluid to produce a stable tissue-like biomaterial [2]. Initial data indicated that the regenerative phenotype of dADSCs was enhanced in EngNT, with elevated levels of NGF, BDNF, VEGF and GDNF compared to cells grown on standard cell culture polystyrene, the key difference being the mechanical environment. It is well established that the stiffness of the matrix environment can affect cell behaviour [3]. The aim of this study therefore was to investigate this phenomenon further in order to create an optimal mechanical environment for dADSCs.

Collagen gels of varying stiffness were constructed using RAFT™ absorbers and their mechanical properties were measured using dynamic mechanical analysis. Gene expression of markers for Schwann cell phenotype (S100, p75, c-jun, Krox20, MBP) were assessed using quantitative real-time PCR analysis. Understanding the effect of the mechanical environment upon dADSC phenotype will enable constructs to be tuned for optimal regenerative performance in nerve tissue engineering.

P278 Stem cell signaling in 3D environments: hydrogels vs cryogels

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Intro:

Paracrine factors that mesenchymal stromal cells (MSCs) secrete can guide a number of biological processes desirable in regeneration. In an injury environment, MSCs communicate with, and respond to, other cells via paracrine signaling. We investigated whether MSC sense and respond to bioactive factors differently when encapsulated in a hydrogel or seeded on macorporous scaffolds.

Materials & Methods:

Alginate was modified using carbodiimide chemistry to present cell adhesive RGD peptides on the cell-material interface. Two types of substrates were prepared: (1) nanoporous hydrogels that encapsulate MSCs, and (2) macroporous cryogels where cells can infiltrate the interconnected structure. MSCs were isolated from bone marrow of Sprague Dawley rats, and used in passage 2-3. Cells were cultured on the two substrates for two days before being stimulated with 50 ng/mL of VEGF. Conditioned media +/- stimulation was collected after 24 hours, and analysed for secreted factors. The functional effects of the conditioned media was tested on myoblasts by measuring their proliferation and differentiation.

Results:

Our data revealed that MSCs cultured on macorporous scaffolds were more sensitive to the VEGF stimulus compared to those encapsulated in hydrogels. After stimulation, higher amounts of secreted cytokines were detected in the scaffold group compared to the hydrogel group. Furthermore, conditioned media from stimulated MSCs in scaffolds significantly improved myoblast function compared to the hydrogel counterpart. Our data suggests that the propensity of MSCs to respond to soluble cues in the injury environment may be defined by what physical environment they are transplanted in.

Conclusion and Implications:

Various 3D hydrogels with information rich environments have been incredibly successful in directing stem cell fate. However, MSCs are also known for their paracrine function and their ability to orchestrate biological processes. The outcomes of our work imply that MSCs transplanted on porous scaffolds may have an enhanced ability to modulate the injury environment than MSCs encapsulated in hydrogels. Further work using mechanically and/or chemically modified hydrogels may improve our understanding of MSC function and lead to improved regenerative outcomes.

P279 Bone marrow mesenchymal stem cells actively participate in the recruitment of murine hematopoietic stem cells in bioengineered minimalistic bone marrows

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INTRODUCTION: Sufficient numbers of hematopoietic stem cells (HSCs) needed for bone marrow transplantations remains a major challenge in medicine today. HSCs expanded *in vitro* rapidly lose their regenerative capacity likely due to the lack of niche-derived signals comprising molecular and cellular components [1]. Identification of critical hematopoietic niche components necessitates both the generation of more tractable *in vivo* models and, in parallel, novel approaches for heightened throughput in screening such systems *in vivo*. We have previously reported on a blank biomaterial that can be decorated with different growth factors and cell types known to be present in the niche *in vivo* [2]. Here, we present a minimalist human bone marrow model based on a synthetic material, and have applied it for use in discovery of critical HSC niche factors.

METHODS: Functionalized biomimetic polyethylene glycol (PEG) hydrogels were laden with human mesenchymal stem cells (hMSCs) and supplemented with or without bone morphogenetic protein-2 (BMP-2). These PEG gels were polymerized directly in the individual wells of novel multiplexing polydimethylsiloxane (PDMS) devices. Next, the screening devices containing PEG hydrogels were subcutaneously implanted in immunocompromised mice. At 8 weeks, the devices were explanted and analysed for bone and bone marrow formation by microCT, histology and FACS. Moreover, human cells were retrieved from the gels and further examined by quantitative RT-PCR.

RESULTS: Screening devices featuring 2mm diameter wells were shown to be the minimal size for niche formation allowing 8 conditions to be screened per implantable site (32 unique conditions per mouse). MicroCT analysis revealed mineralization in all wells containing gels with hMSCs, with or without BMP-2. Histological analysis corroborated these findings. Hydrogels containing hMSCs and BMP-2 developed into bone marrow-like constructs including a typical bone shell filled with marrow and trabecular bone structures. Ultimately, long-term HSC enrichment in the constructs containing cells and BMP-2 compared to only BMP-2 gels was confirmed by FACS analysis on the recruited murine cell population. Intriguingly, qPCR of retrieved hMSCs revealed heightened expression of osteogenic markers in all samples irrespective of BMP-2 addition. These results indicate the formation of a functional ectopic niche in cell-laden and fully synthetic hydrogels.

DISCUSSION & CONCLUSIONS: An implantable screening device was developed to optimize hydrogel conditions, cell type, and soluble factors to support bone marrow niche formation *in vivo*. Results indicated a direct participation of hMSCs in both bone formation and murine HSCs recruitment. This device represents a powerful new tool for heightened *in vivo* screening of tissue engineering constructs with a broad range of applications.

P280 Sequential synthesis of modular poly(ethylene glycol)–peptide hydrogels for controlling stiffness, degradability and biomolecule presentation in 3D artificial stem cell niches

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Introduction On 2D substrates, matrix stiffness[1], degradability[1,2] and biomolecule presentation[1–3] are implicated in directing mechanotransductive processes that influence mesenchymal stem cell differentiation. The shift over the last decade to 3D cell culture models such as hydrogels has played an important role in elucidating the effect of stiffness under more *in vivo*-like conditions, better representing the dimensionality of most tissues. However, the effects of degradability and the clustered arrangement of cell adhesion ligands in 3D remain poorly understood. In the present research, sequential reactions were used to form modular poly(ethylene glycol) (PEG) hydrogels in which it is possible to form clusters of biomolecules, enabling individual or combined control over matrix stiffness, degradability and biomolecule presentation in 3D. **Methods & Results** Custom-synthesised peptides containing or lacking adhesive and/or degradable sequences and with N-terminal Lys (amine) and C-terminal Cys (thiol) groups were first deprotected using 1.5 molar equivalence triethylamine in anhydrous dimethylsulfoxide for 2 h. They were then conjugated to four-arm PEG–nitrophenyl carbonate (synthesised according to ref. 4) via the amine group for 30 min. *DL*-dithiothreitol was used to cleave disulphide bonds between thiol groups, prior to lyophilisation. The conjugate was rinsed in pure ethanol, sonicated and centrifuged three times, in order to remove 4-nitrophenol and *DL*- dithiothreitol impurities. Hydrogels were then formed via addition of four-arm PEG–vinyl sulfone via the thiol group in 50 mM HEPES pH 8.2. Hydrogels with a broad range of stiffnesses were successfully synthesised via this sequential approach, by varying PEG MW or total solid content. Biomolecule presentation was varied by incorporating different proportions of each peptide. **Discussion** Due to their bioinertness, non- immunogenicity and low protein adsorption, PEG hydrogels are ideal for studying effects of ECM properties on stem cell differentiation in 3D. Although diverse and highly innovative PEG hydrogels have been developed over the last 15 years[2], incorporated biomolecules such as cell adhesion ligands or growth factors have typically been conjugated as "pendants", preventing cross-link formation at those sites and therefore reducing orthogonality. The use of sequential reactions to pre-form biomolecule "clusters" which are then cross-linked, without changing other properties such as stiffness and degradability, is a novel concept. The use of peptide cross-linkers containing branched biomolecules overcomes this issue, enabling variation of one or multiple properties with minimal effects on others. These materials are now undergoing biological evaluation to assess the effects of matrix stiffness, degradability and biomolecule presentation on adhesion, spreading and differentiation of encapsulated mesenchymal stem cells in 3D via immunohistochemistry and gene expression analysis. **Acknowledgements** NJW acknowledges Jane & Aatos Erkko Foundation, The Company of Biologists, Journal of Cell Science and Royal Society of Chemistry for funding.

P281 Regulation of mitochondrial transfer in osteocytes

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Osteocytes imprisoned in the mineralised bone matrix are the key coordinator of bone formation and bone resorption and mineral metabolism. To reveal the mystery on how osteocytes are coordinated each other in regulation of bone homeostasis and why they can live a long life within the bone matrix, we reason that osteocytes may rely on mitochondrial transfer to compensate each other on their energy consumption in respond to stress and thus coordinate the balance of cellular activities. Here using real time florescence confocal imaging we showed dynamic mitochondria transfer between osteocytes through their dendritic network. By using two- dimensional co-culture and established three dimensional tetraculture system, we showed that functional dendritic mitochondria preferentially transferred into GC stressed adjacent osteocytes to attenuate the level of accumulated ROS. Administration of glucocorticoid (GC) significantly impeded the distribution and motility of dendritic mitochondria in osteocytes. Specifically, GC stressed ER was accumulated onto mitochondria, and the stress factor MKP1 which induced by GC translocated onto ER-mitochondria contact site to negatively regulate mitochondria function by inducing mitochondrial fission and inhibiting mitochondria membrane potential. Collectively our data demonstrated for the first time that dendritic mitochondrial transfer occurs between osteocytes within their dendritic network to potentially maintain the osteocytes homeostasis, and GC induced MKP1 excessively impeded this processes by regulating ER-mitochondria function.

P282 Long term human skin graft: gold standard model formimic constructs and its capability to react after pressure mechanicalstimulation

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Introduction: Developments in regenerative medicine and tissue engineering have given rise to human skin substitutes as alternatives to skin grafts. However, research in this field has been hindered by a lack of experimental animal models. This study examines dermal extracellular fibrillar proteins in a long-term mouse model of human full-thickness skin graft (hFTSG) before and after applying mechanical pressure.

Material and Methods: Immunosuppressed NOD/Scid mice (n=10) were engrafted with human skin of dimensions 4x3 cm. After 60 days as a permanent graft, a pressure ulcer (PU) was created in the human skin using a compression device. Three study groups were established: native human skin, hFTSG and hFTSG-PU. Evaluations were conducted with visual and histological assessment. In a detailed histological examination, the extracellular matrix protein profiles of samples from each group were compared by immunohistochemical staining for tropoelastin, collagen I and III, fibulins, and lysil oxidases (LOX) among others.

Results: In the native human skin group versus hFTSG group, significant differences were observed only in labeling for tropoelastin, while hFTSG and hFTSG-PU differed significantly in terms of collagen I, III, LOX and fibrillin-1 expression. Protein distributions except for those of fibulins 4 and 5 varied significantly between the native human skin group and the hFTSG-PU group.

Conclusions: Our human skin graft model on mouse revealed specific histologic patterns in the dermal extracellular matrix and dynamic changes produced in response to mechanical pressure injury. Future bioengineering constructs should mimic these characteristics.

Keywords: tissue engineering, human skin graft, extracellular matrix constructs, skin constructs.

P283 Local growth hormone therapy on a human skin mouse model produce ulcer healing after

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Introduction: Pressure ulcer (PU) is a problem of high importance which entails social, health and economic impact. The growth hormone, anabolic hormone par excellence, is involved in skin homeostasis and wound healing. We hypothesize whether it is possible to improve PU healing by locally applying the recombinant human growth hormone (rhGH) in a human skin mouse model.

Material and methods: Non-obese diabetic/severe combined immunodeficient (NOD/Scid) mice (n=10) were engrafted with full thickness human skin graft of 4x3 cm. After 60 days with stable grafts, human skin underwent three cycles of ischemia-reperfusion with a compression device to create a PU. Mice were classified in two groups: rhGH treatment group (n=5) and control group (n=5). In the rhGH group four local intradermal injections, each of 0.15 mg (0.5IU), were applied to the PU edges, once per week for four weeks. Evaluation of the wound healing was conducted with photographic and visual assessments and the healing rate was calculated. Histological analysis was performed after complete wound healing.

Results: The results showed a healing rate twice as fast in the rhGH group compared to the control group (1.25 ± 0.33 mm^2 per day versus 0.61 ± 0.27 mm^2 per day; p-value < 0.05). Kinetics was compared between groups, with faster healing rate during the first 30 days in the rhGH group (1.27 mm^2 vs. 0.37 mm^2 - time in which the hormone was administered once per week). The rhGH group showed thicker skin (1953 ± 457 µm versus 1060 ± 208 µm; p-value < 0.05) in repaired area, with a significant decrease of collagen type I/III ratio.

Conclusion: Local administration of the growth hormone (rhGH) accelerates PU healing in our model. The rhGH may have a clinical use in pressure ulcer treatment.

Keywords: growth hormone; pressure ulcer treatment; wound healing; human skin graft.

P284 Extracellular matrix (ECM) homeostasis in cultures of non-epithelial vaginal cells (NEVCS) from mice with and without pelvic organ prolapse (POP)

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Introduction: Pelvic organ prolapse (POP) is characterized by the downward descent and abnormal protrusion of pelvic organs such as the uterus, bladder, and vagina. Lysyl oxidase-like 1 knockout (LOXL1 KO) mice reliably prolapse with age and increased parity, similar to women. Due to the role of LOXL1 in elastic fiber assembly/crosslinking, we hypothesized that POP results from aberrant ECM and elastin homeostasis in the vaginal wall. We therefore investigated the effects of LOXL1 KO, parity, and POP on *de novo* ECM biosynthesis in NEVC cultures.

Materials and Methods: Whole vaginal tissue was harvested from wild type nulliparous mice (WT, n=5), LOXL1 KO nulliparous mice (N, n=3), and LOXL1 KO multiparous mice with POP (MP, n=4) and without POP (MNP, n=4). NEVCs were isolated by enzyme disassociation and cultured for 21 days at passages 2-4. Cultures were then assayed for elastic matrix (Fastin assay) and DNA content (Hoeschst dye assay) to estimate elastic matrix produced/cell. RT-PCR compared gene expression of key proteins regulating ECM/elastic matrix assembly including tropoelastin (ELN), collagen 1a (COL1A), matrix metalloproteinases-2 & -9 (MMP2, MMP9), tissue inhibitor of MMPs-3 & -4 (TIMP3, TIMP4), fibulin-5 (FBLN5), fibrillin-1 (FBN1), lysyl oxidase (LOX), transforming growth factor beta-1 (TGFB1), and bone morphogenetic protein -1 (BMP1). Western blots compared protein expression for COL1A, TIMPs-1, -4, MMPs-2, -9, LOX, TGF-β1, and BMP-1.

Results: A significant decrease in elastin synthesis was noted with LOXL1 KO ($p<0.001$) and parity ($p<0.001$), but not with POP, suggesting changes to ECM structure rather than elastin amounts in mice with POP. There were a greater number of significant differences in gene expression than western blot data. Conserved significant differences across both assays were increases in MMP-2 production by MNP cells relative to both N ($p<0.01$) and MP cells ($p<0.05$).

Discussion and Conclusion: The increase of MMP-2 with parity highlights birth as a probable trigger for pelvic floor ECM degradation. Reduced MMP-2 production in prolapsed mice suggests a diminished ability to initiate the remodelling process, resulting in cumulative matrix deformation and eventual POP. Ongoing studies are investigating the elastic matrix morphology of vaginal explants via histology to assess structural differences between POP and NON-POPtissues.

P285 Vascular grafts for in situ tissue engineering based on elastin-like recombinamers

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Introduction.

The increasingly high incidence of vascular diseases has resulted in numerous efforts to engineer vascular bioartificial substitutes [1] and current research strives to develop cell-free systems able to support cell infiltration and remodeling once implanted in the body [2]. Such approach, referred to as in situ tissue engineering, entails multiple advantages when compared to cell-based systems, namely: ready availability, easy storage and transport and potentially faster clinical adoption.

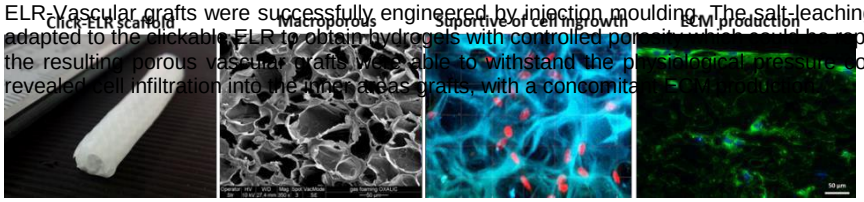
In this work, we developed macroporous vascular grafts for in situ tissue engineering by using Elastin- Like Recombinamers (ELR) [3], a novel class of recombinant biomaterials with tailored biocompatibility, elastic properties and low thrombogenicity.

Materials and methods.

Two ELRs bearing the reactive groups needed to form hydrogels via a click reaction (cyclooctyne and azide) were used [4]. Salt-leaching gas foaming procedure was adapted to create macroporous scaffolds. Dual syringe systems were used to inject the two components in a mould system for the fabrication of vascular grafts with inner diameter of 3 mm. SEM and Two Photon Microscopy were used to investigate the porosity. Cellular studies were carried out with smooth muscle cells (SMCs) isolated from human umbilical veins. Scaffold infiltration and extracellular matrix (ECM) production was investigated by immunohistochemistry. The functionality of the vascular graft was assessed in a bioreactor under physiological conditions. Mechanical properties were evaluated by burst strength measurements.

Results.

ELR-Vascular grafts were successfully engineered by injection moulding. The salt-leaching gas foaming technique was adapted to the clickable ELR to obtain hydrogels with controlled porosity which could be reproducibly tuned. Importantly, the resulting porous vascular grafts were able to withstand the physiological pressure conditions. The cellular studies revealed cell infiltration into the tunica media grafts, with a concomitant [illegible]



Conclusions.

We have successfully addressed the engineering of macroporous ELR-based scaffolds, here proposed for vascular endogenous tissue generation. Notably, the materials and fabrication techniques used here can be employed for a variety of applications in the field of regenerative medicine. Specifically, the capability of creating a desired porosity that supports cell infiltration makes this system of high interest for the development of cell-free systems for in situ tissue engineering.

P286 Antibody-based targeting of matrix-regenerative nanoparticles for abdominal aortic aneurysm wall repair in a rat model

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Introduction: Abdominal aortic aneurysms (AAAs) are rupture-prone aortal expansions resulting from chronic proteolytic breakdown and lack of regeneration of elastic fibers in the aorta wall. We previously reported on the significant pro-elastin regenerative an anti-proteolytic properties of cationic amphiphile-surface functionalized, doxycycline (DOX)-releasing PLGA nanoparticles (NPs) and attributed these effects to both the amphiphiles and released DOX. In this work, we sought to improve specificity of NP targeting by tagging them with antibodies (Abs) that bind to cathepsin K, a serine protease highly overexpressed within AAA tissue. The efficacy of this targeting mechanism was evaluated in vitro, ex vivo cell/tissue cultures and in vivo in an elastase-injury model of AAAs in rats.

Materials and Methods: We compared NP binding, elastic matrix amounts and crosslinking, elastic fiber formation, and proteolytic activity between cytokine-activated cultures of rat aneurysmal smooth muscle cells (SMCs) supplemented with cathepsin K-Ab-modified and un modified DOX-NPs, and investigated transport of the NPs past an activated endothelium in an in vitro model. Enhanced uptake of Ab-modified NPs into the AAA wall was examined following tail vein injection of the NPs ex vivo and confirmed upon tail vein infusion of NPs in the rat AAA model. The NPs were found to be retained at the AAA site for at least 1 week.

Results: Covalent conjugation of cathepsin K Ab to the DOX-NPs enhanced NP binding to AAA SMCs and maintained levels of DOX release, augmented elastic fiber assembly and crosslinking and diminished proteolytic activity. Ab-modified DOX-NPs showed enhanced uptake into the wall of matrix injured vessels. Passive transport of the NPs across the leaky, activated endothelium was enhanced by targeting NPs to αvβ3-integrins overexpressed by these cells.

Discussion and Conclusion: This study has shown that Cathepsin K Ab conjugation is a useful targeting modality for our pro-regenerative NPs. Ongoing studies are investigating safety and biodistribution of these NPs in the rat AAA model, data which will guide studies to investigate their therapeutic efficacy in achieving regenerative repair of the AAA wall matrix.

P287 Matrix regenerative immuno-nanoparticles to improve the lung tumor microenvironment

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Introduction: Non-small cell lung cancers (NSCLCs) are a leading cause of mortality. Response rates to new immune-checkpoint inhibitor therapies are <20%, likely due to a Tumor Micro Environment (TME) that favors immune evasion and pro-tumorigenic phenomena such as macrophage polarization from a pro-inflammatory (M1) type to an immunosuppressive and pro-tumorigenic (M2) phenotype. The TME is also compromised by chronic enzymatic breakdown of alveolar elastic matrix to generate elastin peptides that can promote M1 to M2 phenotypic switch. Preventing elastolysis and stimulating elastin regenerative repair can thus benefit immunotherapy outcomes. This is challenged by poor elastogenicity of adult cells. To address this, we are developing nanoparticles (NPs) designed to a) inhibit proteolysis, b) stimulate elastogenesis, and c) reduce macrophage polarization.

Methods: Biodegradable poly(ethylene glycol)-poly(lactic glycolic acid) (PEG-PLGA) NPs encapsulating doxycycline (DOX) an MMP inhibitor were fabricated via a double emulsion solvent evaporation method, using different cationic amphiphile surfactants, which functionalized the NP surface. Antibodies targeting interleukin 4 receptor (IL4R), to which IL4 binds on M1 macrophages to in turn prompt their switch to an M2 phenotype, were covalently tagged to the NP surface for targeting. NP size and charge (Dynamic Light Scattering and Phase Analysis Light Scattering), NP degradation (Mass loss, SEM), DOX release profiles (spectrophotometry) as a function of NP dose and DOX loading were characterized, as also targeted binding of the NPs to M1 macrophages and their exclusion in the extracellular space. Inhibition of macrophage polarization by exogenous and NP-released DOX, the latter in the presence and absence of IL4R targeting were studied (immunofluorescence, cytokine array). Blank NP and IL4R Ab-DOX-NP stimulatory effects on elastic matrix synthesis, crosslinking, fiber formation, and inhibition of matrix metalloproteases 2 and 9 in lung fibroblast cultures were assessed by a Fastin assay, ELISA for desmosine, TEM, and gel zymography respectively.

Results: NPs formulated with 1% w/v DMAB, a cationic amphiphile exhibited sizes of 200-200 nm that ensured their retention outside cells and non-clearance by phagocytosis. Steady state DOX release was achieved in the low micromolar range at which DOX exhibited significant pro-elastogenic, anti-proteolytic, and anti-polarization effects, and continued for at least 30 days in vitro, at which time ~28% of theoretically loaded DOX was released; steady state release of DOX from the NPs depended on DOX loading (65-90%) and NP concentration (0.5 mg/ml); NPs, surface modified with DMAB showed significant pro-elastin crosslinking, anti-elastolytic and elastin binding properties, that were independent of but synergistic with the effects of DOX. IL4R Ab conjugation to the NPs ensured their targeted binding to M1 macrophages and showed evidence of providing an additional deterrent to macrophage polarization as stimulated by exogenous IL4.

Future work: Future studies will investigate targeted delivery, retention, safety of IL4R Ab-modified NPs to the TME in a C57BL6 mouse tumor model and efficacy as an adjuvant therapy to improving immunotherapy outcomes.

P288 Superior elastic matrix regenerative properties of bone marrow mesenchymal stem cell-derived smooth muscle cells are maintained in a 3D collagenous milieu

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Introduction: Abdominal aortic aneurysms (AAAs) are localized expansions of the abdominal aorta resulting from chronic proteolysis of medial collagen and elastin. Poor elastogenicity of adult SMCs challenges restoration of elastin homeostasis in the AAA wall, necessary to arrest or regress AAA growth. We previously identified specific phenotypic subtypes of SMC progenitors (cBM-SMCs) differentiated from rat mesenchymal stem cells (BM-MSCs) that were significantly more elastogenic than healthy adult SMCs (RASMCs) and BM-MSCs, and also uniquely provided pro-elastogenic and anti-proteolytic effects to aneurysmal SMCs, all in 2D culture. In this work, we investigated the ability of these derived cells to maintain their unique phenotypic characteristics in a 3D collagenous tissue culture milieu, evocative of the de- elasticized AAA wall, and further their matrix regenerative properties and pro-elastogenic/anti- proteolytic effects on co- cultured aneurysmal SMCs.

Methods: Acid solubilized type-I collagen was mixed with 5×DMEM and 0.1N NaOH to titrate the mixture to neutral pH and cBM-SMCs, RASMCs, BM-MSCs alone and 1:1 ratio of all of these cells with aneurysmal SMCs (EaRASMCs) were added to the above solution to obtain a final concentration 2mg/ml of collagen, 250k cells/ml, 20% v/v serum and 1% v/v Penstrep in DMEM-F12. After gelation, they were cultured in 10% media for 21 days and contractility was measured using Image J. IF staining was done on the cryosection of the gel for elastin homeostasis marker and MAGP for terminal differentiation in-vivo. Morphometric analysis was done for quantifying the density of elastin produced and multiphoton imaging technique was used for qualitative assessment of the matrix on paraffin embedded, modified HART stained sections. Expression level of MMPs was confirmed using western blotting and desmosine crosslinks were quantified using ELISA.

Results: Quantity, quality, contractility and crosslinking of elastin was significantly augmented in cBM-SMC-seeded cultures compared to other cases. Also the significantly higher expression of MAGP-1 shows these cells are terminally differentiated in-vivo. Higher contractility and greater amount of structurally intact elastin production in co-culture of this cell with EaRASMCs in 3-D further confirms its significance in elastin regenerative repair. Co-culture of the cBM-SMCs with EaRASMCs significantly improved elastic matrix deposition, mature fiber formation, and attenuated matrix metalloprotease (MMP 2&9) production and activity, with increases in elastic moduli.

Discussion and Conclusion: Our study strongly supports the utility of cBM-SMCs for cell therapy aimed at restoring elastic matrix homeostasis in the AAA wall, which is being investigated in rat AAA models.

P289 Development of humanised 3D liver metastasis of pancreatic origin: the role of tissute-specific exctracellular matrix in tumor progression and chemoresistance

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Background and aims: Over 50% of patients with pancreatic cancer are diagnosed at the metastatic stage and die because of the debilitating metabolic effects of their unrestrained growth. Despite efforts in the past 50 years, conventional treatment approaches have had little impact on the course of this aggressive neoplasm. Therefore, the development of new treatment strategies to control cancer metastases is of immediate urgency. Fulfilment of this difficult task relies on our knowledge of the cellular and molecular biology of both primary and metastatic pancreatic cancer and the use of relevant 3D extracellular matrix models will certainly help define individual and collective aspects of this complicatedprocess.

Methods: Our model is based on the utilisation of decellularised human livers (n=4) and pancreases (n=4) that have previously been characterised for cellular material elimination and preservation of extracellular matrix (ECM) proteins and micro-architecture. Both metastatic tumour cells (PK1, derived from a liver metastasis from pancreatic origin) and primary pancreatic tumour cells (PANC1) were seeded onto 5 mm3 liver and pancreas scaffolds, as well as 2D culture systems. Histological analyses were used to confirm cell attachment and migration. Further, gene expression after 7 and 14 days was evaluated by qRT-PCR. Additionally, AlamarBlue assay was performed to test chemo-resistance in both 2D and in 3D scaffolds upon treatment with 0.5 µM of Doxorubicin and Gemcitabine.

Results: Behavioural and molecular differences were observed between cancer cell lines in the different decellularised tissues. PK1 metastatic cancer cells were able to exclusively migrate and invade the liver scaffolds, and only attached superficially onto the pancreatic scaffolds. Whereas, PANC1 primary cancer cells were able to migrate and invade the pancreas scaffolds but only attached superficially onto the liver scaffolds. These differences were corroborated by significant deregulations in gene expression, for MMP9, COL1A1, TIMP1, WNT1 and β-CATENIN between 3D scaffolds and 2D cultures. Interestingly, both primary and metastatic cells were found significantly more resistant to treatment with Doxorubicin and Gemcitabine in the 3D models when compared to the same treatment on 2D cultures ($n=4$, $p<0.001$).

Conclusion: Our results suggest that primary and metastatic pancreatic cancer cells manifest a conserved invasive behaviour depending on the 3D ECM structure of origin. Moreover, there is an evident alteration in cell response to different cancer chemotherapy in the presence of a natural ECM niche. These observations provide a proof of concept for the development of an effective bio-engineered model characterised by a well-defined 3D ECM microenvironment.

P290 Engineered 3D vascularized skeletal muscle environment inducing the muscle-specificity of the endothelium

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The generation of functional vascular structures represents a key aspect to promote the integration of the engineered tissue within the host body but also to analyze the complex interactions occurring between the vasculature and the tissue of interest [1]. In this field, several attempts were performed to biofabricate vascularized skeletal muscle constructs [2]. However, these models lacked similarity with the human physiological muscle tissue architecture, which is characterized by long fascicle-like structures covered by a continuous and fibroblasts-rich endomysium and surrounded by a structured vasculature. In addition, no *in vitro* model was able to induce the organ-specificity of the endothelium, a feature only described by *in vivo* observations. For these reasons, there is an urgent requirement to develop novel models recreating the complex skeletal muscle environment in order to analyze the heterogeneous interactions contributing to the muscle homeostasis. Here we employed a meso-scale system [3] to enable casting of three non-planar bundles (600 um diameter) of differentiated human muscle cells next to each other and subsequent addition of human endothelial cell (EC)-laden hydrogels to form a millimeter scale, physiologically relevant vascularized muscle model (Fig.1A). Computational simulations of oxygen consumption/diffusion demonstrated that the oxygen level was above the critical threshold for cell survival. ECs self assembled into patent microvascular networks which surrounded and interacted with muscle bundles (Fig.1B). Noteworthy, homogeneously dispersed muscle fibroblasts progressively migrated towards the muscle bundles and finally adhered to the outer part of the bundles generating a sheath enveloping the fiber (Fig.1C) and resembling the *in vivo* localization of muscle fibroblasts. Surprisingly, similar results were not obtained when muscle cells were dispersed within the matrix rather than organized into bundles. Finally, single cell populations were collected for gene expression studies. Besides the standard endothelial markers, we demonstrated that ECs in contact with the muscle acquired organ-specific features with closely mimic the phenotype of *in vivo* myosin. We believe the future exploration of this new model for regenerative medicine applications and development of new therapies for muscle disease.

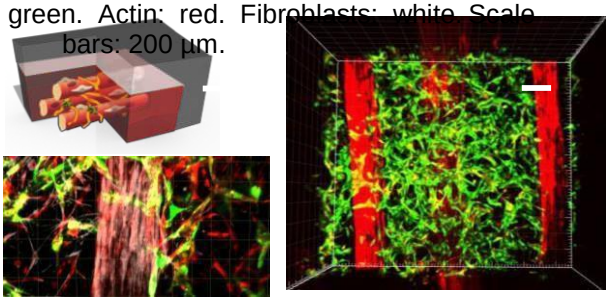

Fig.1 A) Configuration of the vascularized muscle environment. B) Confocal imaging of the whole construct. C) Fibroblast sheath covering a vascularized muscle bundle. ECs: green. Actin: red. Fibroblasts: white. Scale bars: 200 µm.

P291 Measuring micro-mechanics of soft biomaterials in physiological conditions

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The micro-mechanical properties of materials play an important role in tissue engineering or regenerative medicine. Similarly to biochemical cues, the mechanical properties of the cell niche also regulate cell behaviour, a process known as mechano-transduction. Although the characterization of (bio)materials used in tissue engineering is well established, the local characterization of very soft materials and materials while immersed in liquids proves not established and very difficult to perform. In this research, we describe a new experimental method to derive the local micro-mechanical properties of soft materials at the cell level, such as biomaterials, scaffolds, tissues or cells themselves, in a non-destructive way with great accuracy and precision while being immersed in a solution (e.g. PBS or culture medium) and kept at physiological temperature so as to mimic in-vivo physiological-likeconditions.

The method involves a novel nano-indentation instrument that uses a unique opto-mechanical force transducer. As this force transducer is fully optical, it can be operated while fully immersed in a liquid. This provides the unique ability to measure the micro-mechanical properties of samples while alive (e.g. tissues or cells) and in fully hydrated condition (e.g. tissues, hydrogels, biomaterials) at typical cell length-scales.

Both load- and displacement-controlled user-defined experiments can be performed with this novel nano-indenter enabling a wide range of mechanical tests, from quasi-static experiments to derive classical elastic modulus values, to step response tests (e.g. creep, stress-relaxation), dynamic mechanical analysis (DMA) and constant strain rate tests (i.e. epsilon-dot method) to investigate (bio)material viscoelastic behaviour.

In this study, the viscoelastic properties of gelatin samples at two different concentrations were characterised both via load- (i.e. creep) and displacement-controlled (i.e. stress-relaxation and epsilon dot method) experiments. Experimental load-indentation data within the linear viscoelastic region (LVR) were then used to derive lumped viscoelastic parameters for Generalised Maxwell (GM) model, from which both instantaneous and equilibrium (or relaxed) elastic moduli as well as characteristic time constants were computed. The results obtained with the three different testing methods were finally compared, discussing their pros, cons and limitations for testing very soft viscoelastic (bio)materials.

The testing and analysis method proposed in this study provides a unique framework to characterise (bio)material mechanical properties at typical cell-length scales in physiological like environments, thus providing a relevant enabling technology for most researchers involved in the tissue engineering and regenerative medicine field.

P292 Validation of corneal bioreactor

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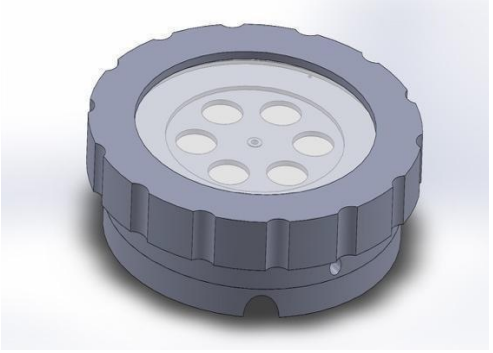
The demand for donor corneas exceeds the supply, and the population of eligible donors diminishes as the popularity of LASIK surgery grows. To address this need and provide a model for research, we have designed and manufactured a bioreactor for culturing Rabbit Corneal Fibroblasts (RCFs) in a collagen fiber matrix to yield a tissue engineered corneal equivalent. The bioreactor, shown in Figure 1, serves four primary functions: culture RCFs, impart strain upon cells, expose cells to electromagnetic signals, and allow for imaging without interrupting cell culture. The bioreactor imparts strain on cells by pressurizing a sealed chamber to inflate a membrane. A COMSOL finite element model was developed to relate chamber pressure to membrane strain. Teflon tape (PTFE) was used to prevent leaks. Initial pressure testing was done on a 3D printed prototype with promising results. Even media flow across each well region is critical for consistent cell culture. Fluid flow within the bioreactor was simulated using SolidWorks FloXpress software. Finally, material selection is a key component of the bioreactor design. The bioreactor is primarily manufactured of PEEK because it is machinable, autoclavable, and biocompatible. Makrolon GP Polycarbonate was chosen for the transparent components because of its spectral properties, strength, low cost, and ease of manufacturing. Its biocompatibility was validated using an MTT cell viability assay. The results, shown in Figure 2**,** depict a clear drop off in cell viability after 10 autoclave cycles. Future work will include experimental validation of strain and flow modeling, and biocompatibility characterization of PTFE.



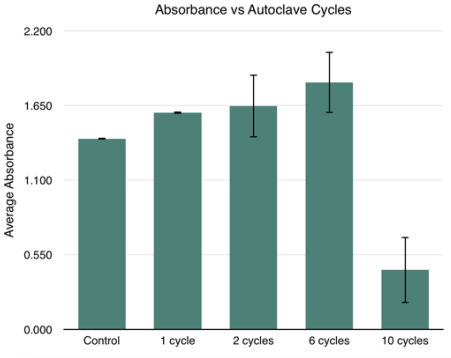


Figure 1 (left): Illustration of assembled bioreactor. Cells are seeded on membrane in circular pattern of six 'wells' and exposed to cell culture media flow. Membrane is pressurized from below.

Figure 2 (right): Polycarbonate MTT assay. Results show sharp reduction in cell metabolic activity when exposed to polycarbonate autoclaved ten times compared to the lower cycles.

P293 Bioluminescence monitored microtissue assembly in a perfused bioreactor

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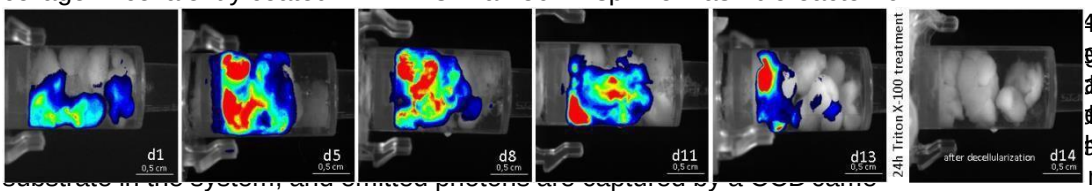
Any tissue engineering approach requires long incubation periods to obtain clinically relevant constructs. Monitoring of the living cells in the scaffolds throughout the construct formation is of upmost importance. Here we have developed a modular approach which allows us to obtain big constructs by firstly creating microtissues from seeding luciferase expressing cells on polylactic acid (PLA) microparticles (MPs) and then assembling those in a perfused bioreactor. Bioluminescence, which consists in the emission of light photons from luciferase-catalysed reactions, is used to track cell viability during the whole microtissue assembly process.

Renilla reniformis luciferase (RLuc) expressing mesenchymal stem cells (hMSC) are seeded on collagen I covalently coated PLA MPs in a 250mL spinner flask bioreactor. 6h-



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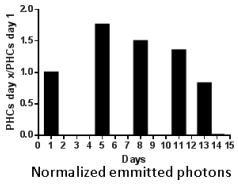

Living cells are localised within the microtissues and followed up during 13 day. Although photons decrease, and therefore cell number does, due to some handling and infrastructural issues, we are able to monitor cells, their viability and location. Moreover, we are able to track cell survival when after 2 weeks of macrotissue formation, a decellularization process is applied in the same perfusion system, without manipulating the scaffold. Right after decellularization, *Photinus pyralis* luciferase (PLuc) expressing cells, which use a different luciferase substrate, are seeded on the decellularized scaffold. Bioluminescence showed how cells attach mostly at the beginning of the construct. Longer experiments will let us know how is the survival of the reseeded cells and their adaptation to the scaffold.

This results confirm bioluminescence is a feasible method to help monitor scaffold formation in tissue engineering approaches. Furthermore, it offers the possibility to track decellularization and repopulation processes without hampering the scaffold viability and sterility conditions. MINECO is acknowledged for financial support (MAT2012-38793 and MAT2015-68906-R).

P294 Improved cell adhesion and proliferation on synthetic star shaped poly(D,L-Lactide)-b-Gelatin - silica hybrid three dimensional scaffolds

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Biodegradable polymer, poly(lactic acid) (PLA) has been widely used to make scaffolding material for tissue engineering applications. PLA lacks the cell adhering functional group on the surface which limits its successful application in tissue engineering. The aim of this present study is to improve the biological property of star shaped 4 arm poly(D,L-Lactide) (ss- PDLLA) by making copolymer with gelatin, ss-PDLLA-*b*-Gelatin (ss-PG). Chemical coupling of gelatin to ss-PDLLA arm altered the surface chemistry which also increased the hydrophilic nature of the polymer as evidenced by ^{1}H NMR, FTIR and contact angle measurement. Further, we fabricated ss-PG - silica hybrid 3D scaffolds by freeze drying technique, to achieve the cell growth throughout the scaffolds which may not be achieved by conventional surface modification techniques. Hybrid gels were made by crosslinking ss-PG with 3- glycidoxypropyl trimethoxysilane (GPTMS) and crosslinking was confirmed by FTIR. SEM results showed that the hybrid ss-PG and unmodified ss-PDLLA scaffolds were porous to support the cell growth. Enhanced hydrophilicity of the copolymer ss-PG, increased the BSA adsorption on the hybrid scaffolds. Cell viability of fibroblast cells (3T3) on the hybrid 3D scaffolds for 7 days showed a significant increase in cell number in hybrid scaffolds compared to the unmodified ss-PDLLA as demonstrated by MTT and live/dead cell assay. Hemocompatability result showed that the fabricated hybrid and unmodified scaffolds were compatible to blood RBCs. Altogether, these results showed that enhanced cell viability with optimal cell adhesion on ss-PG-silica hybrids due to their surface chemistry. We cogitate that these hybrid scaffolds can be used for successful soft tissue engineeringapplications.

P295 Fibrous scaffolds for soft tissue regeneration

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Regenerative medicine aims to reconstruct functional tissue through stimulating the body's own healing mechanisms to repair damaged tissues, which would potentially solve the problem of the shortage of donated organs and transplant infection. Designing a precise local microenvironment, which provides mechanical and biological signals temporally and spatially, to control and guide cellular behaviors is a potential approach in regenerative medicine. Chronic wounds are representing one of the most significant unmet healthcare burdens in the world today and a main complication of diabetes, and deficiencies in extracellular matrix (ECM) components are significant characterizations of non-healing wounds. An introduction of a functional construct which recruits nearby skin cells into wound area may significantly contribute to tissue regeneration. Thus, we hypothesize that a scaffold stimulating cell migration could help chronic wounds repairing. In fundamental studies, the topographic effect of scaffolds was studied on cell guidance. It was demonstrated that electrospun fibrous scaffolds (fiber diameter ranging from 300 to 2000 nm) in native ECM fibrils scale could promote cell migration comparing with fiber in tens of micrometers and flat film, and the orientation of fibers could direct cell migration. Moreover, human dermal fibroblasts cultured on oriented fibrous matrices greatly expressed alpha smooth muscle actin, representing the enhanced contraction. *In vivo* models showed that chronic wounds treated with fibrous scaffolds had a faster healing rate, and fibrous scaffolds promoted neoepidermis formation from histomorphometric analysis of wound sections. These results indicate that the fibrous scaffolds may be a potential wound dressing for chronic wound repair.

P296 Comparative evaluation of adipogenesis of human adipose stem cells in microgravity and gravity culture conditions

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The aim of this study is to comparatively evaluate adipogenic differentiation of human adipose stem cells (ASCs) in a rotating wall vessel bioreactor simulating microgravity conditions, and in standard 2D culture. During long orbit missions, astronauts experience loss of bone mass and increase in fat mass. Similarly, culture studies performed in osteogenic culture medium and simulated microgravity report decreased osteogenesis with increased adipogenesis. Microgravity conditions lead to a sharp decrease in mechanotransductive forces, while cell-cell and cell-medium interactions increase, causing the formation of 3D tissue organoids that resemble living tissue. Adipogenic microgravity conditions on ASCs have not been comprehensively investigated. Adipogenesis was investigated using histochemistry, immunohistochemistry and PCR methods. Differentiation into preadipocytes and adipocytes was assessed by evaluating changes in marker expression levels. Thus, useful information was obtained to elucidate fate decisions of human ASCs under adipogenic 3D simulated microgravity and 2D standard culture conditions. Plastic-adherent cells were isolated from the waste adipose tissue of adult patients collected during elective operations under ethical approval. ASCs were expanded in DMEM-LG containing 10% FBS, Pen-strep, 1% L-glutamine at 37°C, 5% CO2-95% air. Spheroids were formed inside STLV vessels. The medium was then exchanged with the adipogenic medium, with or without cis-9,cis-12 linoleic acid. After 11 days of culture, we evaluated the expression of PPARγ2, leptin, adipsin and adiponectin by immunohistochemical stainings and by real-time PCR techniques. cis-9,cis- 12 linoleic acid increased the expression of PPARγ2, and decreased the expression of leptin, adipsin and adiponectin. Furthermore, adipose-derived stem cells cultured in the Rotating Wall Vessel displayed spherical shape and formed three-dimensional structures upon 72 hours of culture under microgravity conditions. Microgravity conditions increases adipogenesis by altering cell shape and induces the formation of three-dimensional spherical cell clusters.

P297 Towards bone biomimetic in vitro osteocyte models using micro-3D printing

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Introduction: Osteocytes, the most abundant cells in bone, are responsible for sensing the strains that bone is subjected to [1]. *In vivo*, they reside in a system of cavities and sub-micron channels in the bone matrix termed lacuno-canalicular network (LCN), and this three-dimensional (3D) environment strongly influences the mechanical signals that osteocytes perceive [2,3]. However, this important aspect is not captured by conventionally used systems to investigate osteocytic mechanosensing such as parallel plate flow chambers. To overcome these limitations and study the influence of the 3D environment on osteocyte mechanosensing, we aim to establish an *in vitro* system for functional live cell studies in biomimetic 3D structures. ***Methods:*** Structures resembling the LCN were fabricated by micro-3D printing with the Nanoscribe Professional GT, a system based on two photon polymerization, using OrmoComp, an inorganic–organic hybrid polymer, as the printing ink. IDG-SW3 osteocytes [4] were grown on planar, collagen-coated OrmoComp substrates and cell viability was determined by LIVE/DEAD staining. Cells were reseeded on tissue culture plastic following collagenase/trypsin digestion. ***Results:*** Networks of channels and cavities were successfully fabricated by micro-3D printing (Fig. 1A). Channel diameters down to 2.5 µm for a channel length of 20 µm could be achieved (Fig. 1B). On OrmoComp, IDG-SW3 cell survival was consistently high (>95%) over 35 days of culture. IDG- SW3 cells reseeded after 35 days exhibited an osteocyte-like dendritic morphology (Fig. 1C). ***Discussion:*** Micro-3D printing of OrmoComp was found to be suitable for creating structures resembling the LCN. Furthermore, the material enabled long-term culture of osteocytic IDG-SW3 cells and observation by fluorescence microscopy. Live cell imaging of osteocytes in the printed structures is currently addressed in ongoing studies.

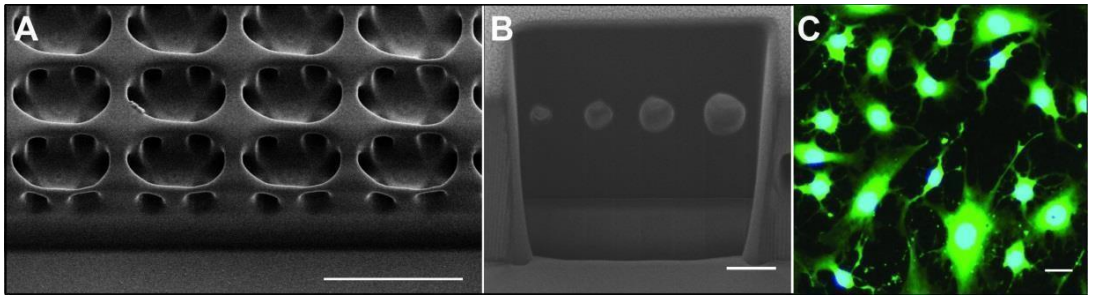

Figure 1: A Micro 3D-printed cavity and channel structure. B Scanning electron micrograph of a focused ion beam cross-section from the center of a 20 µm channel in OrmoComp. C Dendritic morphology of IDG-SW3 cells after reseeding (green: cytoplasm (Calcein AM), blue: nuclei (Hoechst)). Scale bars: A, C: 30 µm, B: 5 µm.

P298 Tenogenic phenotype maintenance and trans-differentiation / differentiation using macromolecular crowding and mechanical loading

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Cell-based tissue engineering strategies for tendon repair have limited clinical applicability due to delayed extracellular matrix (ECM) deposition and subsequent prolonged culture periods, which lead to tenogenic phenotypic drift. Deposition of ECM *in vitro* can be enhanced by macromolecular crowding (MMC), a biophysical phenomenon that governs the intra- and extra- cellular milieu of multicellular organisms, which has been described to accelerate ECM deposition in human tenocytes. A variety of cell sources have been studied for tendon repair including tenocytes, dermal fibroblasts (DFs) and mesenchymal stem cells (MSCs) and various biophysical, biochemical and biological tools have been used to mimic tendon microenvironment. Therefore, we propose to assess the combined effect of macromolecular crowding and mechanical loading on different cell sources to determine their suitability for the *in vitro* fabrication of tendon-like tissue.

The uniaxial strain induced differential cell orientation based on the differentiation state of the cells: tenocytes and DFs, both permanently differentiated cells exhibited alignment perpendicular to the direction of the load, similarly to what is seen in native tendon environment. Immunocytochemistry showed that, when MMC is used, the DFs and MSCs showed increased deposition of collagen type I, one of the main components in tendon ECM. It is also seen that the ECM deposited follows the alignment of the cell cytoskeleton. However, for tenocytes, deposition of collagen type I is only seen when MMC is used in combination with mechanical loading (Figure 1), indicating that mechanical loading led to increased synthesis of collagen I, suggesting maintenance of the tenogenic phenotype. Other collagen types relevant to native tendon composition were also analysed, including types III, V and VI, and their deposition was also shown to be modulated by the use of MMC and mechanical loading. This appears to recreate the events of tendon tissue formation during development, where these collagen types are involved in regulation of collagen I fibrillogenesis and fibril diameter. Preliminary results for expression of tenomodulin by tenocytes using western blotting showed expression only when mechanical loading was used. Although this appears to indicate the suitability of this multifactorial approach for tenogenic phenotype [illegible] retion and deposition, further analysis is required to a[illegible]s.

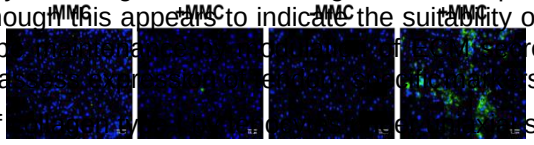

Figure 1: Deposition of [illegible] strain for 3 days (12 hours / day).

P300 An in vitro chondro-osteo-vascular triphasic model of the osteochondral complex

Riccardo Gottardi[1,2], Alessandro Pirosa[1,3], Peter Alexander[1], Dario Puppi[3], Federica Chiellini[3], Rocky Tuan[1]

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Introduction: The development of therapies against osteoarthritis (OA), a main cause of disability in the US, is hindered by the lack of veritable in vitro osteochondral (OC) models (1). In this work, we have developed a chondro-osteo-vascular (COV) triphasic model of the OC complex within a recently developed 3D printed microphysiological tissue system (MPS) bioreactor (2) that allows the separate flow of specific media to the chondral and osseous components while maintain them in contact and allowing tissue-tissue communication (3).

Materials and Methods: PCL (CAPA 6500, 50000 g/mol) fibrous scaffolds manufactured by computer-aided wet-spinning (CAWS) (4) were combined with methacrylated gelatin (gelMA) scaffolds (3). Human bone marrow mesenchymal stem cells (hMSCs) were seeded on PCL scaffolds at 8×104 cells/construct, expanded for 10 days, then placed in the bottom chamber of the bioreactor (Fig. 1A), or suspended in 10% gelMA/0.15% LAP (w/v)/PBS at 1×107 cells/ml, poured in the upper part of the insert and photopolymerized in situ. Chondrogenic/osteogenic medium (3) were supplied through the upper/lower conduits, at 1 ml/day. After 2 weeks, PCL scaffold pores were filled with a hMSCs:GFP-HUVECs 1:4 suspension (1×106 cell/ml) in 5% gelMA. Osseous constructs were incubated for 2 more weeks with 1:1 OM:endothelial growth medium.

Results and Discussion: At 4 weeks, histology showed chondrogenic (alcian blue) and osteogenic differentiation (alizarin red) in gelMA and PCL construct, respectively. Similarly, RT-PCR of individual OC components showed upregulation of chondral (COL2, ACAN, SOX9) and osseous genes (RUNX2, BSPII, OPN). HUVECs formed interconnected capillary-like networks, induced stronger alizarin red staining and enhanced osseous gene expression vs. no-HUVECs control. These results suggest that the engineered COV-OC construct mimics native OC tissue in terms of structural architecture and gene expression profile.

Conclusion: The COV-OC model recapitulating three different tissue types of the OC unit could represent a key step towards an effective in vitro analog of the OC unit to understand the biology of cartilage and bone development and regeneration, and to develop high throughput screening approaches for drug development.

Acknowledgements: Commonwealth of Pennsylvania, NIH (1U18 TR000532), Ri.MED Foundation.

P301 An advanced 3D model to study and develop materials for skin wound healing

Chiara Griffoni, Markus Rottmar, Samantha Chan, Katharina Maniura-Weber *Empa, St Gallen, Switzerland*

Introduction: Due to the increasing occurrence of chronic wounds in the aging society, preclinical investigation of novel wound healing therapies is of growing relevance. Despite the clinical need, one of the major issues in wound care is the absence of models enabling the assessment of novel treatments or material concepts designed to improve healing. Indeed, the involved biological mechanisms are still not completely understood and the in vitro models used so far poorly translate to the human physiology[1].

Our aim was to establish a method for the creation of a full thickness skin model (FTSM) including the integration of a wound and to improve the physiological relevance via integration of an immune component into the model.

Methods: FTSM was established encompassing a dermal compartment consisting of primary human fibroblasts, and an epidermal compartment consisting of primary human keratinocytes differentiated with air-lift culture. Excisional wounds were created, and then a fibrin hydrogel was applied to the wound site. The effects of fibrin on the healing process were evaluated by means of histological analyses and ELISA quantification of inflammatory cytokines (IL-6, IL-8). Preliminary studies were performed to identify the suitable parameters for a co-culture of fibroblastic and epithelial primary cells with THP-1 derived macrophages. Cell viability, proliferation and morphology were evaluated and a cell culture medium was selected.

Results: FTSM derived from human primary cells had a structure similar to the native skin, showing the presence of a stratified epithelium already after 7 days of air-lift culture. After fibrin hydrogel inclusion in the wound the area appeared transparent, becoming opaque by day 7 due to epithelial cells infiltration (Fig. 1). Wound area measurement showed no significant change in the healing rate, as well as no significant change of interleukin expression in presence of the fibrin hydrogel. All cell types remained viable and maintained a normal morphology over 7 days of culture in the selected medium. Fibroblasts and keratinocytes additionally showed increasing proliferation rates.

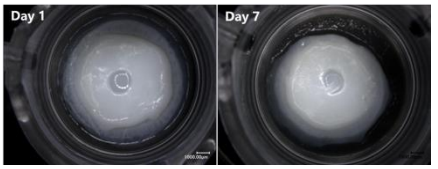

Fig. 1: Top view of the wounded FTSM created by a 2mm biopsy punch and filled with a fibrin hydrogel, at day 1 and 7 after wounding

Conclusion: A FTSM with an excisional wounding method was established, and the use of fibrin as a wound filling material resulted in no significant change in wound closure and inflammatory molecules release, thus serving as soft materials control in future studies. Furthermore, the medium composition for integrating THP-1 derived macrophages into the FTSM to obtain an immunocompetent skin model was established.

(P302)

P302 Fabrication ~~of~~ a bilayered scaffold comprising gelatin/polycaprolactone nanofiber electrospun on microfibrous eggshell membrane for wound healing applications

Preetam Guha Ray[1,3], Pallabi Pal[2], Santanu Dhara[2], Piyali Basak[3], Somenath Roy[1]

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The modern era of skin tissue engineering focuses on engineering and fabrication of scaffolds that can closely mimic the extracellular matrix and its microenvironment so as to facilitate cell adhesion and proliferation. The objective of the present study is to design and fabricate a porous, mechanically stable, biocompatible, non-immunogenic and cost effective bilayered scaffold for wound healing applications. In order to do so, microfibrous eggshell membrane (ESM) along with nanofibers of Gelatin/ Polycaprolactone (PCl) was deployed. In addition to a rich source of collagen, ESM also contains several growth factors and GAGs, essential for the regeneration process. A homogeneous blend of gelatin and PCl was electrospun onto the ESM to decorate it with randomly arranged nanofibers, followed by the cross-linking of these nanofibers (200 nm diameter) to the microfibers (1 µm diameter) using NHS/EDC coupling (EG_PN). The surface topography of the 230 µm thick bilayered scaffold was explored using FE- SEM and AFM, while the porosity was evaluated using BET analysis. The spectroscopic analyses using FT–IR and XPS reveal successful crosslinking between the nanofibers of PCl/ Gelatin and microfibers of ESM in the scaffold which corroborates to the FESEM and AFM results. The matrix exhibits considerable enzymatic degradation after 27 days, 70% wettability, tensile strength of 8 MPa and substantial anti–microbial activity. In addition, MTT, Rhodamine– DAPI assay and FESEM studies demonstrates excellent cell adhesion and proliferation of human dermal fibroblast (hDF) cells. Furthermore, the samples presented enhanced wound healing characteristics when grafted over a full thickness wound on a rat model. Moreover, the histopathological examination of the treated wounds at different time intervals revealed fast epithelization and in t x. Owing to its excellent collagen deposition he extracellular matri microstructural, physicochemical and bio com patible properties, along with its superior wound healing efficacy, the micro/ nano architec tured EG_PN mats could be a potential wound healing matrix for clinical skin regeneration.

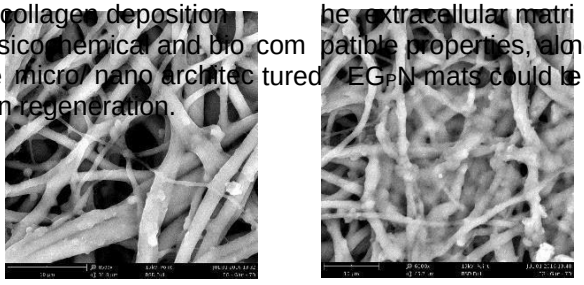

Fig 1: (a) FESEM Micrograph of the micro/ nano architecture EG_PN mats (b) hDF cells loaded EG_PN mat after 1 day.

P303 The effect of hypoxia and macromolecular crowding on matrix deposition and phenotype maintenance of human adipose derived stem cells

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The fabrication of matrix-rich tissue equivalents is becoming of increasing interest for the tissue engineering and regenerative medicine field. Adipose derived stem cells (ADSCs), characterised by their self-renewal and multi-differentiation potential, are a favoured cell population for future tissue engineering applications. *In vitro,* recapitulation of the *in situ* microenvironment with biophysical, biological and biochemical cues aims to provide cells with relevant stimuli to support their phenotype and function. Among them, hypoxia, through the activation of HIF-1*α* and HIF-2*α* has been shown to increase collagen synthesis, while macromolecular crowding has been shown to recapitulate the dense *in vivo* microenvironment of the extracellular area, and enhance matrix deposition *in vitro*. Here, we assessed the effect of hypoxia and macromolecular crowding on enhanced ADSC matrix synthesis and deposition. Carrageenan was used as a crowder. Cells were plated in 24 well plate at a density of 25 000 cells/cm². 24 hours after the seeding, the media was changed to include different concentrations of carrageenan (0, 50, 75, 100, 250 and 500 *μ*g/mL). Various oxygen tensions (0.5, 2, 5, and 21 %) were subsequently used in combination with the optimal concentration of carrageenan.

Cell morphology (phase contrast microscopy), viability (Live/ Dead®), proliferation (PicoGreen®) and metabolic activity (alamarBlue®) were assessed. SDS PAGE was used to detect collagen type I deposition by ADSCs. We characterised the cell trilineage differentiation potential (osteogenic, chondrogenic and adipogenic lineages) and expression of CD34, CD90 and CD, 105 as well as the absence of CD45 by flow cytometry to ensure phenotype maintenance.

Optimisation of the concentration of carrageenan demonstrated optimal collagen I deposition at 50 µg/ml (Figure 1). Cell viability was not affected by MMC. We hope to obtain a matrix-rich ADSC sheet by combining this with hypoxic conditions. In conclusion, mimicking the *in vivo* environment with MMC appears to hold great promise for future applications in tissue engineering.

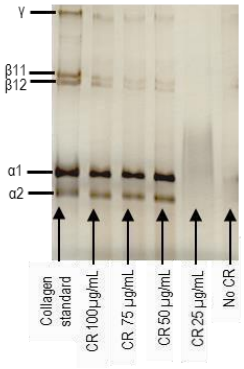

Figure 1: SDS-PAGE analysis of collagen type 1. Different concentration of carrageenan have been tested: 100, 75, 50, 25 µg/mL and the control does not contain carrageennan. Collagen standard contains 0.5µg/mL of collagen type 1.

P304 In-vitro vasculogenesis to interconnect organoids in a multi-organ-chip platform

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The Multi-Organ-Chip platform is one of the first microphysiological systems developed to evaluate the toxic effects of drugs, cosmetics, chemicals and alike in a sub-systemic mode. At the scale of a microscope glass slide, it comprises several compartments for any co-cultivation of 3D tissue constructs. These organoids are physically separated, yet, interconnected through a microfluidic system. The system contains a minute volume enabling crosstalk between the organoids. The resulting tissue-to-fluid ratio is more physiological-like than in comparable systems. The cultures are, however, not sufficiently vascularised to overcome limitations in size and complexity. We summarise our efforts to recreate a continuous endothelium and link it to advances in modelling the vascular and endosteal niches inside the bonemarrow.

Three major aspects were addressed: (1) Implement a near-physiological, pulsatile flow. It should provide an in vivo-like shear stress regime, which is needed for a phenotypical behaviour of the incorporated cell types. Therefore, the complex fluid dynamics created by an on-chip micropump were characterised and optimised using micro particle image velocimetry (µPIV). (2) Create an endothelial lining within the chip's microfluidic system. The optimised flow promoted vitality and the expression of typical markers. The cells could be cultivated for more than 106 days. (3) Establish capillary-like vessels as a direct route to the organoids. Fibrin hydrogels containing an endothelial / stromal cell co-culture enable the self-organised formation of microcapillaries. Eventually, connecting the capillary network to an organ equivalent such as the bone marrow model showed general feasibility of the approach.

Basic features of blood vessels could be emulated inside our platform. A continuous endothelium is crucial for physiological-like interactions, regulation and homeostasis within organoid (co-)cultures and is essential for long-term tissue cultivation in future applications. Moreover, it is a requirement for replacing medium with a full blood surrogate and to enable immunological queries.

P305 Biochemical matrix composition, stiffness and compliance orchestrate multipotent differentiation behaviour of human mesenchymal stem cells

Ricarda Hess, Anna Jäschke, Gabriel Jurado, Dieter Scharnweber

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Research Motivation

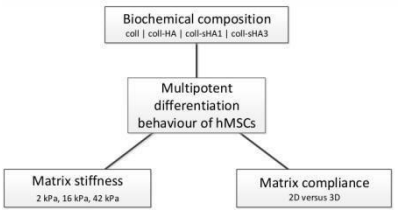

Fig. 1: Overview of studied parameters

A key challenge for cell-based therapies is to learn how to guide the differentiation of stem cells toward specific lineages in order to regenerate functional tissue. Although the influence of biochemical stimuli provided by soluble factors and the insoluble extracellular matrix have long been known to regulate cell function, also physical attributes of the ECM has now emerged as a mechanism equally important for controlling cellular functions. The aim of this study was to determine how various biochemical and physical stimuli in their effect alone and in combination influence multipotent differentiation of human mesenchymal stem cell (hMSCs) (Fig. 1).

Materials and Methods

Polyacrylamide (PAM) hybrid sandwich gels with stiffness of 2 kPa, 16 kPa and 42 kPa were prepared according to Fischer *et al*.[1]. Additionally, matrix chemistry was altered by incorporation of hyaluronan (HA) and HA-derivatives varying in their degree of sulfation (sHA1; average degree of sulfation of 1 and sHA3; average degree of sulfation of 3). Multipotent (adipogenic, chondrogenic and osteogenic) differentiation of hMSCs was analysed via biochemical (ALP-activity), histochemical (Oil-Red O staining, Alcian-Blue staining, ALP staining, Alizarin Red staining) and molecular biological (qRT-PCR) methods.

Results and Discussion

Biochemical composition, compliance as well as matrix stiffness significantly influence multipotent differentiation behaviour. On 2D collagen based coatings osteogenic and chondrogenic differentiation of hMSCs is enhanced in the presence of sulphated HA-derivatives. Contrary, adipogenic differentiation is reduced. These effects are not as dominate in 3D environments. Further, soft matrices promote adipogenic and chondrogenic differentiation meanwhile stiffer substrates enhance osteogenic differentiation. Combination of selected matrix compositions with varying stiffness shows enhanced expression of adipogenic and osteogenic marker depending on the interplay between substrate stiffness and presented ECM components.

Conclusion & Outlook

Data emphasize the complex and dynamic regulation of cellular commitment. Further work will focus on cell signalling pathways to achieve a better insight how multiple cues are sensed and integrated simultaneously and get converted into a coherent signal.

P306 Three-dimensional adipogenic differentiation model using human adipose-derived stem cells

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Introduction: Attempts to construct an adequate *in vitro* model simulating metabolic dysfunction have been inadequate due to lack of physiologically-relevant behavior and appropriate cellular organization. We aim to meet these challenges by using chemical modification of biopolymer substrates to create three-dimensional (3-D) *in vitro* tissue models that achieve enhanced survival and biological function versus conventional cultures. Specifically, we created a 3-D spheroid culture system atop positively charged elastin-like polypeptide-polyethyleneimine (ELP-PEI) coating to study the differentiation and maintenance process of lineage committed 3T3-L1 mouse preadipocytes and pluripotent human adipose-derived stem cells (hASCs). 3T3- L1 preadipocyte are commonly used to study the adipocytic differentiation process and one aim of this study was to compare their kinetics and extent of differentiation against hASCs.

Materials and Methods: To induce 3-D spheroid formation of 3T3-L1 preadipocytes and hASCs, ELP-PEI conjugate (5mg/mL) was coated on TCPS surfaces. hASCs were isolated from elective liposuction aspirates under an IRB-approved protocol. 3T3-L1 cells were purchased from ATCC. Typically 26,000 cells/cm^2 were seeded and differentiated for 3 days in media containing 1 µM dexamethasone, 1 µM indomethacin, 0.5 µM IBMX, and 1U/mL insulin. Subsequently, the differentiated cells were given adipocyte maturation media (10% v/v FBS and 1U/mL insulin) for up to 15 days. Spheroids were also matured in presence of linoleic acid (500µM) that induces oxidative stress. Intracellular protein, triglyceride content, expression of CD36, and expression of adipocytic genes were analyzed.

Results and Discussion: Time-lapse optical microscopy showed increasing spheroid size during the culture period for both 3T3-L1 cells and hASCs. However, the linoleic acid treatment generally resulted in slightly smaller hASC spheroids (~38 m diameter) compared to those cultured in the control medium (~45 m). 3T3-L1s showed no such effect. Triglyceride accumulation increased with increasing spheroid size and linoleic acid treatment in both 3T3-L1 cells and hASC spheroids. Both 3T3-L1 cells and hASC spheroids showed enhanced CD36 expression, indicating cell competency for consuming extracellular fatty acids. hASC spheroids, however, showed slower differentiation compared to the 3T3-L1 spheroids, with the spheroid size, CD36 expression, and triglyceride content being ~2 fold lower than the 3T3-L1 spheroids. 3T3-L1 spheroids indicated significant differential expression of genes related to adipogenesis, including adipocytokine signaling (14 genes), fatty acid metabolism (15 genes), PPAR-γ signaling (37 genes), and ECM interaction (27 genes).

Conclusion: We have shown the adipogenic differentiation of patient-derived hASC spheroids and lineage committed 3T3-L1 cells, which elucidated key kinetic features of their adipogenesis process. The materials and methodology developed in this research can be used as a “platform technology” to prepare 3-D *in vitro* models for drug delivery and tissue engineering applications to simulate tissue development in normal and disease states allowing scientists to test various hypotheses, study metabolic pathways, and facilitate development of new therapeutics.

P307 Metabolite-sensing in 3D bioengineered kidney tubules stimulates indoxyl sulfate secretion

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Recently, we established 3D bioengineered kidney tubules capable of active metabolites (e.g. uremic toxins) secretion through the concerted action of essential renal transporters, *viz.* organic anion transporter-1 (OAT1), breast cancer resistance protein (BCRP) and multidrug resistance protein-4 (MRP4)[1]. We hypothesize that renal cells can sense elevated metabolite levels and can stimulate their renal excretion. In this study, we studied the metabolite-sensing renal excretion pathway using bioengineered kidney tubules.

Bioengineered renal tubules were exposed to indoxyl sulfate (200 µM), a known substrate for the OAT1 uptake transporter and BCRP and MRP4 efflux transporters, or control medium for 24h. Subsequently, tubules were perfused with indoxyl sulfate (100 µM) using a microfluidics system (6 $ml.h^{-1}$). Exposure to indoxyl sulfate tends towards an increased secretory clearance (123 ± 18%) as compared to control ($p=0.37$). This was accompanied by increased gene expressions of OAT1 (5-fold, $p<0.001$), BCRP (3-fold, $p<0.001$) and MRP4 (1.7-fold, $p<0.001$) compared to control. An Arylhydrocarbon receptor (Ahr) luciferase reporter assay showed that indoxyl sulfate is an Ahr ligand (2.6-fold enhanced compared to positive control TCDD) and stimulated nuclear translocation of the aryl hydrocarbon receptor nuclear translocator protein (ARNT; 150 ± 11%, $p<0.001$), that forms a nuclear complex with ligand-bound Ahr to bind responsive elements in genes and initiate transcription. Enhanced transport, as detected by a fluorescent probe based assay, was inhibited by an Ahr antagonist CH-223191 (10 µM; 31 ± 4%, $p<0.001$) as well as an EGF receptor ligand cetuximab (500 µg/ml; 48 ± 4%, $p<0.001$). As such, it has been reported that indoyxl sulfate binds to the EGF receptor[2]. Here, we show that indoxyl sulfate exposure in the presence of cetuximab counteracts ARNT translocation (37 ± 6%, $p<0.001$), thereby attenuating the upregulation in transporters. Elucidating the EGFr signaling pathway further using focused gene array analysis revealed involvement of the MAPK pathway.

The Ahr-ARNT complex activated by indoxyl sulfate binding and concomitant EGFr signaling plays a pivotal role in the metabolite-sensing regulatory secretion pathway in renal epithelial cells. This mechanistic insight provides opportunities for the development of novel therapeutic avenues to preserve kidney function in patients suffering from kidney disease.

P308 Microenvironmental control of interstitial fluid flow to develop *in vitro* 3D pancreatic islet model

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Native pancreatic islets are not only interacted with neighboring cells by establishing 3D organization but also surrounded by perfusion at an interstitial flow rate. However, the flow effects are generally ignored in islet models in vitro, even though cell perfusion has the potential to improve the cell microenvironment and eliminate many of the problems found in static cell culture systems. We propose to develop functional pancreatic islet spheroids using microchip-based three-dimensional (3D) cultural systems under dynamic condition. Pancreatic islet spheroids with controlled size and shape were cultured in concave-shaped microwell arrays integrated with an osmotic micropump system to investigate the flow effects. The changes in islet characteristics were thoroughly investigated by comparing static and dynamic culture conditions for 2 weeks. We observed the flow enhances not only islet health but also maintenance of non-endocrine cells such as islet endothelial cells in vitro. Our platform also optimized effective flow conditions for islet culture and showed its potential application in drug testing. The proposed model can be used for islet preconditioning prior to transplantation, and as a pancreatic islet-on-a-chip for the study of diabetic drugs.

(P309)

P309 Electrohydrodynamic printing as a method to micropattern large titanium surfaces

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[1]Contipro a.s., Dolni Dobrouc, Czech Republic, [2]PROTIP MEDICAL, Strasbourg, France, [3]INSERM UMR 1121, Strasbourg, France, [4]Masaryk University, Faculty of Science, Department of Chemistry, Brno, Czech Republic

In implantable systems, most of the surfaces have to be treated to become biologically active. Herein, we use the electrohydrodynamic printing method (EHD) that is based on the same principle as the electrospinning technology for the modification of implantable surfaces with biocompatible polymers with microscale precision.

Application of polymeric micropatterns on implantable surfaces improves their interaction with the host tissue, enables the delivery of growth factors, antibiotics, anti-inflammatory cytokines etc. from the implant surface and controls the immune responses to the implant via controlling the attachment of immune cells, such as macrophages. Surface patterns with a resolution of less than 50 µm can be created using an electrohydrodynamic printing, a template-free and single- step process. Thanks to the similarity of the EHD process to electrospinning, the 4SPIN® laboratory device for nanofibrous layers preparation was modified for patterning of primary conductive, but in some cases, also for non-conductive, substrates. Reduction of distance between the electrodes (emitter, collector) to only several millimetres causes that the gradient of electrostatic field preserves only the stable phase of the standard electrospinning process and enables us to deposit periodic and regular structures. Process parameters as well as solution properties play key role in the patterning process.

In recent work, we used EHD for the deposition of parallel stripes of photocrosslinkable, cell adhesive polymeric composites with spacing of around 20 micrometres onto medical grade titanium substrates. We focused especially on optimisation of the process parameters - the solution, voltage, feed rate and collection speed. Using a very rapid movement of the grounded rotating collector we were able to prepare polymeric stripes as well as angled multilayer structures on titanium substrates. These patterns have highlyprecise periodic distances between individual stripes. A titanium foil patterned by mixture of modified polyethyleneglykol and modified gelatin was used for in vitro tests.

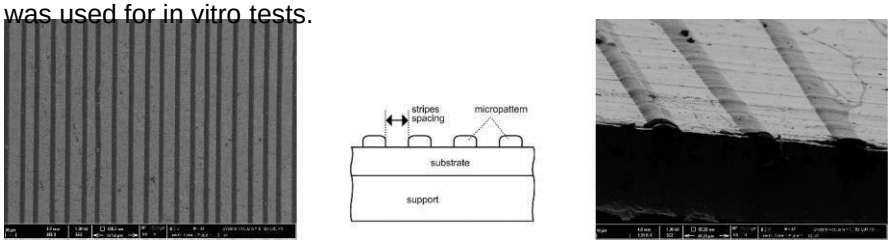

Fig. 1 The SEM image of a patterned Ti surface, the schematic illustration of pattern structure and the SEM image of a cross section of patterns.

"This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no. 602694 (IMMODGEL)."

P310 Bone tissue engineering: a proof-of-concept to derive physiological relevant tailor-made bone constructs in vitro

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Research Motivation (Figure 1)

1. To expand and achieve multipotent hMSC at low passage phase.

2. To recapitulate early processes of endochondral ossification in customised perfusion bioreactor; for long term cultivation of artificial bone constructs.

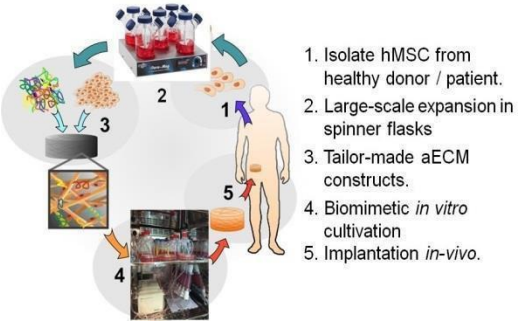

Figure 1: Processes to generate patient-specific bone grafts *in-vitro*.

Materials and Methods

Large-scale hMSC expansion: 1 x 10^6 hMSC was seeded on 0.3 g Cultispher®-S microcarrier (Sigma Aldrich) and cultured in 250 ml spinner flask (NDS glass) with continuous stirring at 90 rpm, for 12 days.

Cultivation in customized perfusion bioreactor: Tailor-made cylindrical collagen I constructs (0.55 cm^3) were seeded with 3 x 10^6 hMSC and cultured for 60 days. Two differentiation protocols were used to recapitulate *in vitro*, the early stages of: 1) intramembranous ossification, and 2) endochondral ossification. This was done by applying 1) only osteogenic differentiation for 60 days (OS), and 2) chondrogenic differentiation for 30 days, followed by osteogenic differentiation for subsequent 30 days (CSOS).

Figure 2: End-products after 60 days.

Results:

Large-scale hMSC expansion: Within a single passage phase, we achieved 4 x more hMSC in a spinner flask than a T175 flask. Further, the multipotency of these hMSC is retained.

Bioreactor culture: The two-step differentiation protocol (CSOS) that mimics early processes of endochondral ossification produced mineralised and more robust constructs (Figure 2) after 60 days.

Conclusion:

- Efficient large-scale hMSC expansion in single passage phase with spinner flask. Subdequently, these hMSC can be utilised in further tissue engineering applications.

- Initial endochondral ossification processes is recapitulated in the perfusion bioreactor, and it is proven critical for deriving more robust bone constructs *in vitro*.

- Customised perfusion bioreactor supports long term *in vitro* cultivation of engineered bone constructs to attain end-products with enhanced mechnical properties.

(P311) -

P311 Substrate stiffness greatly affects phenotypes of cells; in-vitro culture of human umbilical vein endothelial cell

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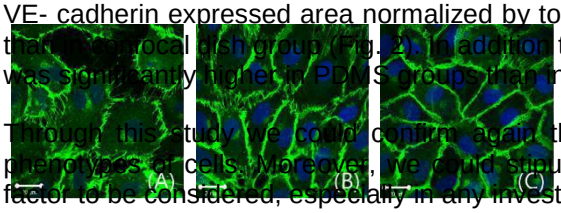
It is widely recognized that the substrate stiffness can modulate or affect phenotypes of the cells, especially in in-vitro culturing of any cell types. The stiffness of blood vessel depends on age and pathological conditions. However, most studies have investigated the characteristics of endothelial cells (ECs) cultured on commercial plates.

This study investigated the effects of substrate stiffness on ECs phenotypes. For this we had three different substrates set: confocal dish and two different polydimethylsiloxane (PDMS) membranes. The stiffness of PDMS membranes were adjusted by varying the ratios of silicon to hardening agents. They were 10:1 and 25:1. The stiffness of each substrate was 3.0GPa, 1.0MPa, and 0.5MPa (approx.) for confocal dish, 10:1 PDMS, and 25:1 PDMS, respectively. Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza and were used at passage #5. The media used was endothelial cell growth media (Lonza CC-3162). The cells were cultured for up to day 7.

For the evaluation of the effects on phenotypes of HUVECs the expression of vascular endothelial cadherin (VE-cadherin) using immunofluorescence staining and their morphology were measured based on confocal images. Utilizing digital image processing the expression of VE-cadherin was quantified. For this MATLAB (Mathworks Ver.R2012a) was used.

As expected the morphology of HUVECs on PDMS membranes was observed closer to that in-vivo. However, their shape on confocal dishes looked round. The cell junction, which is one of the typical characteristics of HUVECs, was found unstable in confocal dish group (Fig. 1). The VE- cadherin expressed area normalized by total area was significantly larger in PDMS groups than in confocal dish group (Fig. 2). In addition the average intensity of expressed VE- cadherin was significantly higher in PDMS groups than in confocal dishgroup.

Through this study we could confirm again that the stiffness of substrate greatly affects the phenotypes of cells. Moreover, we could stipulate that substrate type, i.e., stiffness, is the first factor to be considered, especially in any investigation of HUVECs.



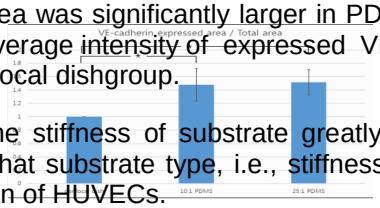


Fig. 1: (A) Confocal dish (B) 10:1 PDMS (C) 25:1 PDMS [Scale bar=20 μm]

Fig 2: The ratios of VE-cadherin expressed areas [5frames/group] *p<0.05

Acknowledgement: This research was supported by a grant of the Korea Health Technology R&D Project through the KHIDI (HI16C0362, the Ministry of Health & Welfare, ROK) and by Priority Research Centers Program (2010-0020224, the Ministry of Education, ROK).

P312 Oral epithelial cell responses to dentine matrix coated cultureware

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Objectives: Dentally derived bioactive molecules such as amelogenin can be used to aid the repair of the epithelial-dentine interface following periodontal disease. The aim of this study was to evaluate the effect of dentine derived molecules on oral epithelial cells, in an *in vitro* experimental model of periodontal regeneration.

Experimental Methods: Extracted-dentin matrix proteins (DMPe) were obtained from human dentin slices after a consecutive process of milling, demineralization, dialysis and freeze-drying. Cell culture plasticware were divided into 4 groups and subject to different coating procedures: type I-collagen (group 1), laminin (group 2), DMPe (group 3) and uncoated (group 4) as a control. 8.7 mg/mL collagen, 4 mg/mL laminin and 1 mg/mL DMPe aqueous solutions of these protein solutions were incubated with cultureware overnight at 4º C and washed prior to cell culture. Plasticware surfaces were analyzed by Coomassie Blue staining and scanning electronic microscopy (SEM) after the coating procedure. H400 cells (oral epithelial cells) were used in chemotaxis assays performed at 3 hours using chemotaxis transwell plates with cells seeded in the upper chamber. The lower chamber was included basal culture medium which was supplemented with 10% FCS as the positive control. Growth of H400 cells were assessed using the MTT assay when cultured in the presence of 30 µg/mL collagen, 50 µg/mL laminin and 0.1 µg/mL DMPe for up to 48 hours.

Results: Coated surfaces demonstrated surface modifications as compared with uncoated surfaces as shown by SEM and Coomassie Blue staining. Plasticware coated with dentin matrix molecules exhibited increased chemotaxis assay compared with uncoated plasticware. DMPe coating resulted in 31.9% increased chemoattraction compared with control. Collagen and laminin coating resulted in 11.6% and 5.8% increases in chemoattraction, respectively. H400 cells in contact with laminin after 24 hours, and collagen after 48 hours compared with the control exhibited higher MTT responses.

Conclusion: Data indicates that the presence of different components of dentine could improve oral epithelial cell movement and growth. The experimental model developed could provide an interesting tool for studying periodontal tissue regeneration *in vitro*.

Supported by: Birmingham Dental Hospital & School of Dentistry, UK

P313 Three dimensional model of the human periodontal ligament to be used as a middle-throughput test system

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INTRODUCTION: The periodontal ligament represents a very complex tissue structure between the tooth cementum and the alveolar bone. The matrix of the periodontal ligament is deposited by periodontal ligament fibroblasts (PDLF) and consists primarily of fibrillary collagen (collagen I and III) and elastic fibres (fibrillin I). As the soft tissue as well as the alveolar bone is degenerated in case of periodontitis, biomaterials are needed that support tissue regeneration. To achieve this, a degradable, suitable matrix has to be placed into the periodontal gap. In order to evaluate the capacity of a library of new materials to promote tissue regeneration, a middle throughput and biologically relevant test system was realized (Figure 1). Self-assembled peptide nanofibers moved into the focus of several research groups, as they exhibit adequate characteristics of a matrix for tissue regeneration processes. In order to evaluate the feasibility of SAPs for periodontal regeneration, the aforementioned 3D test model was implemented.

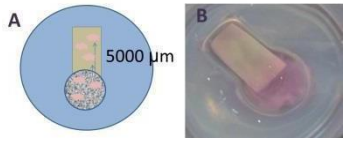

Figure 1: In vitro *model of the periodontal pocket of 5 mm. (A) Schematic drawing, (B) periodontal model with PDLF containing collagen hydrogel (pink) and dentin surface (yellow) surrounded by agarose.*

RESULTS: Visual determination of dentin-matrix interaction showed significant attachment of the self-assembling peptides to dentin surface. The peptide hydrogels not only covered the surface, but leaked into dentin channels. Human primary periodontal ligament fibroblasts (PDLFs) were able to attach to the dentin surface, but also to SAP covered ones, making preferences to certain peptide hydrogels and peptide concentrations apparently. ATP content increased over time and allowed conclusions concerning cellular proliferation. The investigated peptides supported adhesion and proliferation differently, so that some candidates could already been excluded. The feasibility of migration and effective coverage of the plane dentin surface was evaluated in the 3D model. Interestingly, there was a very different migration pattern apparent for each peptide hydrogel. Degradation of the hydrogel is due to dissolution of the structure, but is not increased by the action of neutrophilic elastase and reaches equilibrium after 24h.

CONCLUSIONS: Effective treatment of periodontitis has emerged to one of the most important issues in dentistry and the need for uncomplicated and straight forward therapies is obvious. Within the study we were able to establish an *in vitro* model of the periodontal ligament and the results supported the proof of concept of this approach. Based on the obtained results, 2 candidates of SAPs were chosen for an animal trial to proof the efficacy of periodontal regeneration.

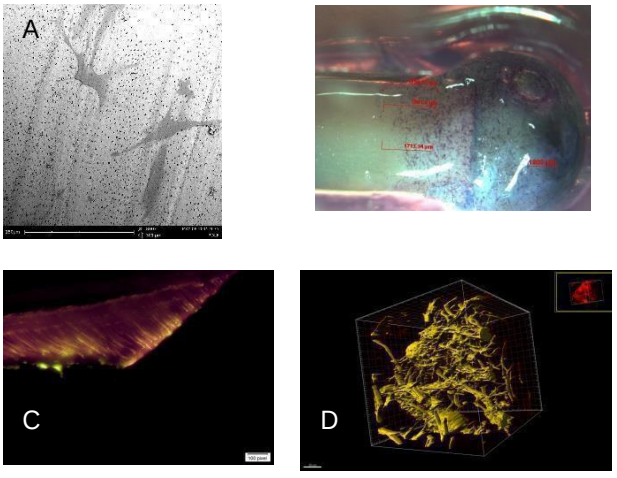

Fig. 1: PDLFs adhesion on pure dentin (A), in 3D periodontal model on SAP hydrogel (B), diffusion of fluorescent SAP into dentin channels (C) and distribution in SAP hydrogel (D)

P314 Tuning the architecture of 3D collagen-based hydrogel constructs using polyvinylpyrrolidone macromolecules

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The architecture of native extracellular matrix (ECM) plays an important role in modulation of cellular behavior; the complex microstructure (fiber diameter and porosity) of the collagen fibers across the thickness of our native skin influences the cellular behavior. Numerous strategies that have been employed to tune the microstructure of collagen fibers include manipulation of polymerization pH, temperature, or even matrix compression. Although the mentioned strategies can be used to tune the collagen microstructures, it is still challenging to achieve a gradient in the microstructure across the entire construct.

Macromolecules have been utilized to tune the architecture of collagen-based hydrogel constructs; we hypothesized that bioprinting of macromolecules would enable us to achieve a gradient in microstructure across the collagen constructs. In this work, a microvalve-based bioprinting system (RegenHu Biofactory, Switzerland) was used for drop-on-demand (DOD) bioprinting of collagen droplets and macromolecules. The bioprinter comprised of multiple microvalve-based print-heads (nozzle diameter of 100 µm), which were used to simultaneously deposit collagen precursor, cross-linker and macromolecules.

We optimized the printing parameters (droplet volume, spacing and platform speed) to facilitate the pH-dependent crosslinking of each thin collagen layer (~ 20 µm thickness). Next, we printed 6-layered graded collagen constructs (~120 µm thickness) in a single-step bioprinting approach by varying the number of PVP droplets at each respective region (top, middle and bottom). We first investigated the influence of PVP concentration on polymerization kinetics of collagen hydrogel; the presence of the macromolecules (resulted in a faster collagen polymerization rate) influences the rate of collagen nucleation and fiber growth, which affects the fiber diameter and porosity of printed collagen constructs. We also performed FE-SEM analysis to analyze the microstructure of collagen architecture across the different regions; an increasing PVP concentration resulted in the formation of thicker fiber diameter (from 37.1 ± 10.7 nm at top region to 68.9 ± 19.1 nm at bottom region) and the lower porosity (from 46.7 ± 1.9 % at top region to 7.4 ± 1.5 % at bottom region) of the printed collagen constructs. Here, we demonstrated a novel single-step bioprinting approach to tune the architecture (fiber diameter and porosity) of printed collagen-based hydrogel constructs in a controlled manner. This work would enable us to investigate the 3D cellular response within an engineered environment in a highly repeatable manner.

P315 Cellulose nanowhiskers for skeletal muscle engineering

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Aims

Volumetric muscle loss (VML) is a life-changing disability which can result from the loss of skeletal muscle after injury. The development of biomaterials to facilitate muscle regeneration is therefore of great interest. A key aspect of muscle regeneration is the deposition of an organised, tissue-specific extracellular matrix (ECM) which provides the appropriate spatial and biochemical cues for muscle differentiation and maturation.

Prior work has shown that spin-coating tunicin cellulose nanowhiskers (CNWs) onto a glass surface creates a highly oriented surface that supports the adhesion, spreading and proliferation of myotubes. Building on this work, this project aimed to develop culture surfaces with biologically active topography in order to better mimic the native cell niche and to direct the differentiation of mesenchymal stem cells.

Methods

Cellulose nanocrystals from *Ascidiella spp* were isolated and used to create chitosan-based polyelectrolyte multilayers using a combination of two well-established, low-cost and facile production methods, dip-coating and spin-coating.

C2C12 myoblasts were cultured on test and control substrates and the ECM components in C2C12-derived ECM were qualitatively and quantitatively analyzed via liquid chromatography/tandem mass spectrometry LC/MS–MS Decellularised substrates were additionally utilised as a cell-modified template to guide and encourage the myogenic differentiation of bone marrow derived mesenchymal stem cells.

Results

C2C12 cells cultured on CNW-Chitosan polyelectrolyte multilayer substrates produced ECM that was well-aligned. Proteomic analysis of the ECM produced by C2C12 cells in response to the CNW-Chitosan polyelectrolyte multilayer substrates showed that the material altered the expression patterns of these proteins towards a phenotype consistent with an early developmental stage of muscle.

The response of bone marrow stem cells to the substrates showed that mesenchymal stem cells were contact guided by the CNWs. When cultured on substrates, both as produced and also pre-conditioned by C2C12 cells, MSCs expressed myogenin, suggestive of a potential myogenic differentiation.

P316 Biomimetic bioreactor characterization of scaffolds based on gellan-gum hydrogels with nano-particulate bioactive-glass

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Biomimetic bioreactors can provide reliable characterization of new biomaterials under physiologically relevant conditions with the ultimate aim to decrease the extent of necessary animal studies. However, each tissue and organ in the body is exposed to different physical and biochemical signals, imposing the need for customized bioreactor design. Hydrodynamic shear stresses were indicated as a key physical signal enhancing growth of tissue engineered bone *in vitro*, while dynamic compressive loadings were shown to affect cell metabolism, structure and biomechanical properties of articular cartilage both *in vitro* and *in vivo*. Accordingly, in the present study, we have used perfusion bioreactors providing shear stresses and a bioreactor with dynamic compression to evaluate gellan gum (GG) scaffolds with dispersed nano-particulate bioactive-glass (BAG) attractive for use in bone and osteochondral tissue engineering. Two production methods were applied resulting in porous scaffolds with closed and open pores. The rehydrated scaffolds in the form of discs (10 mm diameter, 5 mm thick) contained 2 % w/w GG and 2 % w/w BAG (composition: 70 n/n % SiO_2, 30 n/n % CaO) while 2 % w/w GG samples served as a control. The scaffolds were investigated for up to 14 days in simulated body fluid (SBF) under (i) continuous perfusion (1.1 ml min^{-1} flowrate and 240 μm s^{-1} superficial medium velocity), (ii) dynamic compression (5 % deformation, 0.68 Hz frequency, 337.5 μm s^{-1} loading rate, 1 h on/ 1 h off) and continuous perfusion (1.1 ml min^{-1} flowrate and 240 μm s^{-1} superficial medium velocity), and (iii) static conditions as a control. Hydroxyapatite (HAp) formation was examined at day 7 and/or day 14 by FEG-SEM and RAMAN analyses. Furthermore, mechanical properties of GG-BAG samples were monitored in the bioreactor with dynamic compression over time. Results of these studies have shown HAp formation in all GG- BAG samples (Fig. 1). In the scaffolds with closed pores HAp formation was similar under all conditions, implying that this process is kinetically controlled. However, in the scaffolds with open pores, the effects of cultivation conditions were evident showing smaller uniformly distributed HAp crystals under the influence of medium perfusion. Furthermore, GG-BAG samples preserved structural integrity over 14 days under physiologically relevant dynamic compression indicating potentials for use as cell scaffolds in bone and osteochondral tissue engineering.

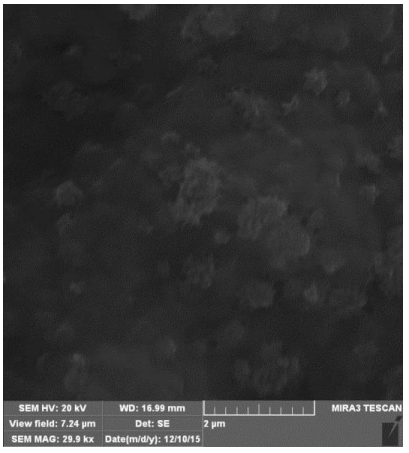

Fig. 1. SEM micrograph of a GG-BAG scaffold (cross-section) after 14 days under dynamic compression and medium perfusion

P317 Development of decellularized somatic and multipotent cell culture-derived extracellular matrices for regenerative applications

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Tissue engineering aims to generate biological equivalents of the living tissue/organ under in-vitro conditions; thus the key parameter is to design and produce scaffolds having close similarity to three-dimensional (3D) in-vivo architecture and to the native extracellular matrix (ECM). The active contents of the native ECM can be maintained in 3D by applying decellularization methods based on the removal of cells and cell remnants from tissues or organs, however this approach may have some disadvantages regarding its use in clinical applications, which is still a controversial issue. Recently, instead of using whole tissues/organs, researchers have focused on isolating the ECM directly from two dimensional (2D) cell cultures by removing cells and the nucleic acid content by applying tissue decellularization methods. In our study, natural cell- derived ECMs have been isolated from different cell types, i.e. human dermal fibroblasts (DFs) and human bone marrow-mesenchymal stem cells (BM-MSCs) by developing/optimizing three different decellularization protocols. Efficiency of decellularization protocol was evaluated by DNA content, SEM and histochemical analyses. The DNA levels were significantly reduced (~90%) compared to control groups by using Protocol III, which involved the use of a combination of 20mM ammonium hydroxide and 1%Triton-X-100. H&E stainings and SEM analyses showed that, while cells were evident in the culture surface, they were removed especially after employing Protocol III, leaving cell-derived ECMs. In the second part of the study, the ECM components of the two types of decellularized cell-derived ECMs were examined by sulphated GAG (sGAG) content and immunofluorescence analyses (i.e. Fibronectin, Laminin, Collagen I etc.). sGAG quantification analysis of cell-derived ECMs showed that our optimized protocol resulted in a yield of ~65% sGAG content in both cell types. Immunofluorescence stainings showed that hDFs expressed different ECM proteins at varying levels. On the other hand, Col I and III were highly expressed in multipotent BM-MSCs. The next step will be to comparatively evaluate the use of the developed somatic and multipotent stem cell-derived ECMs, as stem cell culture and differentiation substrates.

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P318 Linking bioreactor technology with optical coherence elastography for characterisation of engineered tissues

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Introduction: The maturation of engineered tissues is promoted through the use of bioreactors mimicking the physical *in vivo* growth environment. Monitoring the maturation of these constructs during culture and characterising them prior to implantation into the patient is important for defining their quality and performance in the clinic. Key properties include scaffold integrity and mechanical properties. Hence, novel non-invasive three-dimensional imaging modalities are required providing rapid results and translational solutions. Optical coherence elastography (OCE) has been coupled with bioreactor technology in order to monitor engineered tissue constructs during manufacture and to overcome drawbacks of tradition elastography approaches.

Method: Agarose hydrogels were prepared to final concentrations of 0.5%, 1.0% and 1.5%. hMSC ($1x10^5$) were incorporated before gelation to prepare cellular gels. Scaffolds were imaged utilizing OCT during mechanical stimulation at 1, 5, 10, 15 and 25 kPa at 1Hz frequency in the hydrostatic force bioreactor[2]. hMSC organoids were cultured over a duration of 12 days. Matrix deposition and changes in displacement were monitored. Displacements maps were generated using elastography algorithms[1].

Results: Improved sensitivity allows phase-resolved OCE to detect small displacements in heterogeneous tissue phantoms caused by either differences in gel concentration or the presence of cells. Clear differences in displacement were detected for hybrid hydrogels prepared from 0.5% and 1.5% agarose compared to 1.0% and 1.0% hydrogels. Interfaces between stiffer and softer gels can be identified. A novel dynamic OCE technique with cyclic compression as the external excitation generated by hydrostatic pressure was established. Phase-resolved OCE algorithms were applied to determine the scaffolds displacement in OCT phase-based images of various tissue phantoms. Our novel approach allows real-time non-invasive monitoring of the displacements tissue phantoms. It enables the investigation of scaffold degradation, material interfaces and heterogeneity as well as changes in scaffold porosity. It further allows the investigation of the effect of mechanical forces on cellular activities during dynamic culture. Future experiments will investigate the maturation of tissue engineered constructs during dynamic culture.

Acknowledgments: The authors would like to acknowledge the EPSRC Centre for Innovative Manufacturing in Regenerative Medicine and the UKRMP Hub for funding.

P319 Contraction and alignment of bioengineered anchored fibrin constructs as functional skeletal muscle models

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We aim to study biomaterial instructed differentiation in the generation of connective tissue with the ultimate aim of developing functional skeletal muscle fibres. A preliminary model using human dermal fibroblasts within fibrin hydrogels is used to prove and study cell-mediated contraction and alignment. These constructs are produced with defined geometries (e.g. discs and tubular) and fibrin concentration (with variation of mechanical properties, varying the elastic modulus up to 2.3 kPa). In particular, different seeding strategies were studied to optimise the construct contraction, exploring the role of 2D vs 3D in vitro systems cultured for up to 2 weeks.

An additional variable to these constructs, was the treatment TGF-β (supplementing media with 10 ng/ml of TGF-β for 2 days), evaluating its effect on contraction and on fibroblast-myofibroblast differentiation. Light-microscopy and image analysis are used to measure the contraction and alignment of these constructs over time. The expression of markers associated with myofibroblastic differentiation (e.g. α-smooth muscle actin and ED-A fibronectin) are investigated using immunofluorescence imaging and qPCR. Masson's Trichrome and Picrosirius red histological staining are used to study the collagen deposition within the constructs. Having profiled the optimal cell culture conditions e.g. fibrin concentration, seeding method, cell density, ±TGF-β, time points, we are now progressing to the development of functional skeletal muscle constructs using human muscle progenitor cells.

P320 Influence of 2D and 3D microenvironments on osteogenic differentiation, stemness and angiogenic factor expression under different oxygen tensions

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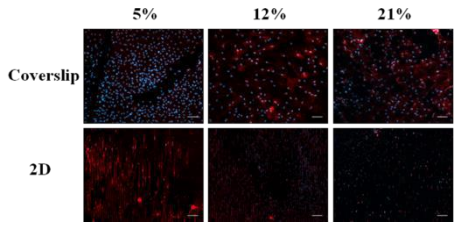
Introduction: The influence of 3D microenvironment on stem cell behaviour and metabolism has not been fully understood. This vital issue holds a particular importance especially in tissue engineering applications such as that of bone that requires regulation of a number of complex parameters including osteogenic differentiation, vascularization and oxygen transport inside the 3D matrix. Hypoxia was shown to promote the osteogenic differentiation of mesenchymal stem cells (MSCs) on tissue culture polystyrene (TCPS) [1]. In this study, 2D and 3D collagen-fibroin scaffolds with microchannel patterns were designed for bone tissue engineering and tested at hypoxia (5% O_2), arterial O_2 pressure (12%) and ambient O_2 tension (21%).

Materials and methods: MSCs isolated from human bone marrow were seeded on crosslinked 2D and 3D collagen-fibroin blend scaffolds and cultured in osteogenic medium. Cell attachment and proliferation were determined on Days 0, 1 and 3 with Picogreen. O_2 levels between the layers constituting the 3D scaffold was monitored postseeding by using fibre-optic oxygen probes (Oxford Optronix, Oxford, UK). MSCs were immunostained for RUNX2 on Day 3 and RT-PCR was carried out to measure relative expression of HPRT1, RUNX2, osterix, BST1, CD90, VEGFA genes. Mechanical test was performed on Day 35 on the unseeded and seeded 2D and 3D scaffolds that were subjected to hypoxia.

Results and discussion: Arterial O_2 pressure (12%) resulted in a lower expression for all markers with respect to hypoxia and normoxia. While the hypoxia favoured the preservation of osteogenic differentiation (Fig. 1), stemness and angiogenic factor expression on TCPS and 2D scaffold, it was the normoxia that presented these characteristics with the 3D scaffold. Tensile properties of unseeded 2D scaffolds decreased after hypoxic treatment. Normoxia, on the other hand, resulted in an improvement in the ultimate tensile strength and Young's modulus of MSC seeded 2D and also at regions of 3D scaffolds which might be have benefited from O_2 availability within the matrix.



Conclusion: Our study showed that in contrast to the 2D constructs, the use of 21% O_2 is better in preparing bone tissue engineered 3D constructs.

Figure 1. Fluorescence microscope images of MSCs on coverslip and 2D scaffolds after exposure to different oxygen levels for 3 days. Red signal belongs to RUNX2 and blue signal shows the nuclei that were stained with Hoechst 33342 (scale bar: 100 µm).

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P321 Biomimetic three-dimensional cultures in a nanofibrous scaffold promotes the generation of salivary lobular organoids from human clonal salivary gland stem cells

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INTRODUCTION: Self-organizing properties of stem cells have been exploited to generate organoids, three-dimensional (3D) structure containing organ-specific cell types. Bioengineering techniques to drive effective organization from stem cells can be promising. In this study, we introduce a 3D culture technique using nanofibrous polycaprolactone (PCL) microwells without addition of exogenous niche factors to generate salivary organoids from human salivary gland stem cells (hSGSCs).

METHODS: We examined the self-assembly capacity of hSGSCs in terms of morphological changes, salivary stem or epithelial gene/protein expression, and salivary secretory function and compared it with different culture conditions including 2D plastic culture and traditional 3D cultures such as Matrigel overlay and floating method. Microwells were fabricated by photopatterning of poly (ethylene glycol) (PEG) hydrogel in the presence of an electrospun PCL nanofibrous scaffold.

RESULTS: hSGSCs cultured in Matrigel, floating dish, and PCL microwells aggregated to form 3D spheroids with the greatest spheroid-forming potency being observed in the microwells. Salivary stem cell markers (Lgr5 and CD90) and pluripotency markers (Oct4 and Nanog) increased in 3D spheroid cultures relative to 2D plastic culture. Upon a differentiation induction, the 3D lobular structures assembled in the microwells expressed higher levels of salivary acinar markers (α-amylase and AQP5) and tight junction proteins (ZO-1 and occludin) along with decreased stem cell markers relative to other 3D cultures. Furthermore, they showed higher levels of α-amylase secretion and intracellular calcium concentration in the presence of adrenergic or cholinergic agonist respectively, suggesting more robust and functional salivary lobular organoid formation.

DISCUSSION & CONCLUSIONS: The results showed that hSGSCs cultured in the microwells could self-organize into salivary acinar-like lobular structures and this bioimimetic 3D culture technique could be effectively harnessed in generation of stem cell-based 3D organoids.

P322 Detrusor bioengineering using compressed collagen, adipose-derived stem cells and smooth muscle cells

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• Introduction

Conditions impairing bladder function in children and adults often need urinary diversion or augmentation cystoplasty as when untreated they may cause severe bladder dysfunction and kidney failure. Currently, the gold standard therapy of end-stage bladder disease refractory to conservative management is enterocystoplasty, which despite providing functional improvement is associated with significant long-term complications. Therefore, there is a strong clinical need for alternative therapies for these reconstructive procedures. The aim of this study is to develop functional smooth muscle tissue for the detrusor muscle repair combining various cell types in hydrogel scaffolds.

• Materials and Methods

Rat bladder smooth muscle (SMC) and adipose-derived stem cells (ADSC) were isolated and characterized using flow cytometry. ADSC were pre-differentiated into SMC-like cells (pADSC). Cells were combined in ratios 1:1, 1:2 and 1:3 (SMC:pADSC) and embedded in compressed collagen (CC). After 1, 2 and 3 weeks, cells in CC scaffolds, direct and indirect 2D co-cultures were analyzed for viability, proliferation, morphology, SMC-marker expression and functionality.

• Results

Cell growth conditions have been optimized and cells have shown high viability and good proliferation in the CC scaffolds. Interconnected microtissues and cell layers have developed all over the CC already after 1 week of co-culture. At 1 and 2 week timepoints cells in CC showed strong expression of the SMC markers calponin, MyH11 and smoothelin. Direct cell co-culture resulted in significantly increased cellular proliferation. Microtissues consisted of a SMC-core surrounded by pADSC. Indirect co-culture resulted in an increased pADSC survival and ratio-dependent increase in SMC-proliferation. pADSC proliferation rate also improved, but remained unaffected by the cell ratio, with 1:1 showing the most consistent results. SMC-marker expression normalized between the different ratios after 2 weeks of co-culture and reached almost the SMC monoculture expression levels. The 1:1 co-culture contracted significantly better than the other ratios after 24h.

• Conclusion

We have shown that a SMC–pADSC co-culture results in an improved cell survival, proliferation, microtissue and cell layer formation without any significant changes in phenotype and functionality. The combination of SMC and pADSC with CC may help to engineer functional detrusor muscle tissue by solving the major issues of tissue engineering, namely poor cell survival, proliferation, phenotype instability and functionality.

P323 Effect of hyaluronic acids from deep-sea organisms in the reconstitution of islet-like tissues using primary islet cells

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Donor shortage is a serious problem in islet transplantation, which is a fundamental therapy for type 1 diabetes. It is expected to apply insulin secretion cells from iPS cells to islet transplantation. In the case of transplantation, organization of three-dimensional (3D) islet-like tissue will be essential because of low insulin secretion activity of single pancreatic β cells. We reported a rapid reconstruction method of islet-like tissue comprising of mouse alpha and beta cell lines [Kojima et al., Transplant. Proc., 2012]. In the method, the 1 µl of basal medium containing suspended cells is injected into 3% methyl cellulose (MC) medium, and suspended cells aggregate within 30 min. We tried to apply the method to reconstitute islets from mouse primary islet cells. However, we found that the cell viability of the reconstructed islets was very low. To improve the viability, it might be effective to use extracellular matrices (ECMs). Our laboratory reported that the aggregation method was also useful to form hepatic spheroids filled with thin-layered ECM by adding ECMs to the basal medium at a low concentration [Tao et al. International conference on Biofabrication 2015]. In this research, we aimed to search ECMs which maintained cell viability in the reconstruction of 3D islet-like tissue using primary islet cells. Islets from 8-weeks-old C57BL6 male mice were digested by treatment with 0.25% trypsin/EDTA. We reconstructed these mouse primary islet cells to islet-like tissue by the MC medium. At that time, we added ECM to MC free medium containing suspended cells which were adjusted to 2000 cells/µl. The used ECMs are Matrigel, type 1 collagen, type 3 collagen, and hyaluronic acid (HA-Matrix isolated from porous-head eelpout (*Bothrocara hollandi*), Agcell Laboratory, Tottori, Japan). Using this rapid reconstruction method, islet-like tissues filled with thin-layered ECM were formed. After islet-like tissues were reconstructed, we measured cell viability of these islet-like tissues by Live Dead assay. We used descriptive statistics and standard deviation to report cell viability. When islet-like tissue was reconstructed with only primary islet cells, the cell viability was 45%. The cell viability of each islet-like tissues filling with Matrigel, type 1 collagen, type 3 collagen, and hyaluronic acid was 58%, 24%, 54%, and 70%, respectively (Table1). We confirmed that ECM affects largely on cell viability of islet-like tissue and hyaluronic acid has the significant cell protection effect.

Table1. ECM effect on the cell viability of reconstructed islet-like tissues

	Control	**Hyaluronic acid**	**Matrigel**	**Type 1 collagen**	**Type 3 collagen**
cell viability [%]	45.1 ±11.4	69.6 ±12.7	57.9 ±1.0	24.4 ±0.5	54.1 ±2.6

P324 Development of laser machined and hydrofluoric acid etched funnel-shaped glass pores to facilitate directed growth of neural networks

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It is desirable to be able to control the growth of neurons and the formation of neural networks, both in dish culture models and on the surface of neural implants. Soft lithography, bio-printing and micro-contact printing of extracellular matrix proteins have been successfully used to direct cell growth in-vitro and create cellular patterns on a two-dimensional surface. However, these patterns can rapidly degrade or become blanketed by secreted proteins, allowing cells to extend unconstrained beyond the bounds of the patterned surface. Neuronal growth is also sensitive to physical cues and micromachining can be used to create surfaces to control neurite growth over longer timeframes.

We report on the generation of angled edges that guide neurite growth, restricting or permitting neurite exploration. Increasing the steepness of the feature surface angle was found to result in a decrease in movement across the surface.

Funnel shaped micropores were created using laser ablation of glass coverslips. Hydrofluoric acid etching was optimised at 55 minutes and 60 minutes respectively for 60° and 90° angled funnels. Characterisation of the funnel micropore surface using Alicona profilometry revealed that the laser ablative technique resulted in a surface roughness (Ra) between 3 µm and 5 µm. Annealing at 600°C for 3 hours was used to smooth the surface however Ra values remained on the order of 3 µm to 5 µm, with the conclusion that either a different surface smoothing technique or a different machining process would be required to create funnelled pores with roughness measurements on the order of 100 nm, sufficient for in-depth and conclusive cell seeding experiments. Investigative cell seeding experiments indicated increased cell interaction with the shallower angle and decreased cell interaction with the steeper angle, as was expected.

P325 Tissue-engineered vascular grafts as in vitro models of vascular disease

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Introduction: The clinical need for small-caliber vascular grafts has driven the effort to develop vascular substitutes biologically and mechanically equivalent to native healthy ones. Besides their clinical use, such constructs can serve as in vitro vascular models enabling the study of patho-physiological processes in a more accurate way compared to e.g. 2D settings. We here propose fibrin-based tissue-engineered vascular grafts to investigate the influence of the endothelial layer's integrity on the vascular smooth muscle cells' proliferation and extracellular matrix synthesis when exposed to shear stress.

Materials and methods: Tissue-engineered vascular grafts were fabricated using a fibrin scaffold encapsulating smooth muscle cells/fibroblasts (SMC/FB). The luminal surface of the constructs was either confluently seeded with endothelial cells (EC) or partially seeded to simulate an incomplete, disrupted endothelium. Additionally, tissue-engineered vascular grafts without any endothelialisation were fabricated. The constructs were transferred into bioreactor systems and conditioned under arterial flow (10 dynes/cm²) and pressure (80/120 mmHg) conditions. Disruption of the endothelial layer was also achieved by non physiological flow conditions such as low shear stress and oscillating flows. Tissue analysis included immunohistological stainings, biochemical quantification of extracellular matrix components and a thrombogenicity assay.

Results: Thickening of the vascular wall and consequent narrowing of the lumen were evident in vascular grafts where the endothelial layer was either absent or incomplete. This was due to flow-induced hyperproliferaion of the SMC/FB and the deposition of neo extracellular matrix on the luminal surface as confirmed by immunohistological staining against collagen I (figure 1). Intimal hyperproliferation could also be induced by pathological flow conditions.

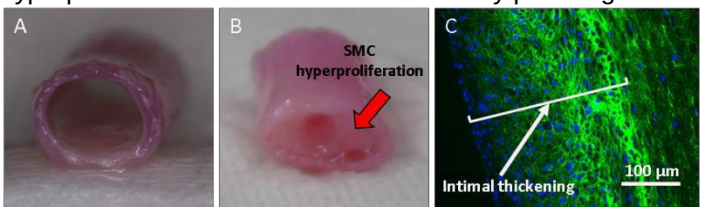

Figure 1. Endothelialized (A) and non-endothelialized (B) TEVG exposed to arterial flow and pressure conditions. (C) Immunohistological staining against collagen I of TEVG presenting wall

Discussion and conclusion: An in vitro model for intimal hyperplasia was presented where the effects of shear stress on vascular SMC in the presence of a disrupted endothelial layer were recapitulated. Such model could be used for testing new stent designs and delivery systems as well as for drugs screening. Although being simplifications of the native systems, living in vitro models hold potential for investigation of vascular (patho)physiological processes.

P326 Intracellular modelling of hypoxia-mediated stem cell commitment to proliferation

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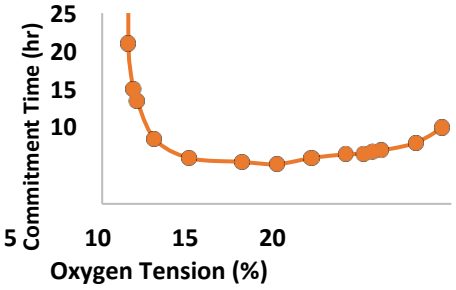
Literature has shown low oxygen conditions (hypoxia) may enhance or suppress mesenchymal stem cell proliferation from experimental results. However, the definitive effect of hypoxia on impacting cell growth is not clearly understood. As well, there lacks a mechanistic model to quantitatively link oxygen level to population doubling time in order to determine the optimal culture condition which maximizes bioreactor productivity. In this work, a comprehensive mathematical model that combines cellular oxygen sensing and hypoxia-mediated cell cycle progression has been constructed. The model is able to estimate the time required for a stem cell to reach the restriction point and commit to proliferation, in late G1 phase under hypoxia. It predicts enhanced proliferation under mild hypoxia and cell quiescence under severe hypoxia. A range of optimal oxygen level is identified which allows the quickest cell cycle commitment to proliferation. Quasi-Monte Carlo simulation results demonstrate that parameters variation has negligible effect on the range of optimal oxygen tension and the distribution of minimum commitment proliferation time. Derivative-based global sensitivity measure (DGSM) were implemented to assess the relative significance of each parameter in affecting the objective function. The significance of parameters that dominate proliferation commitment time was found to be dependent on the severity of hypoxia. Our work provides guidance for prioritizing future studies and the design of experiments to characterize hypoxia-mediated proliferation. Additional systems (metabolic, cell-to-cell contact, etc.) will be incorporated in the future, in order to establish an integrated model to predict stem cell response in a bioreactor culture environment.



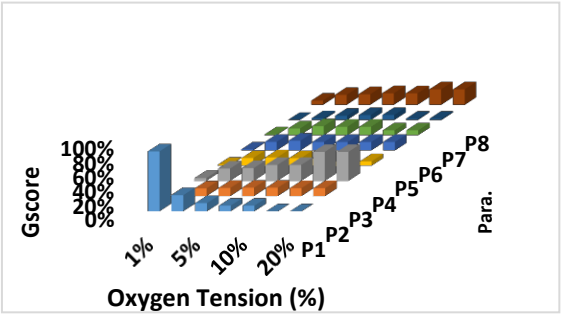


Figure 1 (left): Simulated proliferation commitment time under different hypoxic oxygen levels. Figure 2 (right): Derivative-based global sensitivity measure (DGSM) results for the selected model parameters. Relative parameter global sensitivity is shown as the calculated G score on the z-axis. Eight different parameters have been evaluated under seven different oxygen conditions.

Acknowledgement: This work is financial supported through studentship by China Regenerative Medicine International Limited.

P327 Dental Pulp Cells Secretome Regulates Endothelial Cell Adhesion and Morphology

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Background: Dental pulp cells are promising cell candidates for tissue regeneration and repair, as they exhibit excellent paracrine/trophic effects to alter host microenvironment. Among these paracrine properties, DPCs promote angiogenesis through secreting a spectrum of bioactive molecules, defined as DPCs secretome, and includes growth factors, chemokines and cytokines. Endothelial cell (EC) adhesion sites recognize soluble ligands and insoluble extra cellular matrix (ECM) proteins and their interaction regulates cell responses such as cytoskeleton formation. The focal adhesion formation is important to direct EC migration, proliferation and differentiation, and to maintain tissue homeostasis. Thus, EC adhesion is a crucial step for angiogenesis.

Objective: Although, the paracrine effect of DPCs has been investigated on different functional aspects of angiogenesis, its effect on EC adhesion is yet to be elucidated. Thefore, the current study aims to investigate EC adhesion process and morphologic alterations after DPCs secretome delivery.

Materials and methods: Human dental pulp cells (hDPCs) were harvested from third molars scheduled for surgical removal. hDPCs secretome was collected as hDPCs-conditioned media (hDPCs-CM). The effect of hDPCs-CM on adhesion and morphology of human umbilical vein endothelial cells (HUVEC) was evaluated by using microscopy, real-time cell kinetic monitoring and colorimetric assays.

Results: hDPCs-CM accelerated and enhanced HUVEC adhesion phases from sedimentation to cell attachment and spreading. The effect of hDPCs-CM on HUVEC cell cycle showed to be an initial event. Cells in hDPCs-CM groups also showed superior morphology compared to other experimental groups.

Conclusion: hDPCs secretome has promising potential to regulate cell adhesion process *in vitro*.

Keywords: Dental pulp cells; endothelial cells; angiogenesis; cell adhesion; secretome; conditioned media

P328 In vivo performance of a bilayered supramolecular scaffold for in situ vascular tissue engineering

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Introduction

In situ vascular tissue engineering (TE) holds great potential as an alternative for current small-diameter vascular replacement therapies, which often fail as a consequence of thrombosis and stenosis. This in situ TE approach aims to induce endogenous regeneration directly at the site of implantation using biodegradable synthetic scaffolds [1,2]. Here, we present a small-diameter, biodegradable, bilayered tubular scaffold based on a supramolecular elastomer. Scaffolds consisted of a porous inner layer to allow cellular infiltration from the bloodstream, and a dense outer layer to restrict transmural ingrowth from the adjacent tissues. Scaffolds were evaluated in vivo for arterial remodeling during 5 months in a rat model.

Materials and Methods

Tubular bilayered scaffolds (inner diameter 1.5 mm) were obtained by electrospinning of the supramolecular elastomer. Scaffold morphology and mechanical properties were characterized in vitro by scanning electron microscopy (SEM) and equibiaxial tensile tests. The scaffolds were then implanted in 20 healthy male Lewis rats as an interposition graft into the abdominal aorta. Grafts were explanted after 1 month (n=6), 3 months (n=7), and 5 months (n=7) for histological assessments and SEM.

Preliminary results

Electrospinning resulted in tubular bilayered scaffolds, with a porous inner layer (wall thickness 270 ± 17 µm) and a dense outer layer (wall thickness 85 ± 26µm) (Fig. 1). Equibiaxial tensile tests were performed to obtain the elastic modulus. This resulted in an elastic modulus of 2.63 ± 0.43 MPa in the longitudinal direction, and an elastic modulus of 1.96 ± 0.27 MPa in the circumferential direction (Fig. 2).

Grafts were explanted after 1, 3, and 5 months. All grafts remained patent with no signs of occlusion. However, most grafts demonstrated aneurysmal changes, ranging from saccular to fusiform. Histological analyses further revealed the local presence of calcium (Fig. 3). Histologically and by SEM, only in 1-month explants scaffold could be observed.

Conclusion

In this in vivo study, we have evaluated the remodeling of a bilayered supramolecular scaffold. The results of the study reveal major aneurysmal changes with a large interindividual variation, possibly related to fast scaffold degradation. Further analyses of the explants are currently performed to identify possible causes of the aneurysm formation in order to optimize scaffold designs for next generation scaffolds for in situ vascular TE.

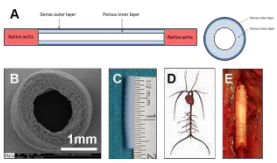

Fig. 1

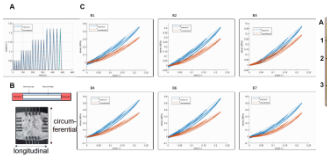

Fig. 2

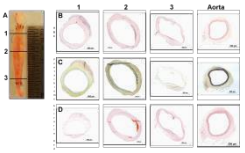

Fig. 3

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P329 First successful in vivo application of a novel regenerative stent device

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Continuous technological improvements have led to distinct generations of stent devices vastly improving patients' clinical outcomes. However, stents often fail as a consequence of thrombosis or (re)stenosis. Furthermore, the traditional rigid and long-lasting stents restrict restoration of the natural vascular function and lack the capacity of growth and remodeling.

The novel technology we propose is based on a porous synthetic biodegradable vascular support graft (or stent) capable of inducing endogenous regeneration at the site of implantation. Once deployed, the graft not only functions as a stent to restore blood flow, but also acts as a 3D scaffold for the homing of endogenous cells capable of producing and remodeling extracellular matrix in the hemodynamic environment. Over time, the support graft will be gradually resorbed, leaving behind a living autologous artery.

This study evaluates for the first time the in vivo potential of the regenerative vascular support grafts using a minimally invasive approach in the rat aorta. Preliminary results show the successful deployment of stents using a minimally invasive approach and a regenerative capacity already after two weeks.

When successful, the technology can bring unique game-changing vascular treatment solutions to the patient using a minimally invasive approach. As such it can be used for various clinical indications to: (1) instantly open stenotic lesions and trigger reinforcement of the vessel wall in atherosclerotic peripheral as well as coronary indications, (2) minimally-invasive creation of new arteries that can take over the load of aneurysmatic vascular walls, (3) radically improve treatment of pediatric patients suffering from congenital heart defects, where biodegradable stenting and vascular regeneration could allow further growth.

P331 Proceedings towards the generation of a three-layered tissue engineered fibrin based vascular graft

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Introduction: Fibrin-based vascular grafts are promising alternatives to allogeneic or xenogeneic scaffolds. Fibrinogen can be easily isolated from autologous blood plasma and polymerized by the addition of thrombin and factor XIII (FXIII) to generate fibrin scaffolds. However, the biomechanical stability of fibrin is not sufficient to withstand physiological blood pressure. Recently, a molding technique was developed to generate stable tubular-shaped fibrin based vascular grafts in a semi-automated process. Here, this technique was optimized to increase stability, uniformity and reproducibility of vascular grafts. Moreover, to mimic the structure of native vessels first steps towards the generation of three-layered vascular grafts were undertaken by seeding the grafts with endothelial cells (EC; *Tunica intima*), smooth muscle cells (SMC; *Tunica media*) and capillaries (*Tunica adventitia*) promoting undisturbed blood flow, vessel tone regulation and connection to external blood supply.

Methods: Fibrinogen was isolated by cryo-precipitation from blood plasma of healthy volunteers. Fibrin grafts with 70-100 mg fibrinogen, 70-250 U thrombin and 7-10 U FXIII per graft were fabricated and the biomechanical stability was tested by uniaxial tensile tests. For cellularization, SMC were incorporated in the grafts during the fabrication process, compacted grafts were seeded with EC onto the luminal side or with EC plus adipose tissue-derived mesenchymal stem cells (ASC) onto the outer side to form capillaries. Seeded grafts were cultivated under static or dynamic conditions for 4-12 days before cell viability and elongation under laminar flow were analyzed.

Results: Uniform fibrin grafts consisting of 70 mg fibrinogen, 70 U thrombin and 7 U FXIII achieved a tensile strength of 0.075 MPa which is consistent with a blood pressure of 523 mmHg. Stability was further increased with 100 mg fibrinogen, 100 U thrombin and 10 U FXIII resulting in a tensile strength of 0.096 MPa (721 mmHg). In contrast, higher concentrations of thrombin (250 U) led to decreased stability (0.032 MPa = 243 mmHg, $p<0.001$). SMC tolerated well the manufacturing process as shown by widely outstretched cells after static and dynamic cultivation for 5 days. Seeding the inner side of the graft with EC resulted in a confluent monolayer 4 days after seeding which aligned in the direction of flow under dynamic conditions. Co-culturing EC and ASC on top of the graft led to the formation of capillary-like structures for up to 12 days ofcultivation.

Discussion & Conclusion: The molding technique enables the generation of fibrin based vascular grafts with a biomechanical stability (721 mmHg) able to withstand arterial blood pressure. By seeding the graft with vascular cells of all three vessel layers, first steps towards the generation of tissue engineered vascular grafts similar to natural vessels were performed which may help to prevent thrombosis and infections and accelerate graft integration into the existing blood circulation.

P334 Convergence of notch signalling pathway and the elastin receptor complex (ERC) in fibrin-based vascular tissue constructs.

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Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Canada

The prevalence of coronary heart disease is a major problem in Western societies not only for the elderly population, but also for paediatric patients. Tissue-engineered vascular substitutes could solve this clinical problem since they can be used as coronary artery bypass grafts. In addition, engineered vascular tissues can be used as model tissues to test drugs, medical devices and can also be used as templates to study pathological basis of diseases.

As a natural biomaterial, fibrin gels have been utilized to fabricate vascular tissues and have shown favourable results; however, they also exhibit some shortcomings. The main problems facing engineered vascular tissue made of natural polymers, such as fibrin gel, are their initial weak mechanical properties and the lack of full functionality. We attribute this to the knowledge gaps to control, coordinate, and direct functional vascular tissue formation during maturation. In order to bridge these gaps, we sought out to study the effect of modulating the Notch signalling pathway in the 3D environment of fibrin-based tissue constructs. Notch signalling is a central pathway in the development of the cardiovascular system, but it has not been given much attention in the development of vascular tissue constructs. We believe that by modulating the Notch signalling mechanism in smooth muscle cells (SMC) embedded in fibrin gels, engineered vascular tissues with improved functionality of can be fabricated.

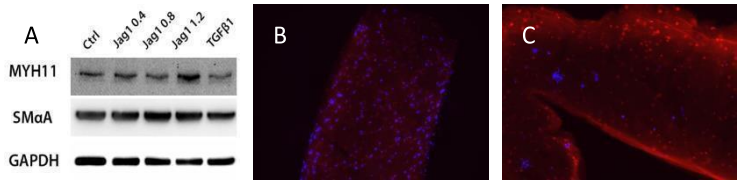

Figure A: effect of Jag1on SMC contractile protein expression. **Figure B:** fibrin gel construct control. **Figure C:** Fibronectin-Fibrin gel (probed for elastin)

Furthermore, the weak mechanical properties of fibrin-based tissue constructs are due to the lack of sufficient extracellular matrix proteins specifically elastic fibres. To that end, we studied the importance of the elastin receptor complex (ERC) in elastic fibre deposition in fibrin constructs, and the effect of Notch signalling on regulating the ERC function. It is known that the Notch signalling regulates the development of the cardiovascular system even at the embryonic stage. It is also well known that elastic fibre deposition is most active at the foetal stage in development and thus we hypothesised that there is a link between the Notch and the ERC signalling mechanisms that have not yet been identified.

In our studies, we emphasized the importance of exploring the signalling mechanisms in engineered tissue constructs to improve our understanding of tissue development and to improve the quality of engineered tissues that closely mimic native tissues and eventually be used clinically to treat diseases.

P337 Improving endothelialization of decellularized tissues by Fibrin Layers

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[1]Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [2]Institute of Macromolecular Chemistry AS CR, Prague, CzechRepublic

The biocompatibility and lifetime of heart valve bio-prosthesis made from decellularized pericardium and decellularized vessels could be supported by a suitable chemical surface modification of the prostheses. The purpose of this study was to prepare bioactive fibrin layers with growth factors supporting endothelialization. Fibrin layers were prepared with/without covalently-bound heparin and with adsorbed or chemically via heparin-bound basic fibroblast growth factor (FGF2) and/or vascular endothelial growth factor (VEGF). The optimum concentrations of FGF2 and VEGF were evaluated using their solutions of 0, 10, 100, 1000 ng/mL, and 10 µg/ml for binding to heparin. On these assemblies, metabolic activity of human saphenous endothelial cells (HSVEC) was measured using resazurin assay on day 1, 3, 6, and 12. Immunofluorescence staining of von Willebrand factor, PECAM (CD31), VE-cadherin, laminin, and type IV collagen was performed on the assemblies. The release of growth factors from fibrin was measured using ELISA. The expression of vinculin, PECAM-1, von Willebrand factor, and alpha$_V$ integrin were measured by qRT PCR on days 1, 7, 14, and 21. The HSVEC seeded on the tested fibrin layers grew better than on control surface polystyrene and reached confluency. Cell growth was dependent on the concentration of individual growth factors or their combination. Maximum expression of endothelial differentiation markers PECAM-1 and von Willebrand factor was found on fibrin with both FGF and VEGF bound.

Fibrin layers with/without heparin and with adsorbed or chemically bound FGF2, VEGF or their combination improved endothelialisation in vitro and could be useful for endothelialization of cardiovascular prostheses.

Supported by the Ministry of Health, grants No15-29153A and 15-32497A, and the Grant Agency of the Czech Republic, project No. P108/12/G108.

P338 To fabricate an small sized vascular by autologous biotube with PVA

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In current the commercial synthetic vascular graft made by including Dacron and ePTFE for small diameter vascular (<6mm), with limited reendotheliaztion and less compliant, often result in thrombosis and intimal hyperplasia again. Even though, the use of autologous artery and vein also had the problem of size-mismatched to do the fistula or cardiovascular bypass surgery on the clinical. Therefore, small-diameter self-growing vascular grafts have beenproposed.

Biotube is an in vivo tissue engineered approached to grow autologous graft by implanting cylindrical inert materials subcutaneously. The capsulation tissue are including collagen, myofibroblasts, and mesothelium. In this study, we embedded 2mm-diameter PVA tube into New Zealand white rabbit's dorsal subcutaneous to grow biotubes for 2 weeks. Poly (vinyl alcohol) (PVA) is a synthetic and polymer which is used on various of biomedical applications. However, PVA has the characteristic of hydrophilic to support cell attachment. Through the formation of tubular capsulation tissue around PVA, it could be speculated to modify the inner surface of PVA tube to promote cell attachment. The cell viability is observed through LIVE/DEAD assay *in vitro*. On the other hand, the mechanical property by material test system is used to evaluate PVA biotube function.

Moreover, the PVA biotube was implanted into the carotid aorta of rabbit for 1 month. The patency rates are observed by angiography, and the endothelialization of inner surface are investigated by the fluorescence staining, including endothelia cell marker of CD31 and vWF, smooth muscle cell marker of α-SMA. Masson was also investigated for collagen fibers.

In the previous results, the H&E stain showed that part of tissue grown into the wall of PVA tube after embedding in the dorsal subcutaneous for 2 weeks. On the other hand, the mechanical properties of PVA biotube was sufficient for withstanding the blood pressure *in vivo.*

P339 Electrochemically aligned miniaturised tendon organoids for controlled co-culture cellular organisation and crosstalk in a 3D environment

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Hierarchical collagen constructs present a very interesting approach for tissue engineering as they combine the anisotropic and mechanical properties of aligned fibres with the potential for emulating the *in vivo* extracellular matrix anchorage properties of hydrogels, thus enabling the creation of miniaturised tendon organoids for controlled cell organisation and the study of cellular crosstalk in a co-culture 3D system.

Potentially, these constructs could have a broad range of applications, such as models to investigate injury and regeneration, or as first line drug screening tools.

We hypothesised that electrochemically aligned miniaturised tendon organoids, through optimal contact guidance, can provide a conducive environment for controlled cell organisation and cellular crosstalk in a 3D co-culture system. Herein, we investigated the influence of voltage applied to the system, collagen concentration and crosslinking in order to determine the optimal fabrication parameters.

Scanning electron microscopy and birefringence measurements were used to quantify the degree of alignment of crosslinked (4S-PEG 0.5 mM) and non-crosslinked collagen fibres when compared to randomly oriented collagen films; crosslinked samples exhibited a significantly higher degree of alignment. Higher concentrations of collagen lead to an increase in the alignment of the produced fibres (evidenced by a brighter coloration at 45º and more uniform extinction at 0º) (Figure 1). Following the seeding of these hierarchical collagen constructs with tenocytes, a high degree of cellular alignment was achieved, with cells being capable of migration across the different layers, thus confirming the potential of this technology for the production of tendon organoids for the study of cellular crosstalk in tendon regeneration.

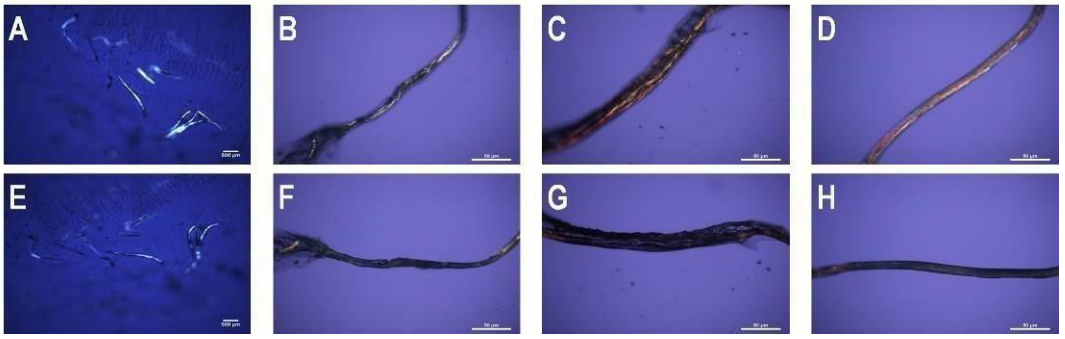

Figure 5 - (A, B, C, D) Polarised microscopy imaging of randomly aligned collagen films and electrochemically aligned 2, 4 and 8 mg / ml collagen fibres respectively (45º inclination); (E, F, G, H) – Polarised microscopy imaging of randomly aligned collagen films, electrochemically aligned 2, 4 and 8 mg / ml collagen fibres respectively (0º inclination).

P340 Vascularization and in vivo biocompatibility of electrospun polycaprolactone fiber mats for rotator cuff tear repair

Sarah Gniesmer[1], Anna-Lena Hoheisel[2], Michael Bode[2], Birgit Glasmacher[2], Henning Menzel[3], Andrea Hoffmann[4], Ralph Brehm[5], Mathias Wellmann[4], Frank Tavassol[1], Nils-Claudius Gellrich[1], Andreas Kampmann[1]

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Shoulder pain, weakness and loss of motion are common symptoms related to rotator cuff tears, which are the third most cause of musculoskeletal diseases and are the most frequent tendon injury in the adult population. Despite current improvements in surgical techniques and the development of grafts, the failure rates following tendon reconstruction remain unacceptably high. Therefore new therapies, which aim to restore the topology and functionality of the interface between the muscle, the tendon and the bone are required. For a successful repair the graft has to integrate into the graft side rapidly. This process needs a rapid ingrowth of cells and tissues, depending on a fast vascularization of the construct. Therefore rapid vessel ingrowth is the key factor for successful incorporation of transplanted tissue engineered constructs.

We investigated the *in-vivo* incorporation of modified electrospun polycaprolactone (PCL) fiber mats in the dorsal skinfold chamber of the Balb/c mouse and the femur chamber in the Lewis rat. By using these models we were able to mimic the conditions at the tendon side of the construct (soft tissue, dorsal skinfold chamber) and the bone side of the construct (hard tissue, femur chamber).

By means of repetitive intravital fluorescence microscopy over a time period of 20 days, we quantified angiogenesis, microhaemodynamics, leukocyte-endothelial cell interaction, and microvascular permeability. This study included four different groups: a commercial PCL-polyurethane-urea patch (control), electrospun PCL fiber mats with different porousness, electrospun PCL fiber mats with a modified surface, and electrospun PCL fiber mats with protein releasing nanoparticles. At the end of the experiments, histological examinations of the bone/implant-interface (femur chamber) and the soft tissue/implant-interface (dorsal skinfold chamber), respectively, were performed and the samples were stained with haematoxylin and eosin. Capillary ingrowth inside the graft was detected immunohistochemically using CD34 as a marker for endothelial cells (Figure 1).

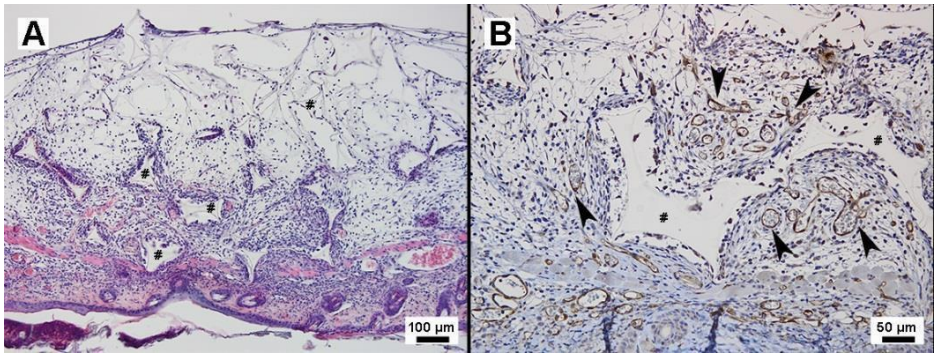

Figure 1: **A** Overview of the control patch in the skinfold chamber, day 14, HE-staining, remaining elements of the patch are marked (#). **B** Detection of capillaries (CD34, arrows) inside the control patch, remaining elements of the patch are marked (#)

(P341)

P341 Biomimetic scaffolds for tendon tissue engineering based on controlled extrusion of dense collagen solutions

Lise PICAUT[1,2], Léa TRICHET[2], Guillaume DUCOURTHIAL[3], Olivier RONSIN[1], Marie-Ange Bonnin[4], Delphine DUPREZ[4], Marie-Claire SCHANNE-KLEIN[3], Tristan BAUMBERGER[1], Gervaise MOSSER[2]

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Tissue engineering reveals high potential for tendon repair, which still remains an important clinical challenge. Several strategies either based on synthetic or natural components have been already used, among them electrospinning, freeze-casting, extrusion.

We have developed a home-made set-up to extrude complex fluids of high viscosity, which allows “in live” follow-up and control of the flow exit. We use concentrated acidic collagen solutions (up to 60 mg/ml) in order to associate liquid-crystal properties and physical constraints to generate threads that best mimic the hierarchical organization of collagen found in native tendon. The threads are generated in absence of any crosslinkers or post processing treatments.

The solutions are extruded in a neutralizing buffer that was previously optimized and which triggers their fibrillogenesis. In a few hours threads, produced in a reproducible and standardized manner, can be handled. We characterized the collagen threads with different techniques. Transmission electron microscopy reveals collagen fibrils with the 67 nm spacing that confirms the fibrillogenenis occurrence. By scanning electron

Table 1: Tensile testing results for collagen threads at 30 and 60 mg/mL performed in wet conditions, at room temperature.

C° (mg/mL)	Young’s Modulus (MPa)	Ultimate Tensile Strength (MPa)	Nb. Meas.
30	0.20 ± 0.06	0.081 ± 0.016	7
60	**1.66 ± 0.63**	**0.31 ± 0.05**	**33**

ably
ng’s
1.7 MPa were reached for the more concentrated condition.

Second Harmonic Generation (SHG) microscopy reveals that collagen fibrils tend to align at a sharp angle with the thread axis inside a shell of age-dependent thickness.

Collagen threads put under tension were seeded with mesenchymal stem cells C3H10T1/2. After three weeks of culture, cells colonized the matrix and aligned along the thread axis (Figure 1).

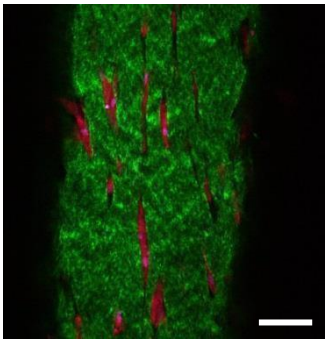

Figure 1: SHG image of a collagen thread at 60mg/mL, 30 µm under the surface, with C3H10T1/2 cells (green: fibrillar collagen; red: cell fluorescence), scale bar=50 µm.

To approach even closer to the native tendon organization and improve those already promising results, we are currently developing a new method based on flow instabilities to reproduce the crimp structure exhibited by tendons. The crimp confers the mechanical properties at low strain, when collagen fibers are aligned while no stress is generated.

P342 Characterisation of collagen type I for biomedical applications

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Collagen, as the fundamental structural component for largely connective tissues, plays an important part in keeping the structural and biological integrity of ECM structural design. Animal derived collagen has been intensively used in industry for the fabrication of scaffolds for biomedical applications. However, commercial collagen is mainly extracted from porcine and bovine skin which will not suit all applications, as mechanical properties will differ among tissues. The collagen source was investigated as a function of species (porcine, bovine), tissue (skin, tendon) and gender (male, female). Collagen type I was extracted and collagen sponges were fabricated via freeze drying method. The structural (SEM), biochemical (ninhydrin), thermal (DSC), mechanical (compression) and biological (collagenase, human adult dermal fibroblasts) properties were assessed. Human adult dermal fibroblasts were seeded on to collagen sponges at a density of 30,000 cells per sponge. Morphology (DAPI/ Rhodamine Phalloidin), viability (LIVE/DEAD®), proliferation (PicoGreen®) and metabolic activity (alamarBlue®) were assessed. Quantitative morphometric analysis was carried out using ImageJ. We show that porcine collagen has a higher level of crosslinks and possesses higher resistance to thermal and enzymatic degradation compared to bovine collagen. However, bovine collagen had inferior mechanical properties compared to porcine collagen. Sponges fabricated from bovine collagen supported cell growth and did not affect cell viability up to day 7. Therefore, collagen type I properties are species, tissue and gender dependent.

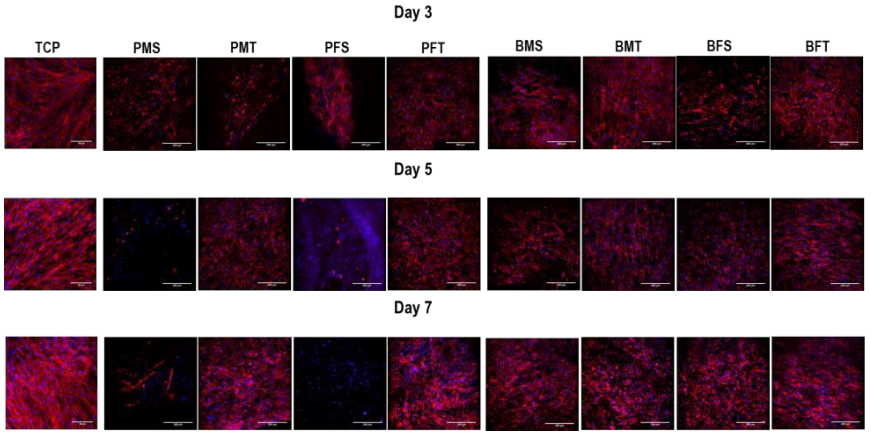

Figure 1: Sponges fabricated from bovine collagen I support cell growth up to day 7. (TCP: tissue culture plastic; PMS: porcine male skin; PMT: porcine male tendon; PFS: porcine female skin; PFT: porcine female tendon; BMS: bovine male skin, BMT: bovine male tendon; BFS: bovine female skin; BFT: bovine female tendon).

P343 Adipose-derived mesenchymal stem cells cultured in tenogenic serum-free medium express tendon-specific markers

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Tendon injuries are common and present a clinical challenge, as they often respond poorly

to treatment and result in long-term functional impairment. Poor tendon healing responses are mainly attributed to insufficient or failed tenogenesis. For optimal treatment, enhanced understanding of tendon physiology is necessary. Among others, growth factors (GFs) and cytokines modulate the differentiation of tendons during embryogenesis and the healing process of injured tendons. Cell-based therapy using mesenchymal stem cells (MSCs) in combination with GFs and biomaterials seems to be the most promising approach to heal tendon injuries. Adipose-derived MSCs (ASCs) are multipotent and immunoprivileged, making them ideal candidates for therapeutic purposes. Moreover, providing safe and regulated cell therapy products to patients requires adherence to good manufacturing practices (GMP), and GMP guidelines should be adhered to throughout the process of isolating, expanding and differentiating MSCs. For these reasons, the aims of this study were: i) to investigate the effect of several GFs already known to be involved in tendon development/healing process on human ASCs proliferation and expression of tendon-related markers; ii) to develop a tenogenic GMP-compliant serum free medium. Subcutaneous fat was obtained from 5 healthy donors by lipoaspiration, after written consent. Primary cultures of the stromal vascular fraction were established and characterized by flow cytometry analysis to evaluate cell viability (7AAD(-) and SYTO 40(+) expression), and ASC surface marker expression (CD45(-), CD146(-) and CD34(+)) and then cryopreserved. After thawing, ASCs were expanded until P3 culturing in a commercial human platelet lysate-supplemented culture medium (hPL) or in a well-defined serum free medium (SF) developed in our laboratories. At P4, tenogenic induction was performed: ASCs were cultured in 6-well plates coated with the tendon matrix protein type-I collagen and in tenogenic medium (TENO) consisting in hPL or SF medium supplemented with 100ng/ml CTGF, 10ng/ml TGFβ3, 50ng/ml BMP12 and 50µg/ml Ascorbic acid (AA) for 1, 3, 7 and 14 days. Cells cultured without any supplementations at the same time points were used as control (CTRL). Morphological appearance (optical microscopy), cell proliferation (lactate assay), gene (RT-PCR) and protein (immunofluorescence, SIRIUS-RED staining) expression were performed in all groups at all time points. Both SF-TENO and hPL-TENO cells appeared more rounded and with more cytoplasmic content and proliferated faster than respective CTRL. Tendon-marker genes (*SCX*, *COL1A1*, *COL3A1, TNC, MMP3*, *MMP13)* were significantly upregulated already after 3 to 14 days of differentiation in respect to CTRL without any significant differences between hPL and SF groups. In the meantime, stem cell gene (*KLF4, NANOG, OCT4*) expression decreased in TENO cells vs CTRL. SCX protein expression and the increase of collagen-matrix deposition were also observed in all TENO cells vs CTRL. These results demonstrate that ASCs possess tenogenic differentiation ability when exposed to CTGF, BMP12, TGFb3 and AA in both hPL and SF medium providing insights of the earliest events of tendon development and move forward the GMP-compliant approaches needed for cell-therapy strategies.

P344 New insights into tenocyte-immune cell interplay under inflammation *in vitro*

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There is clear evidence that inflammation plays a role in tendinopathy development. However, it is still poorly understood how the tissue microenvironment and innate immune cells support the development of chronic tendon disorders. Therefore, more basic knowledge is needed to understand a possible interaction between tenocytes and immune cells *in vivo*. The aim of the present study was to analyze the response of human tenocytes to an inflammatory milieu and to uncover their interplay with human macrophages *in vitro*.

Tenocytes from degenerative ruptured supraspinatus tendons (n=10, obtained with informed consent and ethics approval) were stimulated for 3 days with either a factor mixture derived from CD3/CD28 activated peripheral blood mononuclear cells (containing IL-6, IL-8, IFNγ and MCP-1) or recombinant cytokines (IFNγ, TNFα, IL-1ß). Analyzing the surface marker expression after stimulation with the factor mixture by flow cytometry, a significant increase in the expression level of adhesion molecules (CD54, CD106) and both MHC class I and class II molecules (HLA- ABC, HLA-DR) was detected compared to unstimulated tenocytes. Moreover, enhanced secretion of IL-6 release was detected by a multiplex assay. Stimulation with TNFα significantly increased CD54 and CD106 expression and HLA-DR increased significantly with IFNγ, whereas IL-1ß did not induce changes in the expression levels. TNFα and IL-1ß triggered enhanced release of IL- 6, IL-8 and MCP-1 from tenocytes compared to the unstimulated control.

When tenocytes were co-cultured for 3 days with macrophages generated from monocytes by 6 days culture with M-CSF, a significant increase was detected in the co-stimulatory molecule CD80 expression level on macrophages. Most notably, the expression level for HLA-DR, a hallmark of macrophage activation and pro-inflammatory polarization, was decreased significantly. Tenocyte/macrophage co-cultures also released more IL-8 and MCP-1 than tenocytes alone. Significant differences were determined using a nonparametric Mann-Whitney t-test between 2 groups, or a Kruskal Wallis ANOVA with Dunn's post-test for 3 or more groups.

Our results indicate that tenocytes are sensitive to an inflammatory environment and respond with an altered marker and cytokine profile, which might trigger macrophage recruitment and interaction. The observed interplay between tenocytes and innate immune cells might impact tendon remodeling and repair processes and indicates possible targets for improved therapies.

P345 Injectable and self-healing dynamic hydrogel as potential artificial nucleus pulposus for intervertebral disc repair

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The intervertebral discs (IVDs) provide unique flexibility to the spine and exceptional shock absorbing properties under impact. The inner core of the IVD, the nucleus pulposus (NP) is responsible for this adaptive behaviour. It is widely accepted that dehydration of NP results in IVD degeneration. Hydrogels which are 3D polymeric network have been considered as replacement material to rehydrate and/or replace damaged NP to treat IVD degeneration. Although covalently cross-linked hydrogel could be designed to match some conventional mechanical properties of the NP (such as stiffness), more special features like frequency-dependent stiffness (or shock absorbing properties) have not yet been achieved. Herein, we evaluate an injectable, self-healing dynamic hydrogel (DH) based on gold(I)-thiolate/disulfide (Au-S/SS) exchange as NP replacement. For the first time we report the application of dynamic covalent hydrogels inside biological tissues. The dynamic exchange between Au-S species and SS resulted in self-healing ability and frequency-dependent stiffness of the hydrogel, as shown by rheology studies. Injection of DH into nucleotomized IVDs restored the biomechanical properties of intact IVDs, including the stiffening effect observed at increasing frequencies for the neutral zone length. Cyclic tension/compression mechanical tests using bovine tail IVD were carried out at different frequencies to determine the parameters responsible for the biomechanical integrity of IVD, namely range of motion (ROM), neutral zone length (NZ length), neutral zone stiffness (NZ stiffness), and creep displacement. Four different groups of 7 specimens each were considered:
i) NP-nucleotomized IVD restored with dynamic hydrogel [DH] and ii) NP-nucleotomized IVD restored with the corresponding covalently-cross-linked hydrogel [CH], iii) NP-nucleotomized IVD as negative control and iv) intact IVD as reference. All the parameters characteristics of healthy NP were recovered when DH was used as NP replacement, especially the increasing NZ stiffness at higher frequencies. On the other hand, CH did not show any improvement of the biomechanical parameters and behaved as the negative control, i.e. NP-nucleotomized IVD. It was shown that such unexpected lack of mechanical improvement was attributed to the damaged caused by the injection process and the lack of self-healing behaviour for this conventional hydrogel. Finally, the persistence of the self-healing hydrogel in the IVD space, confirmed by tomography after mechanical testing, together with its dynamic properties suggest that this material would have long life span as an NP replacement material.

P346 Comparison of two AF injury models in organ culture

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Introduction: A high number of people suffer from low back pain due to disc herniation. In research a reproducible model to study annulus fibrosus (AF) injuries and prospective repair strategies is missing. Here, we aim for a highly reproducible and defined injury model and testing it in an organ culture model under different loading parameters.

Material & Methods: Fresh bovine intervertebral discs (IVD) were isolated and an injury with either a 2mm biopsy punch or a custom designed cross incision tool was induced [1]. Both injuries show a width of 2mm and depth of 7mm. Reaching the centre of the NP in 25% of tested cases. The cross incision tool can be operated with one hand, is fully autoclavable and blades can be exchanged, Fig 1. IVDs (5 per group) were then loaded with one of the following three profiles over 14 days in HG-DMEM with 5% FCS: 1) no loading, 2) static loading of 0.2 MPa or 3) complex loading consisting of 0.2MPa load and 0±2° of torsion at 0.2Hz for 8hrs/day. On day 0 and 14 of culture disc height, DNA and glycosaminoglycan (GAG) content as well as histology were measured.

Results: Disc height under no load and static load showed a trend towards increasing disc height for the cross incision. With applied torsion disc height showed a decrease compared to 2mm punch, Fig 1B. DNA and GAG content do not show a significant difference between the two injury models tested. Also, no difference between the tissue harvesting point near the injury (injured) and the opposite side (intact) could be found, Fig 1C-D.

Discussion: Independent of the injury, a restoration of DNA content could be observed comparing no loading vs. complex loading [2]. The main difference of the two injury models was mainly seen in disc height. This could be explained due to the alignment of the single AF sheets. Cross incision caused a bigger structural damage than biopsy combined with torsion. Also, the cross incision tool allows for a highly reproducible injury due to its fixed dimensions.

Acknowledgements: This project is supported by the Gebert Rüf Stiftung project # GRS-028/13. We thank Eva Roth for her assistance.

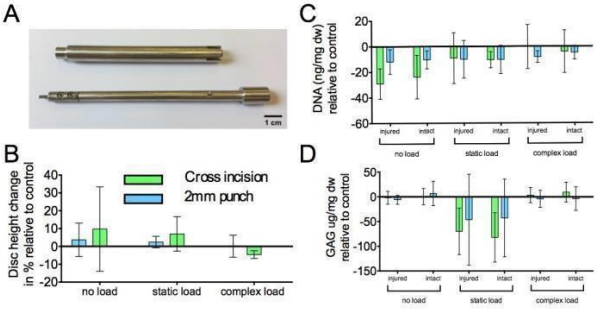

***Figure 1:** **A** Custom designed cross incsion tool, **B** disc height over culture, **C** DNA content and **D** GAG content of n=5, mean ± SD.*

P347 Design and evaluation of electrospun structured biomaterials for *Annulus fibrosus* regeneration

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Intervertebral disc (IVD) herniation is a common and severe disease responsible for up to 20 % of chronic low back pain affecting the global population. Disc herniation is defined as the protrusion of the central part of the disc, the *Nucleus pulposus (NP)*, through the external circumferential part of the IVD, the *Annulus fibrosus* (AF). Current surgical strategies are limited to treating the symptoms and mainly focused on the repair of the NP, at the expense of the structural integrity of the AF. Unrepaired defects in the AF are often associated with postoperative reherniation and further IVD degeneration. In this context, tissue engineering regenerative strategy for the AF has gained interest in the last decade. The aim of this study is the design of an electrospun biomaterial, composed of polycaprolactone (PCL), which mimics the oriented and multi-lamellar fibrous structure of the native AF to properly close the defect, contain the NP and maintain normal IVD biomechanics.

Oriented PCL membranes were produced by electrospinning technology and characterized by scanning electron microscopy (SEM) and uniaxial tensile mechanical analysis. Membranes were then seeded with human adipose stem cells (hASCs) and cultured 2 weeks *in vitro*, in mono- or multi-layer form. Constructs were then observed with SEM and confocal microscopy after YOYO-1 and alexafluor® 568 phalloïdine staining.

Our electrospinning apparatus allowed the production of two sets of fibers with an average diameter of 430 ± 160 nm and 810 ± 290 nm, respectively. SEM observation showed a homogeneous hASCs distribution on the fibrous membrane surface, with an increasing number of cells from day 4 to day 14, suggesting a cell proliferation. Phalloïdine-A568 and YOYO-1 staining allowed visualization of actin filaments and nucleus, respectively, using confocal observation. Human ASCs seeded on oriented PLC biomaterials adopted an elongated morphology and aligned in a parallel manner to the underlying scaffold but did not migrate within the membrane thickness. After 2 weeks of culture, cells formed a homogenous monolayer covering the entire surface of the PCL fibers. In a separate experiment, an AF patch was created by assembly of 2 layers of seeded PCL fibers. After 1 week of culture, the 2 layers were found to be tightly attached to each other, suggesting the feasibility to reproduce the multi-lamellar structure of a native AF.

In this study, we validated the feasibility of using the electrospinning technology to synthesize aligned nano-sized fibrous scaffold mimicking AF native structure and their use for hASCs cell culture. Their potential application will be further investigated with longer *in vitro* study using AF cells and fibroblasts. Finally, biomaterials will be implanted in an ovine model of surgically induced AF defect to assess *Annulus fibrosus* regeneration.

P348 Genipin cross-linked fibrinogen repairs annular defects with restoration of biomechanics in vitro and in vivo

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Introduction: There is an important clinical need for an injectable annulus fibrosus (AF) repair, and large animal validation is an essential step in clinical translation. This study evaluated the performance of FibGen, fibrinogen crosslinked with genipin, to biomechanically repair annular defects in ovine cervical spine motion segments. Comprehensive acute *in vitro* and 1 year *in vivo* tests were performed. **Methods:** *In vitro:* 10 ovine cervical spines were dissected into motion segments and distributed to 2 groups: Injured and FibGen. Specimens were thawed and tested. The loading protocol was 20 cycles of 100/-240N and +/-4° at 0.1, 1 & 2 Hz. Torque range (TR) was calculated from peak to peak displacement and torque. Injured specimens were prepared with 2mm biopsy punch, rehydrated and tested. FibGen specimens were injured identically and then repaired with FibGen. *In vivo*: 10 ovine sheep were subjected to cervical surgery with 2 FibGen and 1 Injured treatments distributed to C2-C3, C3-C4 and C4-C5*. After 1 year, sheep were sacrificed and dissected. Half of the specimens were fixed for histological evaluation and half were tested biomechanically as above (FibGen n=10, Injured n = 5). Statistics: ANOVA for frequency and level, t-test for FibGen.

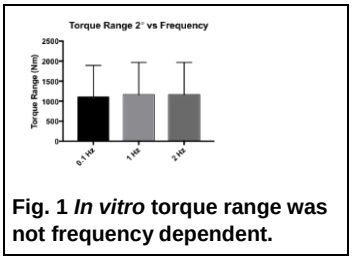

Fig. 1 *In vitro* torque range was not frequency dependent.

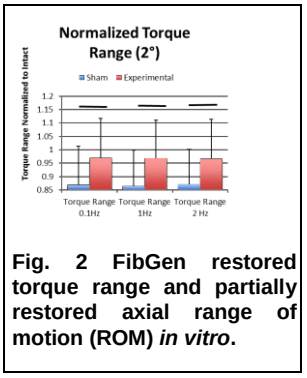

Fig. 2 FibGen restored torque range and partially restored axial range of motion (ROM) *in vitro*.

Results: *In vitro:* TR was not frequency dependent (Fig.1). FibGen restored TR (Fig 2). Ovine cervical spine levels C4-C5, C5-C6 & C6-C7 had different TR than C2-C3 & C3-C4 (Fig 3). *In vivo:* FibGen did not significantly affect TR from Injured (p=0.11) (Fig. 4). **Conclusions:** Acute *in vitro* and 1 year *in vivo* biomechanical results were similar. FibGen increased TR acutely but these trends were diminished and not significant after 1 year. Cervical biomechanics varied with level and frequency. Future work is to test these hypotheses on other variables, such as axial range of motion and stiffness. *with local ethical approval

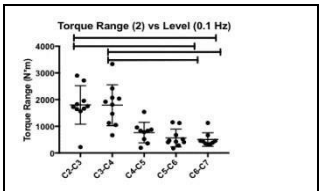

Fig. 3 In vitro axial range of motion (ROM) and torque range varied with level.

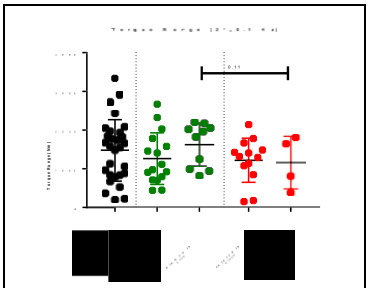

Fig. 4. The FibGen and Injured *in vivo* torque range did not differ from *in vitro* torque range. The FibGen and Injured *in vivo* did not differ significantly (p = 0.11).

P349 Generation and characterization of wharton's jelly stem cell-based stromal microtissues for tissue engineering

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Introduction: Tissue engineering is traditionally based on the generation of 3D substitutes by combining cells, biomaterials and growth factors. Although these engineered tissue models showed promising *ex vivo* and *in vivo* results, novel methods and technologies are in need for the development of more functional and fully biocompatible artificial tissues free from severe immune reactions *in vivo*. To solve this problem, novel biofabrication methods have been developed in tissue engineering, including the novel techniques allowing the generation of natural extracellular matrix produced by cells in culture which are completely natural, biomimetic and biocompatible. In this context, and based on this principle, it could be possible to generate microtissues consisting of biological microspheres for tissue engineering applications. The aim of this study is to generate and characterize novel stromal microspheres by using human Wharton's jelly stem cells (WJSC).

Methods: Human WJSC were isolated from umbilical cords and expanded until passage 5. Primary cultures of human fibroblasts were used as controls. In order to generate stromal microspheres 5 $x10^4$ cells were seeded on agarose chips containing 256 micro-wells with an average size of 400x800 µm. The microsphere formation was controlled daily by phase contrast microscopy analysis. Furthermore, the cell viability (using live/dead, WST-1, and DNA release methods), structure and histological properties of these microspheres were analysed at 4, 7, 14, 21, 28 days of *ex vivo* culture.

Results: Microscopy showed that WJSC and fibroblasts were able to form microspheres from the 4 day onward. Interestingly, important differences between both cell types and over the time were observed using morphometric, cell viability and histological analyses. Histology demonstrated that WJSC produced an abundant extracellular matrix and cells adopted a peripheral distribution. In contrast, control fibroblasts were more homogeneously distributed and generated more compacted stromal microspheres.

Discussion and Conclusions: This study demonstrated that WJSC and fibroblast are promising cell sources for the generation of microspheres for stromal tissue regeneration. Both generated microspheres were morphologically stable and cells were able to produce a complex and abundant natural extracellular matrix in just 28 days. Finally, future in vivo studies are necessary to elucidate the potential clinical use of these microspheres in tissue engineering.

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P350 Selective cell adhesion in microchannels: hydrodynamic structures combined with aptamer binding to improve microfluidic cell selection

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Precise selection of rare cell subtypes is of fundamental importance for different areas, such as cell based therapies and personalized medicine [1]. Microfluidic cell sorters allow to select rare cells from heterogeneous suspensions but not always provide high purity of selected cells. On the other hand, adhesion of target cells on a specifically coated surface presents a good purification efficiency but could be highly time-consuming in standard setups [2]. Thus, we propose a microfluidic sorter based on a cell selective surface coated with modified aptamers for cell capturing and release. The laminar flow in the microfluidic channel could limit the interactions between cells flowing in the channel and the selective surface, particularly at high flow speed, decreasing the sorting efficiency. Thus, aiming at improving cell adhesion to the aptamer-coated surface, we investigated the use of hydrodynamic structures, i.e. Herring-bone structures, which have been proposed as mixing generators [3] and were shown as powerful means for tuning cell trajectories within microfluidic channels [4]. To determine the efficacy of Herring-bone patterned channels in capturing target cells as compared to rectangular microchannels, we used both Computational Fluid Dynamic (CFD) models and experimental validation. We designed different Herring-bone geometries, with different dispositions and angles (from 30° to 60°, features from 80 µm to 400 µm in length and pitch) and evaluated their efficacy in inducing fluid recirculation and in forcing fluid/particles to contact the coated surface. To experimentally validate the results of CFD analyses, we fabricated microfluidic PDMS channels with designed geometries, coated the target surface with biotinylated polylysine and introduced streptavidin coated polystyrene beads with diameter (10-15 um) and density (1.05 g/cm^3) comparable to those of cells, at different flow velocities. Experimental results on a number of geometrical variants show that the presence of Herring-bone structures can help in inducing cells to contact the target floor surface, in particular in a range of flow rates where sedimentation is negligible. Furthermore, to test the possibility to fabricate an highly selective target surface, we modified an aptamer specific for HepG2 cells by binding it to a SAM forming peptide, aiming at increasing cell-binding specificity and introducing the possibility of cell detachment. The aptamer showed a 85% efficiency of HepG2 capture from an heterogeneous mixture, that was further increased up to 95% by multiple passages of catch and release process. In conclusion, with our approach it is possible to increase the efficiency of cell capture by a selective surface within a microfluidic cell sorter, in view of the development of a microfluidic, affinity-based cell sorting strategy.

P351 Biomaterials-based microfluidic devices with embedded tridimensional topographical features

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Introduction: Inside living tissues, cells populate microenvironments in which the extracellular matrix (ECM) provides important mechanical, biochemical and topographical cues critical for the tissue homeostasis and functionality. To mimic this process, micro- and nanostructuring of biomaterials has been explored as a valuable tool for controlling the differentiation of stem cells (1)(2). The aim of this study was to develop a microfluidics-based device having functional biomaterials as a main component. To explore the topographical effects on adhesion and morphology of osteoblast-like cells, the biomaterial surface was micropatterned by pyramid-shaped features.

Materials and methods: Polylactic acid (PLA) substrates were micropatterned by micromolding using a polydimethylsiloxane (PDMS) mold, before closing the device with a PDMS lid, with open inlets and outlets for medium supply. Microstructured PLA bottom was characterized by scanning electron microscopy (SEM). MG-63 human osteosarcoma cells were seeded and cultured for 24h. Cell morphology and orientation were microscopically assessed after Phalloidin/DAPI staining of actin and cell nuclei, respectively.

Results: Microfluidic devices housing PLA surfaces with 3D microscale topographical features, i.e. pyramids consisting of fine or coarse layers, were successfully produced to allow cell culture (Fig. 1a). MG-63 cells adhered and grew on the patterned surfaces, along the sides of the pyramids. Actin and nuclei staining indicated that the surface roughness of the pyramids affected the ability of cells to elongate in the z-direction (Fig. 1b/1c).

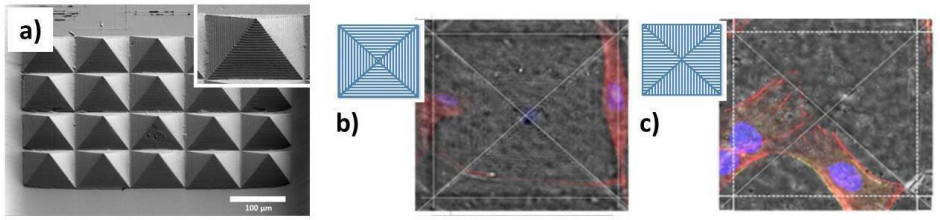

Figure 1 – Characterization of the microfluidic device by SEM (a) and merged fluorescence images of cell actin and nuclei and brightfield image of MG63 cells cultured on microstructured PLA (b, c), with horizontal (b) and vertical (c) slicing planes.

Discussion and conclusion: PLA-based microfluidic devices were successfully engineered to allow cell culture and study the effect of 3D microscale topographical features. The adhesion and elongation of cells in the z-direction on sloped pyramid walls was shown to be dependent on the roughness of the underlying surface.

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P352 Biofabrication of a stenotic vessel model

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Atherosclerosis consists of progressive growth of atherosclerotic plaques in the arterial circulation which, in advanced stages, cause progressive obstruction of the arterial lumen. The rupture of stenotic plaques can further result in formation of a thrombus which may eventually cause life-threatening complications, such as pulmonary embolism, myocardial infarction and stroke [1]. The ability to study stenotic vessel environments and thrombus formation under controlled conditions would therefore be useful in the discovery and optimization of preventive and post-thrombotic treatments. By combining 3D biomanufacturing technologies and medical imaging we have developed a new approach for fabricating in vitro cell culture models which closely mimic architectures found in both healthy and stenotic vessels. In a first step, computed axial tomography (CAT) scans were used to generate high resolution templates by means of digital light processing (DLP) 3D printing of PIC100 resin. The templates were then used as molding chambers where polydimethylsiloxane (PDMS) was casted. After PDMS curing, the PIC100 templates were removed leaving behind perfusable vessel-like shapes (10-15 mm length and 200-400 µm diameter) with high resolution and shape fidelity. The resulting PDMS microfluidic devices were finally coated with 0.1 mg/ml collagen-I and seeded with human umbilical vein endothelial cells (HUVECs) at a density of 4x106 cells/ml by means of perfusion. Live-dead staining showed that almost all cells seeded cells were alive and NucBlue/ActinGreen staining showed high confluency and normal cell morphology. Overall, this methodology overcomes important design limitations found in typical 2D wafer-based soft-lithography microfabrication techniques and shows great potential for applications such as personalized therapy and testing of medical drugs.

P353 Biofabrication of perfusable 3D tissue models

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It is becoming increasingly evident that standard tissue models, such as 2D in vitro cultures and even in vivo animal models, may not be fully representative of native human tissues. Various industries, including pharma and cosmetics are in need of improved and more reliable tissue models in order to generate products more efficiently. Many groups and companies are developing such models independently by resorting to additive manufacturing-based technologies [1]. At the Utrecht Biofabrication Facility, a highly multidisciplinary environment allowed us to realize that most tissue models follow common design principles. We are currently applying such principles to the development of a number of models mimicking healthy and diseased tissue such as i.e. liver, breast cancer and stenotic vessels. One of the most important and overarching design principles is the ability to allow perfusion through construct's pores and/or channels to enable nutrition, accurate exposure to specific compounds and downstream removal/collection of metabolic products. We are currently developing a library of tissue model designs which can be easily customized to address tissue- and user-specific requirements by means of parametric design. This means that 3D-printable device designs can be automatically generated by just defining values for parameters, such as i.e. the desired construct's size and amount as well as construct's vascularization architecture, diameter and density. Due to their underlying design principles, the resulting 3D-printed devices allow to cast and vascularize constructs from virtually any kind of material/cell combination as well as to easily further perfuse the same constructs with fluids and cell suspensions for seeding, culturing and testing purposes. This platform also allows to combine and interconnect different kinds of tissue models into one single device. The platform herein described shows the potential to be easily adopted for tissue model-based research in many fields given its versatility, upscalability and ease ofuse.

P354 Multi-organ-chip developments: towards a paradigm shift in drug development

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The development of drugs is a process obstructed with manifold security and efficacy concerns. Although animal models are still widely used to meet the required diligence they are regarded as outdated tools with limited predictability. Current in vitro cell culture assays, on the other hand, are failing to emulate the human cellular microenvironment and, therefore, lead to a rapid dedifferentiation and loss of function in primary human cell cultures. Microfluidic systems have proven to be a powerful tool for recreating tissue- and organ-like functions. Their ability to host 3D organoid constructs in a controlled microenvironment with mechanical and electrophysiological stimuli enables them to create and maintain homeostasis. These platforms are, thus, thought to be superior tools for testing and developing substances such as drugs, cosmetics, chemicals, and alike.

The new opportunities for the application of human- and organ-on-a-chip systems, as well as important challenges in realizing the full potential of this technology will be addressed. Furthermore, latest result of our multi-organ-chip will be presented. Several combinations of organs have been performed using this platform (e.g. a co-culture of liver equivalents with skin, intestine, pancreatic islets or neuronal tissues). In addition, we present a new 4-Organ-Chip (4-OC) platform for ADME profiling. In this 4-OC platform, a human primary intestinal model and a skin biopsy have been integrated on standard cell culture inserts. A fluid flow connected these barrier models with liver spheroids and a barrier segregating the media flow from fluids excreted by a kidney model. It could be shown, that co-cultures of these organ models were stable over a culture period of up to 28 days. Finally, further developments of multi-on-a-chip devices towards a miniature organism on a chip are presented leading the way towards a paradigm shift in drug development.

P355 High-throughput production of highly homogeneous bioactive ceramic microparticles using droplet microfluidics

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Introduction: For successful treatment of orthopaedic critical-size bone defects, affordable and therapeutically effective biomaterials are required. Calcium phosphate (CaP) ceramics are widely used for bone repair and regeneration, but there is still much room for improvement when it comes to their properties and biological performance. To improve the existing CaP bone graft substitutes, new engineering approaches are needed that provide a fine control of the mineralization process, high chemical yields and versatility in the delivery format. In our work, a microfluidic strategy for production of CaP microparticles inside highly monodisperse droplets is proposed for the controlled synthesis of bioactive ceramic materials.

Materials and methods: Monodisperse droplet emulsions were produced in a PDMS microfluidic device through hydrodynamic focusing. CaP precursor solutions and the oil phase were introduced through independent inflows, resulting in a downstream droplet formation after flow crossing. Droplets were collected to allow mineral precipitation. The analysis of the synthesized materials was performed using optical and scanning electron microscopy, XRD, EDS and Fourier transform infrared spectroscopy.

Results: Highly monodisperse droplets containing CaP solution were produced using droplet microfluidics (Fig. 1, left). After collection on a silicon wafer and sintering at 1000 °C, the droplets were disrupted, exposing mineral particles which were homogenous in size (Fig. 1, center). The EDS analysis revealed the presence of Ca and P on the ceramic particles (Fig. 1, right).

Conclusions: The results of this study suggest that droplet microfluidics is a powerful tool for the production of ceramic microparticles with highly homogeneous properties. This method can potentially be used to produce new ceramic materials and gain deeper insight into their biological performance.

Acknowledgments: This research has been in part made possible with the support of the Dutch Province of Limburg.

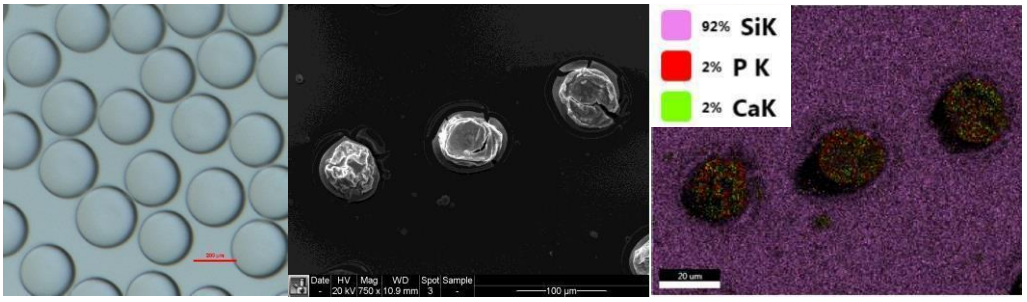

Figure 6. Left: Light microscopy image of monodisperse microdroplets produced in the microfluidics device. Center and right: SEM image of CaP mineral after collection on a silicon wafer and sintering at 1000 °C whereby the oil shell was disrupted and the mineral exposed, and respective EDS image.

P356 Microfluidic osteochondral interface

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Microfluidic systems can provide excellent platforms to generate and host microscale tissue models, which can mimic native organs. Such microphysiological *in vitro* systems (MPS) are capable of providing mechanical stimulations and recapitulating dynamics of the cellular physiology and cellular condensations at various developmental phases. The tunability of micro-tissue composition, size, as well as the high throughput and reproducibility aspects highlight the broad utility of bioengineering platforms for generating functional tissue models. Recently, we have reported novel bioengineering approaches in generating 3D microenvironments, where we can directly differentiate stem and progenitor cells and recapitulate developmental phases of tissue generation (Bouyer et al. 2016, Namkoong et al. 2016). The microfluidic systems we develop provide excellent platforms to investigate the mechanotransductions as well as the physiological response of the cells in native like niches (Guven et al. 2015, Kocal et al. 2016). Here, we demonstrate unique MPS design, which aims to recapitulate the bone-cartilage interface development. We used identical mesenchymal progenitor cells to generate both 3D bone and cartilage tissues, which are direct physical contact. As strategy, we developed the osteogenic compartment through endochondral ossification placed next to the chondrogenic compartment in a unique microfluidic chip. We simultaneously delivered two different differentiation media (osteogenic and chondrogenic) only to corresponding compartment and monitored the cell-cell interactions and developmental phases of tissues at 1, 3, 5 and 8 weeks *in vitro*. The continuous microfluidic flow provides the mechanotransduction signals through shear stress on the cells. This novel microphysiological model can recapitulate the complexity of native tissues and enable precise control over the microenvironment including cytokine and oxygen gradients and becoming essential for pre-clinical tests of therapeutics for osteogenesis.

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P357 Three-dimensional spheroid culture systems of mesenchymal stem cells for tissue regeneration

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Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological functions of tissues. We developed three-dimensional spheroid cell culture system on the hemispherical concave polydimethylsiloxane microwell chips for tissue regeneration, for cells in 3D may better mimic the conditions and properties of our body than 2D culture.

Cell therapy using mesenchymal stem cells (MSCs) have been increasing in several degenerative diseases. Even though ongoing research is attempting to the application of MSCs to the clinical trials, further understanding on their efficacy and optimal condition for therapeutic effect are required.

First, we successfully engineered scaffold-free tonsil-derived mesenchymal stem cells (TMSC) and differentiated into parathyroid hormone (PTH)-releasing cells, demonstrating the therapeutic role in hypoparathyroidism. Cells in TMSC spheroids were highly viable and expressed high levels of intact PTH, the parathyroid secretory protein 1, and cell adhesion molecule, N-cadherin. Furthermore, TMSC spheroids-implanted parathyroidectomized rats revealed higher survival rates over a 3-month period with physiological levels of both serum iPTH and ionized calcium. This is the first report of a scaffold-free, human stem cell-based parathyroid tissue engineering and represents a clinically more feasible strategy for hypoparathyroidism treatment than those requiring scaffolds.

Second, placenta-derived mesenchymal stem cells (PD-MSCs) are engineered for restoring ovary function. In an ovariectomized rat following transplantation. PD-MSCs transplantation significantly increased the estradiol level was significantly and increased efficiency of their engraftment onto ovary tissues. Furthermore, the mRNA expression levels and protein expressions of Nanos3, Nobox, and Lhx-8 as folliculogenesis markers were significantly increased in two weeks. PD-MSCs transplantation can restore ovary function through increased estrogen production as well as enhanced folliculogenesis on a rat model with ovariectomy in a short period.

These findings offer new insights into further understanding of stem cell-based therapeutic mechanisms for organ function regeneration and should provide new avenues to develop more efficient therapies using new 3D cultivation system.

P358 Multi-variate bioreactor with independent or interlinked sensing and actuating mechanisms

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Bioreactors are a broad category of devices used in the areas of bioprocessing and tissue engineering/regenerative medicine, to improve, refine or optimize a biological process. In the context of the field of Tissue Engineering, where we have a scaffold providing substance, shape and support, alongside cells providing biochemical and biological functionality specific to said tissue/organ, the bioreactor may be employed as part of the actual scaffold *fabrication* step, as part of a *conditioning/cultivation* step that may involve living cells, or both.

In this work, we set out to add further functionalities to a proprietary bioreactor platform that enables both these functionalities in a streamlined process workflow. The bioreactor platform should possess the necessary automation and integration of standalone devices that may be categorized as either sensors or actuators. The automation and integration approach seeks to minimize manual handling of the two basic tissue entities, substance and cells, that are to be used in the tissue fabrication and conditioning/cultivation steps. The sensing technologies employed include on-line temperature, pH and flow measurement, which are fed to the central controller. Depending on the configuration, the system may be set to maintain the sensed value around a set point (e.g., constant temperature, pH, flow) or set to a certain regime (e.g., variable temperature, pH and flow). Such a regime may be set to mimic a specific tissue environment, such as cold/warm air fluctuations on respiratory tract tissues. Depending on the setting, and sensed parameters, actuating devices such as perfusion, mixing and dispensing units are engaged accordingly, in a dynamic feedback manner. Remote control of all system functionalities, with visual monitoring through integrated cameras, in addition to the configuration of custom alarms with e-mail notification upon engagement, enable efficient control capabilities to the operator. Data logging may be configured within the same interface as the other system functionalities.

We have developed a flexible platform consisting of a central processing unit fitted with touch-screen technology to produce, condition and cultivate biofabricated tissues, in various shapes - circular, rectangular and tubular. Additive Manufacturing is employed for certain components, enabling specific anatomical features. Hollow organs such as whole kidneys, livers and hearts may be decellularized and recellularized using the same platform with its associated consumables and tissue/organ chambers. Mathematical and CFD models allow for monitoring of wall shear stress on the biofabricated tissues, measured in dyn/cm^2. All components contacting tissues/organs during fabrication, conditioning and cultivation are sterilizable by standard methods. The ease of operation and multitude of definable parameters, set to steady-state or dynamical regimes, where the parameters are either independent or interlinked to each other, enable highly flexible and versatile uses for Tissue Engineering and Regenerative Medicine applications.

Keywords: bioreactors, automation, additive manufacturing, collagen, shear stress

P359 Cell manufacturability for retina epithelial pigment cells derived from hiPS cells

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The manufacture of cultured cells and tissues is still burdened by instability owing to the qualitative fluctuation of cell sources, the risk of biological contamination and skill of experts. Efforts to commercialize cell-based therapies are driving the need for capable, scalable, manufacturing technologies. The development of a processing system is considered to lead to "Safety, Security and cost-Saving (3S)". However, the criterion of process design based on the 3S to date has not been clear, and a novel strategy for practical cell manufacturing is required. “Design for manufacturability (DFM)” is known to be the general engineering art of designing products in such a way that they are easy to manufacture. This concept exists in almost all engineering disciplines, but the implementation differs widely depending on the manufacturing technology. Therefore, the DFM for cell production will lead to facilitation of the consistency and robustness for process as well as reduction cost for the cell manufacturing. The cell processing can be denoted as a “tailor-made process” with low reproducibility, during which a number of manual procedures relying on empirical knowledge and the proficient skill of the operators are included. It can be said in regenerative medicine and cell therapy that “the process is the product”. Consequently, technical development is the key issue for cell manufacturability to realize the3S.

The system consists of input and output for the process, and there are several fluctuations derived from extrinsic noises (environmental errors) against the system, input quality such as starter cells and materials (medium, reagents, vessel and pipet etc.), and intrinsic disorders (in-process errors). Especially, intrinsic disorders cause the difficulty to make consistency and robust process for stable quality because the cells have uncertainty accompanied by time-dependent and time-delay properties. Therefore, environmental, material, and operational standardizations are required to realize consistent process.

In the present study, the stability in the processes through the maintenance cultures of iPS cells and the differentiated cells of the retina epithelial pigment cells was estimated by measurement of the motion fluctuation during the several operations. The variation in motion was revealed between manual and automated operations.

P360 Miniaturized high-content imaging (HCI) of 3D-cultured cell microarrays for mechanistic toxicity assessment

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The key to accurately predicting toxicity and efficacy of drug candidates is to understand complex biological functions and the mechanism of action of a compound. High-content imaging (HCI) assays provide valuable information on complex biological processes with various fluorescent dyes and become an important tool for predicting toxicity and efficacy of drugs. However, the majority of HCI assays have been performed on two-dimensional (2D) cell monolayers for high throughput, which often do not represent the *in vivo* characteristics accurately and therefore reduce the predictability of drug toxicity/efficacy *in vivo*. Three-dimensional (3D) cell cultures provide a clear understanding of morphological and functional features of human tissues, but current 3D cell culture systems are limited by relatively low throughput and difficulty in cell imaging. To alleviate these issues, miniaturized 3D cell culture and high-throughput HCI capability have been demonstrated on a micropillar/microwell chip platform to investigate mechanistic profiles of compound-induced toxicity. As a proof of concept, Hep3B human hepatoma cell line was mixed with alginate and fibrin gel, printed on the micropillar chip, and cultured in 3D over time in the microwell chip containing growth media. The 3D-cultured Hep3B cells were treated with six model compounds, including acetaminophen, lovastatin, rotenone, tamoxifen, menadione, and sodium citrate (control). Mechanisms of toxicity of these compounds were investigated by analyzing multiple parameters such as DNA damage, mitochondrial membrane potential, glutathione level, and cell viability using hoechst 33342, tetramethyl rhodamine methyl ester (TMRM), monochlorobimane (mBCl), and calcein AM, respectively. The IC_{50} values were determined and compared for each parameter and compound to investigate the main mechanism of toxicity. Conclusively, we were able to successfully demonstrate miniaturized HCI assays on 3D-cultured Hep3B cell microarrays for high-throughput investigation of mechanistic profiles of compound toxicity.

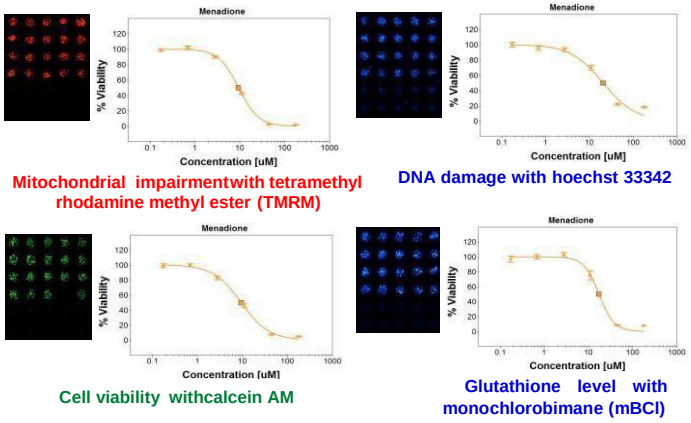

Figure 1. Assessment of mechanistic toxicity of menadione on Hep3B cell microarrays.

P362 Cancer cell tracking using dendrimer nanoparticles uptake in a microfluidic platform

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Labeled nanoparticles (NPs) have been proposed to monitor cells in real time, and consequently improve the prediction of the efficiency of any new cancer therapy *in vivo*. Additionally, when associated to a microfluidic device, such as Vena8 biochip, it's possible to realize functions *in vitro* that are not easily imaginable in conventional biological analysis due to its exceptional *in vivo* like setting. In this study, we aim to validate an improved platform composed of labeled dendrimer nanoparticles and a microfluidic device for real-time monitoring of new therapies. Cancer cells' tracking was achieved by grafting fluorescent label probe Fluorescein-5(6)- isothiocyanate (FITC) to dendrimer nanoparticles allowing the investigation of its uptake by cells and visualization within the microfluidic device by fluorescence microscopy. The physicochemical and biological characterization of recently developed Carboxymethyl-chitosan/poly(amidoamine) (CMCht/PAMAM) dendrimer nanoparticles were performed using TEM, AFM, Zeta Sizer and cytotoxicity screening. Cancer cell lines derived from different tumor types, including HeLa (Cervical Carcinoma), HCT-116 (Colon Carcinoma) and U87MG (Glioblastoma), were exposed to different concentrations of CMCht/PAMAM dendrimer nanoparticles over a period of 3 days. After finding the highest non cytotoxic nanoparticle concentration, cell viability and internalization efficiency were investigated, in both static (standard cultures) and dynamic (microfluidic cultures) conditions using flow cytometry. All three cancer cell types widely internalized nanoparticles and remain viable when cultured in the microfluidic chip Vena8, although higher sensitivity was observed compared to standard cultures (Fig.1). This work demonstrated that the proposed platform allowed real-time cell monitoring, which could be used for the screening of new therapeutics and consequently the improvement of cancer research.

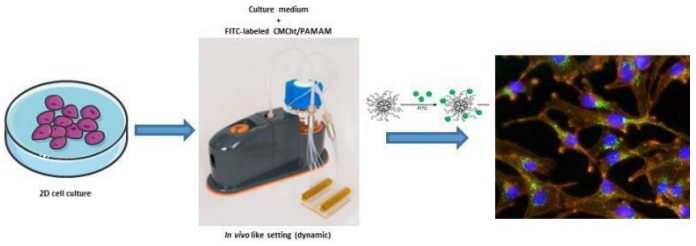

Figure 1 – CMCht/PAMAM dendrimer nanoparticles as a proper tool to track cells in a semi-automated microfluidic platform that can be used to test drug efficiency. (Cellix®, Irland).

P363 Next-generation microfluidic platforms for patterning stem cells

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Tissue patterning occurs during organogenesis, diseases and repair via coordinated cell migration, proliferation and differentiation. While some underlying mechanisms have been decoded, numerous remain elusive. Here we present new microfluidic tools to reconcile directed differentiation of stem cells and control cell patterning processes, which will eventually pave the way to create engineered tissues with increased complexity and functionality. Utilizing biofunctionalized microfluidic systems fabricated with our proprietary micro- and nanoscale polymer film forming and functionalization technology termed 'Substrate Modification and Replication by Thermoforming' (SMART), we study the formation of polarization events in stem cell clusters. We explain the development of tools, e.g creation of well-defined gradients, using which we can interfere with these intrinsically-controlled pattern formations.

In an initial step, we used COMSOL to model the effect of different flow rates and concentrations of morphogens. We varied concentrations of morphogens (TGFß, Activin A and BMP4) known to induce lineage differentiation in mouse embryonic stem cells (mESCs) from 0 to 100 ng/ml. Different flow rates ranging from 100 µl/min to 1000 µl/min were used to create gradients of the abovementioned morphogens. These results will lead to experiments using mESC clusters which will be used to verify the accuracy of the microfluidic simulations. Initial experiments along these lines, including live cell imaging have shown the high potential and capability of these microfluidic tools which could eventually be used to better control and understand complex cell patterning in vitro. Understanding these phenomena even will eventually aid us in the development new techniques to better support and improve current tissue engineering and regenerative medicine approaches.

P364 Modelling of 3D spatially controlled compartmentalized tissues in microfluidics

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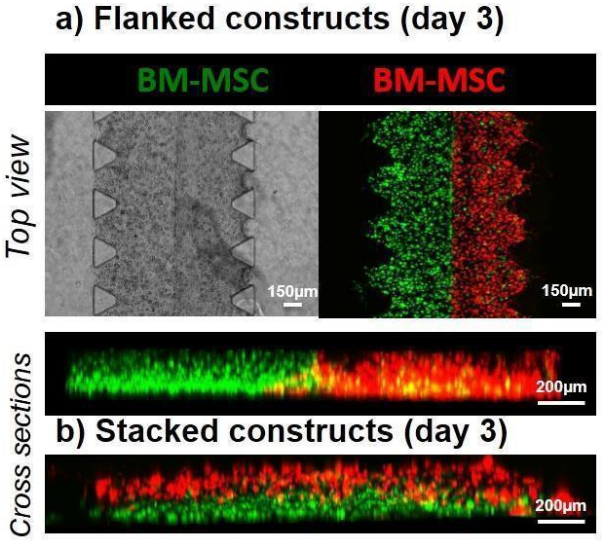
Organs-on-chip have been widely addressed as potential tools for recreating tissue structure and functions within microdevices. In perspective, the possibility of engineering cellular three-dimensional constructs with behaviour similar to physiological tissues or organs is a paramount aim for improving basic research studies and drug screening processes [1]. Biological functional structures are typically characterized by a compartmental architecture where multiple cellular units made up of different cell types and/or extracellular matrix are spatially organized to interact and contribute to biological homeostasis and function. Nevertheless, it is currently challenging to neatly interface multi-compartmental 3D biological constructs within microfluidic systems and there is a need for techniques that allow fine spatial control of 3D cell-laden matrices [2]. We here present a novel microfluidic technique for engineering complex micro-tissue structures made of controlled multi-compartmental three-dimensional cellular constructs. By employing molding PDMS layers, we show how to form pure composites of two stacked or flanking tissue constructs (made of human bone marrow-derived mesenchymal stem cells, Panel A and B) within existing microfluidic systems commonly used for controlled presentation of soluble factors (differentiation factors, drug compounds, etc.) or application of medium perfusion. We then applied this technique to form endothelialized constructs with vessel-like structures within microtissues. We demonstrate cell viability, continuity of composite constructs and endothelial barrier formation. As no confining structures (pillars or phaseguides) are present at the generated interfaces, this technique holds promise for advanced modelling of complex multi-compartmental tissues/organs and ongoing work is aimed at generating micro-tissues of relevant physiological structures (blood-brain barrier and osteochondral interface).



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P365 Breaking balance of cellular forces within the expanding Madin-Darby canine kidney (MDCK) cell monolayer by hepatocyte growth factor (HGF) gradient

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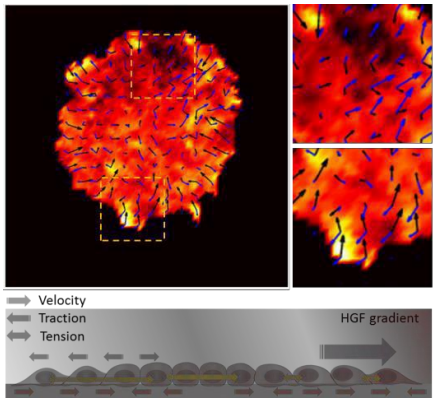
Collective cell migration is a common phenomenon observed in many important biological events such as developmental processes, wound healing, and even cancer metastasis. The collective movement is governed through the orchestration of cellular physical and biochemical interaction by biomolecules, surrounding cells and extracellular matrix (ECM). However, existing analysis methods and experimental models for studying collective cell migration have limitations in quantitatively observing the distribution of biomechanical changes during collective migration under the condition mimicking physiological environment, such as renal regeneration by the interstitial flow with hepatocyte growth factor (HGF) gradient. In this study, we integrated a microfluidic chip with traction force microscopy in order to quantitatively analyze the biophysical aspects of collective migration of the patterned Madin-Darby canine kidney (MDCK) cell monolayer under the gradient of HGF. The results showed that the changes of anisotropic movement and shape of the cellular monolayer were observed toward the source of the highest HGF concentration. The cellular tension in the monolayer was low at the point where the angle difference between the cellular velocity vector and the traction vector was small and the tension was high where the angle difference between the two vectors was large. Converting the result to the angle distribution plots, imbalance of the migration directionality was clearly observed by the HGF gradient, but the distribution of traction force directions showed a balanced ellipse-distribution extended along the axis of biased mobility. The frequency of small angle between the two vectors of velocity and traction was found in the higher concentration of HGF reflecting that the higher the concentration of HGF, the higher the dependence of the tensile force on cell migration. As a result, we can conclude that the local tension generated by the migration deflection caused by the HGF gradient changes the expansion of the cellular monolayer biased, resulting in symmetry breaking.



P368 A hybrid electrospun titanium or zinc oxide-polyurethane as a material for self-cleaning shunt catheters

Davide Erbogasto, Richard Black

University of Strathclyde, Glasgow, UK

Infection is one of the causes of failure of shunt catheters employed in the treatment of hydrocephalus, affecting one in every eight patient. Another major complication is shunt obstruction by cell adhesion and proliferation. Hydrophylic surfaces are effective in both inhibiting bacterial infection and reducing cell adhesion.

Two hybrid electrospun materials are hereby investigated, comprising a medical grade polyurethane (b9 'A' series, Biomer Technology Ltd, Runcorn, UK) and titanium dioxide and zinc oxide, respectively: an *in-situ*, nonaqueous sol-gel reaction was employed to synthesize inorganic oxides of titanium and zinc within the polyurethane matrix.

The microstructure of the polyurethane, which is a segmented polymer, is altered by the sol-gel process, which obtains a good dispersion of the inorganic component within the organic matrix, and results in a homogenous surface, with considerable changes to the surface properties of the polyurethane.

While untreated polyurethane exhibits a contact angle of about 90°, adding titanium dioxide and zinc oxide leads to an increase in hydrophilicity. Likewise, the adsorption of protein (bovine serum albumin) on the modified materials is greater when compared to unmodified polyurethane.

The materials tested negative for cytotoxicity using human glioblastoma astrocytoma immortalized cells (U373). Cell migration studies show how the migration rate and the mobility on treated electrospun materials is considerably higher than on polydimethylsiloxane, which is the standard material currently used for shunt catheters.

Such hybrid materials exhibit photocatalytic properties upon irradiation under UV light, as shown by the reduction of Rhodamine B in solution. Such properties confer antibacterial characteristics on the materials, which may render them effective for a range of applications where self-cleaning surfaces are required.

P370 The effect repeated intrathecal and intramuscular application of mesenchymal stem cells and their secretome in the treatment of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is characterized by degeneration and subsequent death of motor neurons leading to muscle atrophy, paralysis and death due to disorders of the respiratory muscles. Although the mechanisms responsible for motor neuron degeneration in ALS remain unclear, cell transplantation is considered as a promising approach for replacing damaged cells and promoting neuroprotective and neuroregenerative repair.

We investigated the effects of repeated intrathecal application of human mesenchymal stem cells (hMSC) in the SOD1-transgenic rat model of ALS. The aim of this project was to compare repeated intrathecal application of MSCs and conditioned medium (CM) obtained from these cells in the treatment of animal model of ALS disease. hMSC and CM-MSC were transplanted into asymptomatic rats (17 weeks old). Treatment with hMSC was also combined with MSC intramuscular application into hindlimb muscles to prevent dying-back axonaldegeneration.

Lifespan, the course of the disease and functional outcome was monitored behaviourally (BBB, rotarod, grip strength tests). We analysed protein and mRNA expression of apoptosis-related genes (Bak, Bcl-2, Casp-3) and necroptosis-related genes (Rip1, Rip3, Mlkl) using Western blot and RT-qPCR.

We found that repeated intrathecal application of hMSC alone or combined with intramuscular injection ameliorated disease progression, significantly improved motor activity, increased number of spared motor neurons, and prolonged life span by 20 days when compared to the control group. We observed reduction mRNA expression Ripk1, Ripk3, MLKL in the treated ALS rats suggesting that applications of MSC decrease necroptosis. Much less effect was observed after application of CM or intramuscular injection only.

The potential effect of repeated application might be even more beneficial to ALS patients, in which disease progression takes years and not weeks, as it is in the rat experimental model.

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P372 Evaluation of adipose stem cells (ASCs) impact on central nervous system in vitro

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Aims: The main therapeutic attributes of mesenchymal stem cells (MSCs), including adipose stem cells (ASCs) are their ability to migrate into injured sites, promote functional recovery and modulate immune responses. Despite the use of ASCs in a large number of clinical trials, cellular and molecular mechanisms underlying ASCs therapy to enhance neurological outcomes are not fully understood [1]. It is known that ASCs neuroprotective effects are mainly based on anti-inflammatory activities [2], however the therapeutic effect of direct cell-to-cell contact can not be excluded. The purpose of this research was to evaluate ASCs neurological potential, impact of their direct and indirect contact with cells of central nervous system (CNS) and investigate potential of ASCs to improve injuries in CNS.

Materials and methods: ASCs were enzymatically isolated from fat tissue and expanded in vitro until achieving homogenous population. Neurons, isolated from rat cortex were cultured in vitro and mechanical damaged using standard scratch-test technique. ASCs were co-cultured with damaged neurons in order to evaluate ASCs therapeutic potential. Alternatively, the supernatant from ASC cultures was added to culture of damaged neurons. ASCs were also co-cultured with not damaged neurons to assess their potential to differentiate into neural lineage, analyzing expression of Nestin (NES) - neural marker gene.

Results: Addition of adipose stem cells improved healing process in damaged neurons culture. Survival time was longer and damage was smaller than in neurons without ASCs. ASCs co-cultured with neurons had shown higher expression of Nestin, demonstrating their potential to differentiate into neural lineage.

Impact of investigation: The number of patients with neurodegenerative diseases is rapidly growing due to process of societies aging. Currently there are no effective treatment methods for such diseases as Parkinson's disease or Alzheimer disease. Therefore there is a big need to investigate new treatment methods and mesenchymal stem cells (MSCs) seem to be a good research direction. The present study demonstrates that adipose stem cells have neuroprotective potential, they can extend survival time of damaged neurons and they are capable to differentiate into neural lineage. It have been proven earlier that mesenchymal stem cells, including ASCs have anti-inflammatory properties and they can modulate immune response, the addition of neuroprotective potential makes adipose stem cells potential candidate to improve central nervous diseases treatment.

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P373 Generation of vascularized constructs for transplantation in large animal models

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Introduction:

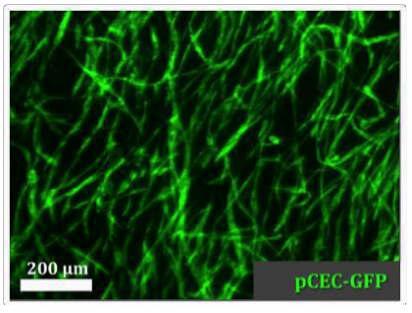
The goal of tissue engineering is the generation of functional tissue *in vitro* for the reconstruction of failing organs. Aiming at the generation of constructs with clinical relevant dimensions, an *in vitro* and/or *in vivo* vascularization is mandatory to supply cells within the construct with nutrients and oxygen. Here, we report the generation of constructs exhibiting endothelial cell networks using porcine cells. Porcine cells are applied for prospective large animal studies. We discuss the influence of cell culture medium, cell composition and cell density on network formation. The resulting construct could be an universally applicable starter matrix for any type of engineered tissue in need of vascularization.

Material and Methods:

Primary porcine endothelial cells (pCEC) were isolated from coronary arteries and cultivated in EGM–2. Primary porcine stromal cells (pASC) were isolated from adipose tissue and cultivated in M199/10%FCS/basic-FGF. Cells were subsequently labeled by GFP or RFP by lentiviral transduction, respectively. Hydrogels based on matrigel and collagen containing different cell ratios ranging from 1:5 to 5:1 (pCEC:pASC) were casted onto decellularized porcine small intestinal submucosa (SIS). Constructs were cultivated in three different cell culture media. Cell densities of 4 up to 12 million cells per mL were investigated. Constructs were monitored via fluorescence stereo microscopy for 14 days. Texas Red-labelled dextran was applied to explore the formation of lumen. The constructs were fixed and cryosections were analyzed by fluorescent microscopy.

Results:

Network formation was supported when constructs were cultivated in M199/10%FCS/basic-FGF. A homogeneous network with long cords was achieved at a cell density of 4 million cells per mL whereas higher cell densities exhibited more filigree and thinner networks. Different cell ratios of pCEC and pASC had a minor influence on network formation. Cryosections revealed first hints for the formation of hollow structures visualized through the accumulation of Texas Red-labeled dextran within GFP-pCEC structures.



Discussion and Conclusion:

Network formation of pCEC in co-culture with pASC was achieved and optimised. First hints suggest formation of hollow structures being a prerequisite for a connected and perfusable network. The application of growth factor gradients, hypoxia or flow may be required for further maturation of the network. Casting of such hydrogel constructs on top of a decellularized biological vascularised matrix (BioVaM) with preserved mesenteric arterial and venous pedicles instead of SIS could lead to a connection of the preserved vessel bed and the network in the hydrogel finally resulting in a vascularised and perfusable conctructs which offers the possibility for anastomosis to the host circulatory system during implantation.

P374 Urokinase system and extracellular matrix produced by cells are crucial for robust functional vascular network formation by cooperation of adipose stromal cells and endothelial cells

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For vascularized tissue engineering the deep insight into the cellular and molecular events involved in the formation of blood vessel network is very important. In this study, we used a two-dimensional co-culture of human umbilical vein endothelial cells (HUVEC) with human adipose stromal cells (ASC) without exogenous matrix addition to study self-assembled network formation(SNF) by HUVEC. We have found that only co-culture of these cells in direct contact (but not out of contact co-culture in transwell) induced SNF by HUVEC. HUVEC start network formation after 14 hours of co-culture with ASC that coincides with synthesis and secretion of extracellular matrix (ECM) by co-culturing cells. However, HUVEC seeded on decellularized ECM produced by co-cultured or monocultured cells did not form SNF in contrast to HUVEC seeded on Matrigel readily starting tubular network formation after 4 hours. We have found that ASC produce fibronectin (FN) more intensively than HUVEC, and after 24 hours of co-culturing Fn cords are clearly visible and create the basis for ECM assembly through binding to other ECM proteins. To confirm the role of ECM in SNF we cultured ASC during 48h so they synthesize enough matrix, and after that we seeded HUVEC on ASC and co-cultured them for additional 24 hours. In this case we observed visible SNF by HUVEC contrary to HUVEC monoculture and coculture during 24 h. Moreover HUVEC form SNF along the Fn cords. Blocking of integrin subunit alpha v by antibodies decreased SNF in this model suggesting possible participation of urokinase system in this process through interaction with vitronectin and its integrin receptors. We observed that co-culture results in up-regulation of urokinase-type plasminogen activator (uPA) expression, its high affinity receptor (uPAR; CD87) and plasminogen activator inhibitor 1 (PAI-1). uPA, uPAR and PAI-1 have been implicated in regulation of several steps of angiogenesis - endothelial cells division, migration, degradation and invasion of the abluminal basement membrane and formation and stabilization of vascular network. uPA promotes pro- angiogenic signaling upon binding to several interacting surface receptors, including uPAR, low- density lipoprotein-related receptor (LRP/a2MR) and specific integrins. uPAR-bound uPA is typically localized on the leading edge of the migrating endothelial and other cells. Upon direct HUVEC –ASC co-culture uPAR was up regulated on the surface of HUVEC and the concentration of uPA increased in conditioned medium. uPAR is essential for SNF since blocking of uPAR by specific antibodies abrogated this process. We also found that endocytosis machinery is important for SNF by HUVEC in presence of ASC because addition of the LRP antagonist - RAP, inhibitor of intracellular protein transport - monensine, or inhibitor of microtubule polymerization - colhicine inhibited this process. Thus ASC provide specific environment for migration of HUVEC and SNF, which includes ECM proteins, alpha v integrin, uPA-uPAR-LRP system.These results enable insights into the process of vascular morphogenesis and should be taken into consideration when vascularized tissue engineering constructs will be developed. The work was supported by RFBR № 16-04-01699 and RSFG № 17-15-01368

P375 Cardiac bone marrow-derived cell-based therapy associated with scaffold for heart regeneration

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Purpose: Currently, no curative treatment exists for patients that survived acute myocardial infarction (MI). MI progresses towards a chronic phase and severe heart failure. The development of patched associating of bone marrow derived cells (BMDC) and a substrate/scaffolds provided evidence of beneficial outcomes. For clinical application, autologous BMDC isolated from the infarcted patients have been used. However, they might have different regenerative potential compared to BMDC cells from healthy donors. To test this hypothesis, in the present study, we compare the regenerative potential of a biological patch composed of BMDC isolated from healthy or infarcted donor using a rat MI model.

Methods: BMDC were isolated for healthy and infarcted male rats. MI was induced in 52 female Lewis rats by Left Anterior Descending Artery ligation. Troponin level at 24 h increased in all animals and cardiac function was measured by high-resolution echocardiography Two weeks post MI. Thirty four animals with an ejection fraction between 35-60% were distributed in different treatment groups, sham operation (Sh, n=9), epicardial application of the biological patches obtained with 2 million cells isolated from healthy donors (HD, n=12), or from infarcted donors (ID, n=7) or substrate only (S, n=6). Four weeks post treatment, heart function, blood analyzes and histology were performed.

Results: Four weeks post MI, heart function decreased was recorded for Sham group (ΔEF_{Sh} = - 1.21±3.2%) and increased in groups with biological patches implanted. Significant improvement of heart function were observed with both treatment compared with substrate only. (ΔEF_{HD} = 1.1±5.6%) vs (ΔEF_{ID} = 0.99±4.9%) vs (ΔEF_{S} = -7.4±6.4%). The regional LV myocardial function using strain imaging demonstrated after treatment an increase in the extent of the non-contractile region for sham and substrate groups and a decrease when biological patches were applied; the Infarct extents before and after the treatment were respectively 10.8±3.3mm and 8.3±4.0mm for HD (p=0,02). Histology showed that compared to sham , the index expansions (EI) decreased with the implantation of the biological patches, and were respectively EI_{Sh} = 0.21±0.11 ,EI_{HD} = 0.08±0.04(p=0.05), EI_{ID} = 0.15±0.07(p=0.47). Additionally, an increased of the systolic and diastolic volume obtained after implantation of the infarcted donor patch was observed.

Conclusion: This on-going study demonstrated that the implantation of a biological patch from healthy donor reduced the infarct expansion and improved heart function suggesting a regenerative capacity. Noticeably, LV volumes increased following infarcted donor patch implantation and therefore questioned the long term effect. Large heterogeneity within treated groups revealed the presence of respondent and non-respondent subjects to the treatment.

P376 Short-term delivery of fibrin-bound VEGF protein in osteogenic grafts: increased vascularization with efficient bone formation

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Introduction: Reconstruction of large bone defects is a major challenge in tissue engineering. Bone marrow mesenchymal stem cells (BMSC) are valuable multipotent progenitors for regenerative medicine. Spontaneous vascularization of BMSC-loaded osteogenic grafts in vivo is too slow to allow survival of the progenitors in constructs larger than a few millimeters. Stimulation of graft vascularization in vivo is needed for cell survival and efficient bone formation. Vascular endothelial growth factor (VEGF) is the master regulator of angiogenesis. However, it was previously found that, while sustained over-expression of VEGF by genetically modified human BMSC was effective to improve vascularization of tissue- engineered bone grafts, it also caused an undesired increase in osteoclast recruitment with excessive bone resorption. Here we hypothesized that short-term controlled delivery of VEGF protein bound into a fibrin hydrogel may improve graft vascularization without impairing bone formation.

Methods: Primary human BMSC were retrovirally transduced to express VEGF linked to a truncated version of CD8, acting as a surface marker. Recombinant VEGF was engineered with the transglutaminase substrate sequence NQEQVSPL (TG-VEGF) to allow covalent cross-linking into fibrin hydrogels and release only by enzymatic cleavage. BMSC were seeded on apatite granules in fibrin pellets. Bone formation and vascularization were determined histologically 1, 4 and 8 weeks after ectopic subcutaneous implantation in nude mice.

Results: One week after implantation, both the constructs with naive BMSC in fibrin-bound TG-VEGF and those with VEGF-expressing BMSC in empty fibrin (VICD8) already displayed increased vascularization compared to the controls with naive BMSC in empty fibrin only. After 4 weeks fibrin gels were completely degraded in all conditions. After 8 weeks both fibrin-bound TG- VEGF and VEGF-expressing BMSC induced significantly increased vascularization compared to naive BMSC only. However, while bone formation was severely impaired with VEGF-expressing BMSC as expected, fibrin-bound recombinant TG-VEGF allowed the formation of bone tissue as efficiently as by naive BMSC alone.

Conclusions: These data suggest that VEGF effects on promoting vascularization and bone resorption can be uncoupled by short-term delivery of recombinant VEGF protein, providing an attractive and clinically applicable strategy to ensure both rapid vascularization and efficient bone formation, while avoiding the safety concerns related to genetic modification of progenitors.

P378 Controlled in vivo angiogenesis in specifically biofunctionalized elastin-like recombinamer-based scaffolds

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One of the most important facts for a tissue regeneration based on engineered scaffolds is a proper angiogenesis. If nutrients are not able to reach all corners within the scaffold, cells will not be able to colonize it or they will die at some point. Many efforts have been done to control angiogenesis, improving it but without obtain aberrant formations as angiomas.[1] Here, we present a bio-activated material which promote or inhibit angiogenesis without the need of external vascular grow factors. Human adipose tissue-derived stromal vascular fraction (SVF) cells are reported to be highly angiogenic thanks to their heterogeneous composition which includes both vascular progenitor and mesenchymal stromal cells. Elastin-Like Recombinamers (ELRs) are protean biopolymers that due to their flexibility in the design, easy chemical modification allowing the introduction of many interesting functionalities. These ELRs have a high potential in tissue engineering [2]. ELRs containing general and specific endothelial cell adhesion sequences (RGD and REDV) have been synthetized to create functionalized gels to enhanced *in vivo* angiogenesis and integration within the host surrounding tissues. In this study, we modulate the angiogenic potential of human SVF cells by simply varying the cell adhesion properties of ELR hydrogel. Non functionalized ELRs were used as negative control. To evaluate the in vitro capacity of pre-vascularization, 2.2 million SVF cells were first encapsulated in not functionalized (NF) or RGD-REDV (RR)- based ELR hydrogels and then cultured either in static or under perfusion conditions. Engineered tissues generated in perfusion culture were further assessed for their *in vivo* angiogenic potential. Four constructs per animal were inserted in subcutaneous pockets created in the back of male nude rats. To assess the cell proliferation in the scaffolds, DNA analyses were performed. Pro- and anti- angiogenic factors were evaluated by ELISA analyses, production of VGEF was measured, histology (H&E) and staining against vimentin, CD31 and CD45 were performed. Quantification of vessel length density (VLD) was carried out. All these results together with scanning electron microscopy (SEM) images showed that ELR gels result in a flexible tool for regenerative medicine applications, by differentially modulating the angiogenic and host integration efficacy of engineered implants, even when generated by highly vasculogenic cells.

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P379 Functionalising a collagen-based scaffold with on-demand drug delivery for diabetic wound healing

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Diabetes affects over 345 million people globally, with diabetic foot ulcers (DFUs) occurring in 15% of all diabetic patients. If DFUs remain chronic, they have the dire consequence of lower- leg amputation. Non-pathologic wound healing is exquisitely well controlled from a temporal perspective; however, this sequence is not observed in pathological diabetic wound healing. To address the DFU challenge, we are developing a collagen-glycosaminoglycan (CG) based wound-healing device with incorporated ultrasound-responsive alginate microparticles that are capable of delivering bioactive agents on-demand. We hypothesise that by mimicking nature's temporal profile for angiogenesis (i.e., sustained VEGF and delayed PDGF signalling), we can drive the sequential healing of DFUs. To control the temporal sequence of factor delivery, we will exploit the precise control possible with ultrasound- responsive alginate that we've previously developed[1,2].

PEGylated gold nanoparticles (PEG-AuNP) were chosen as a model drug and these were incorporated into combinations of high (~250kDa; 0 – 1%) and low (~70kDa; 0 – 1.5%) molecular weight alginate, which was electrosprayed to form microparticles ($CaCl_2$ crosslinked). A CG slurry was blended using type I bovine collagen (5 mg/ml) and chondroitin-6-sulfate (0.44 mg/ ml) in 0.05M acetic acid solution. The microparticles (20% final volume) were mixed with the CG slurry and cast in steel trays for freeze-drying. Scaffolds were crosslinked (by UV, dehydrothermally or by carbodiimide chemistry). Ultrasound was applied (0 – 40% amplitude) to assess AuNP release and SEM was used to assess the affect of microparticle incorporation and ultrasound stimulation on pore structure. PDGF coated AuNPs were then developed and their bioactivity confirmed using an MSC proliferation assay. Finally, optimum PDGF dose and timing was assessed in an in vitro vascularisation assay in the CG scaffolds.

The ability to incorporate PEG-AuNP loaded microparticles in collagen-GAG scaffolds was first confirmed. SEM images revealed local disruption of pore structure neighbouring the microparticles but an open interconnected porous structure in the remainder of the scaffold. Using ultrasound, the ability to release PEG-AuNP from the constructs was confirmed (up to 50% release in <5min); baseline release of AuNPs was negligible. Stable PDGF-AuNPs were then developed and enhanced MSC proliferation (30% in 48hrs). An in vitro vascularisation assay assessed different temporal sequences of PDGF addition, and delayed PDGF addition outperformed all control samples.

These results demonstrate the potential for this alginate-doped collagen-GAG scaffold to facilitate cell seeding and to locally deliver drugs on-demand. By releasing drugs at specific timepoints, the current challenges in healing poorly sequentially controlled wounds may be overcome. Future work will demonstrate the devices' potential to coordinate angiogenesis and direct healing.

P381 Human bone marrow mesenchymal stromal cells, adipose tissue-derived stromal cells and bone marrow mononuclear fraction improve wound healing in diabetic rat model

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INTRODUCTION: The biological properties of mesenchymal stromal cells (MSCs) have the potential to provide a favourable environment for wound healing and angiogenesis. This is highly demanded in diabetic patients, where prolonged and incomplete wound healing result to diabetic ulcer formation, and in many cases even limp amputation. To evaluate the therapeutic effect of MSC from various sources, we explored the process of full-thickness wound healing in diabetic rats treated with human MSCs isolated from bone marrow (BM-MSCs), adipose tissue (ASCs), or bone marrow mononuclear cells (BMC) obtained from diabetic and healthy donors.

METHODS: BMC were isolated using Gelofusine separation, BM-MSCs and ASCs were isolated and cultured according to standard protocols. Diabetes was induced in Wistar rats by a single intraperitoneal injection of streptozocin 64 mg/kg and confirmed 48 h later by a blood glucose level higher than 15 mmol/l. Two full-thickness skin wounds, 0.8 cm in diameter, were created by a punch biopsy at the back of each animal. The cell suspension ($2x10^5$ for BMC, $2x10^6$ for BM- MSCs and ASCS,) was injected into the base of the wound, and its close surroundings. The speed of wound closure and vessel index (VI), assessed as number CD31+ cell number in wound area, were determined after 3, 7 and 14 days.

RESULTS: A significant acceleration of wound healing was seen in all cell treated groups in comparison with controls after 7 and 14 days (BMC $p<0.001$; BM-MSCs $p<0.01$; ASCs $p<0.05$). No difference between diabetic cell- and nondiabetic cell-treated groups was observed. VI was significantly higher in all cell-treated groups in comparison to controls ($p<0.05$). However, lower VI was found after treatment with diabetic BMC in comparison to the nondiabetic BMC and nondiabetic BM-MSCs, ($p<0.05$).

DISCUSSION & CONCLUSIONS: Both diabetic and nondiabetic stromal cells improved healing of full-thickness wounds in diabetic rats. However, better neovascularization was found after treatment with nondiabetic cell source. BMC, BM-MSCs and ASCs represent a safe and efficient allogeneic as well as autologous cell source for improvement of wound healing in diabetic patients.

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P382 Electrospun yarns for vascularization applications in tissue engineering

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Tissue Engineering (TE) offers promising opportunities to treat injuries. TE constructs usually consist of mono-layers of cells. Till date, complex tissue structures are difficult to achieve due to several limiting factors. One of the most significant limitations in tissue engineering is the full supply of the cells throughout a three-dimensional scaffold with oxygen and nutrients. Thus angiogenesis, the formation of capillary-like structures in the tissue, is necessary to form more complex vessel networks in tissue engineered constructs. [4] The successful induction of angiogenesis and subsequent perfusion in three-dimensional cell culture is one of the largest, current challenges in TE.

The approach of this work deals with the integration of highly porous electrospun yarns (E-yarns) into fibrin scaffolds with a length of up to 10 mm. The E-yarns are placed vertically in a scaffold made of fibrin gel. To study the potential of E-yarns for an application either as guiding structures for angiogenesis or as a supply structure for oxygen and nutrients endothelial cells (ECs) and fibroblasts (HDFs) are co-cultured in the modified scaffolds. The aim of this research is to identify if E-yarns are suitable structures for the induction of orientated angiogenesis and the provision of TE-constructs thicker than 3 mm with oxygen and nutrients.

The E-yarns were produced using contrary charged (+ 25 kV/- 25 kV) spinnerets spinning on a rotating (200 rpm) funnel. The spun fibres created a network in the revolving funnel which was drawn off from the funnel (0.1 m/min) onto a spool. Through the rotation of the funnel the E-yarn was twisted.

The E-yarns were produced from blends of Polylactide (PLA) and PEG in varying ratios dissolved in chloroform. The individual fibres of the E-yarn contained both polymers PLA and PEG. The PEG content is used to tailor the pores which account for the liquid transport along the E-yarns. For cell application, ECs and HDFs as well as single electrospun yarns are embedded into a fibrinogen scaffold polymerized to fibrin by thrombin and CaCl2 addition. The cultivations conditions were set to 14 d of static co-cultivation and followed by (immune-) histological analysis.

The cell seeding of E-yarns with ECs showed that cells attached to the surface of the E-yarns and formed a cylindrical coating. Also it was possible to show that the E-yarns have a combination of pores along the electrospun fibers and micropores between the individual fibers in the E-yarn. This combination of pores led to a liquid transport along the E-yarns. The yarn count of the produced E-yarns was between 50 and 325 dtex. The elongation of those yarns was between 58 and 400 % and the measured tensile strength showed values between 1 - 1,8 cN/tex.

After the mentioned preliminary tests, the yarns were investigated regarding their ability to improve the supply with nutrients and oxygen in thicker tissue engineered constructs. The incorporation of E-yarns into scaffolds from fibrin-gel showed that they feature an overall improved nutrient supply.

P383 Developmentand characterisation of a composite skin construct with electrospun membrane andmicrochannel hydrogel

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Introduction

Perfusable three-dimensional skin constructs are important in providing a simulated physiological model for in vitro drug testing and as in vivo skin grafts. Electrospun membranes as an epidermal layer, act as a pseudo-basement membrane allowing for keratinocytes to proliferate and migrate across its surface. Meanwhile, microchannel hydrogels seeded with dermal fibroblasts (HDFs) and endothelial cells (HUVECs) form an endothelialised vascular network within the dermal construct, which allows for perfusion of the composite epidermal-dermal scaffold. In this study, we characterize a PLLA/gelatin hybrid electrospun membrane and used soft photolithography to develop the microchannel hydrogel scaffold.

Methods

8% PLLA/gelatin in HFIP were electrospun and fibre diameters were determined by scanning electron microscopy. Membrane mechanical properties were tested using the Instron universal testing machine. Membrane degradation by mass was measured. Viability and proliferation of keratinocytes seeded on electrospun membranes were determined using Live/Dead assay and MTT assay. Patterned microchannels templates were developed using SU-8 soft photolithography. 20% gelatin Type A was then carefully poured onto the template and crosslinked using EDC-NHS at 4 degrees overnight. The patterned hydrogels were then hydrated and tested for microchannel patency by injecting a red dye into the inlet ports. HUVEC and HDF cells were seeded within the scaffolds and cultured for up to 14 days. 3D imaging of the scaffold was done using confocal microscopy to monitor behaviour and morphology of the endothelial cells and vascularization.

Results

Smooth fibres measuring approximately 1-2 microns in diameter were obtained. Tensile strength of PLLA/gelatin membranes was 6.06 ± 0.50 MPa. Degradation by hydrolysis of the electrospun membrane was negligible but increased with the addition of trypsin. Cell proliferation increased significantly on Day 14 and cell viability was about 80% up to Day 14. The microchannel hydrogels maintained patency throughout the incubation period and endothelial cells proliferated and migrated across channel surfaces forming endothelialized lumens. The hydrogel was able to withstand contractile forces of the HDFs maintaining its microstructure without collapse.

Discussion

In this study, we have developed and characterized a novel bioengineered perfusable skin bilayer using electrospun membrane and microchannel hydrogels as epidermal and dermal components, respectively. Further in vitro and in vivo validation studies is required before translating this platform into the clinical setting.

P384 Alginate/Gelatin hydrogels to coat porous tubular scaffolds for vascular tissue engineering

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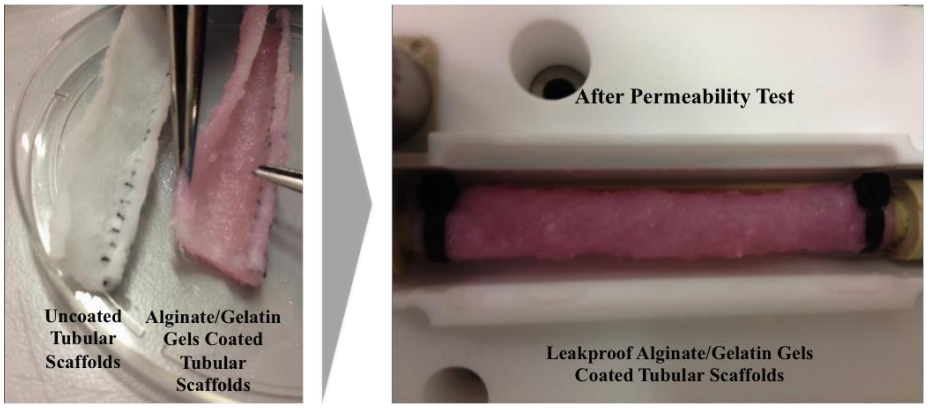
To perfuse porous tubular scaffolds for vascular tissue engineering (VTE) with a controlled flow rate, there is the need to prevent leakage from the scaffold lumen. A gel coating made of 8% w/v alginate and 6% w/v gelatin, at different volumetric proportions (50/50, 70/30 and 90/10), functionalized with fibronectin, was produced using a custom-made bioreactor-based method. Gel swelling and stability, and rheological and uniaxial stretch tests on different percentages of alginate and gelatin demonstrate the gel ability to resist to biochemical microenvironment, to well withstand physiological deformations (~10%) and wall shear stresses (5-20 dyne/cm^2). These are prerequisites to stimulate tubular scaffolds with the proper mechanical stimuli able to maintain the physiological phenotype of vascular smooth muscle cells and endothelial cells (ECs), as in blood vessels. Cytocompatibility shows gel capability to induce ECs proliferation and colonization within the gel, especially in presence of fibronectin and higher gelatin percentage. The custom-designed bioreactor is suitable to create reproducible and homogeneous tubular gel coating, independently from alginate percentage. Permeability test shows the effectiveness of 70/30 alginate/gelatin gel to occlude wadding pores, and therefore prevent leakages. In this regard, the synthetized tubular alginate/gelatin gel with fibronectin represents a promising substrate for ECs, as well as leakproof when the scaffold is submitted to pulsatile perfusion for VTE applications.



P385 Human fibroblast-derived matrix facilitates vasculogenesis in 3D environment and enhances skin wound healing

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Extracellular matrix (ECM) is crucial to many aspects of vascular morphogenesis and maintenance of vasculature function. Currently recapitulation of angiogenic ECM microenvironment is still challenging, due mainly to its diverse components and complex organization. Here we investigate human fibroblasts-derived matrix (hFDM) as an active component in building a 3D vascular construct. hFDM was obtained via decellularization of *in vitro* cultured human lung fibroblasts and analyzed via immunofluorescence staining and ELISA, which detect multiple ECM macromolecules and angiogenic growth factors (GFs). Human umbilical vein endothelial cells (HUVECs) morphology was much more elongated and better proliferative on hFDM than on gelatin-coated substrate. To prepare a three-dimensional (3D) construct, hFDM is collected, quantitatively determined, and incorporated into collagen hydrogel (Col) with HUVECs. Capillary-like structure (CLS) formation at 7 days was significantly better with the groups containing higher doses of hFDM compared to the Col group (control). Moreover, the group (Col/hFDM/GFs) with both hFDM and angiogenic GFs (VEGF, bFGF, SDF-1) showed their synergistic activity on CLS formation and found much larger capillary lumen diameters with time. Further analysis of hFDM via angiogenesis antibody array kit reveals abundant biochemical cues, such as angiogenesis-related cytokines, GFs, and proteolytic enzymes. Significantly up-regulated expression of VE-cadherin and ECM-specific integrin receptors was also noticed in Col/hFDM/GFs. In addition, transplantation of Col/hFMD/GFs with HUVECs in skin wound model presents more effective re-epithelialization, many regenerated hair follicles, better transplanted cells viability, and advanced neovascularization. We believe that current system is a very promising platform for 3D vasculature construction *in vitro* and for cell delivery toward therapeutic applications *in vivo*.

P386 Intestine re-endothelialisation for whole organ bioengineering

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Different congenital and acquired pathologies of the intestine can lead to short bowel syndrome (SBS) with associated significant morbidity and mortality. SBS remains an unmet clinical need and transplantation with engineered functional substitutes would be ideal. In the field of whole organ tissue engineering, regeneration of the vascular network is a major limitation to tissue survival and scaling up for clinical use. In this scenario, the aim of this study is to regenerate a functional endothelium within decellularised rat intestine pre-existing vasculature and to develop a platform for scaling up in larger animal models.

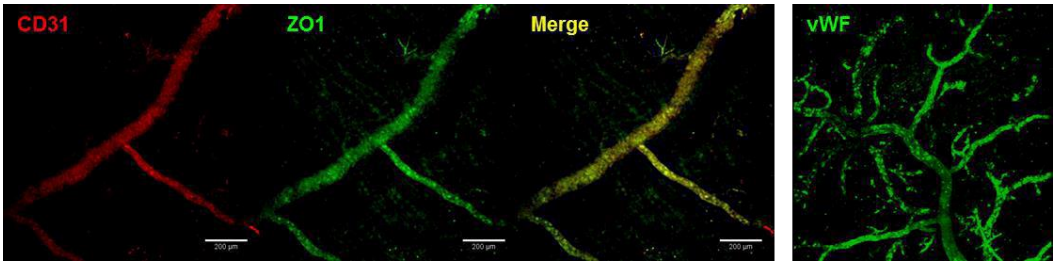
Small intestine segments were harvested from Sprague Dawley rats and decellularised according to a detergent enzymatic protocol previously developed that preserves the integrity of the vascular network. A segment of the small intestine was isolated with a fine-tuned surgical technique preserving the mesenteric vascular plexus via cannulation of the mesenteric artery and vein, obtaining a segment suitable for orthotopic transplantation.

Human umbilical vein endothelial cells (HUVECs) were perfusion-seeded through both artery and vein and cultured in specific growth media with high and low serum, in order to promote respectively proliferation and stabilisation of the endothelial layer. Culture was performed inside a custom made bioreactor specifically designed to provide perfusion through the blood vessels and the lumen of the small intestine scaffold.

After 7 days of culture, the blood vessels patency was confirmed with fluorescent dextran assay, also showing a low hydraulic resistance. Immunofluorescence staining performed on whole mounts showed that HUVECs engrafted till the very distal capillaries of the intestinal scaffold with considerable lumen coverage, expressing functional markers (CD31, von Willebrand Factor) and showing development of a mature barrier function by formation of tight junctions (ZO1) (Figure 1).

These findings represent a crucial step in the engineering and scale up of a whole intestine. The creation of a functional blood network will be essential for the survival of the graft in vivo and the delivery of the digested nutrients.

Figure 1



P387 Electrospinning of bi-layered vascular grafts resembling the architecture of the natural blood vessel

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Introduction - In the field of maxillofacial surgery, autologous bone grafting (*e.g*. iliac crest or free fibula flaps) is regarded as the gold standard treatment to restore bone defects[1]. Nevertheless, the use of autologous bone grafts is associated with drawbacks[1]. Regenerative techniques hold the promise to develop bioengineered osteogenic constructs with functional vasculature, which is desired for nutrient and oxygen supply of the developing bone tissue as well as for anastomosis[2]. Most blood vessels consist of a single intimal layer of endothelial cells and a secondary medial layer containing smooth muscle cells[3]. Therefore, the aim of this study was to engineer an electrospun small diameter vascular graft with a bi-layered architecture inspired on the natural vessel.

Material and Methods - Bi-layered electrospun scaffolds were fabricated using two fabrication processes. Solution electrospinning (solES) enabled the generation of nonwovens and melt electrospinning writing (MEW) was used to generate oriented fibers. Endothelial Colony Forming Cells (ECFCs), isolated from human cord blood, and Multipotent Mesenchymal Stromal Cells (MSCs) derived from human bone marrow were seeded on the inside and onto the outer layer, respectively. Samples were cultured for at least 7 days, after which they were analyzed by immunofluorescent labelling of the endothelial markers VE-cadherin and Von Willebrand Factor (vWF), and of a smooth muscle marker, α-smooth muscle actin (α-SMA). Confocal microscopy was used to visualize the fluorescently labelled markers and the cellular orientations.

Results and discussion – The feasibility of combining MEW and solES techniques to engineer one coherent construct was demonstrated. First, a randomly orientated inner layer (fiber thickness ~1-1.5 µm) was made with solES onto a 3 mm diameter grounded collector, after which a second, orientated layer of MEW was deposited (fiber thickness 20-30 µm). Fiber orientation could be controlled by varying the collector rotation and translation velocity[4].

A confluent monolayer of VE-cadherin and vWF-positive ECFCs was found on the inner layer. The electrospun intimal layer was therefore shown to be sufficient in steering the process of endothelialization. On the outer layer, a circumferential layer of α-SMA-positive cells was found throughout the thickness of the medial layer. The orientation of the α-SMA-positive cells was resembling the situation in native vessels.

Conclusion - The combination of two different electrospinning methods in one construct was shown to be a successful approach for vascular tissue engineering. The multi-layer approach provided a scaffold that fostered the infiltration of the smooth muscle-like cells while also supporting endothelialization. Future directions are aimed at applying fluid shear stress at the luminal side of the vascular scaffold, via culturing in a flow-perfusion bioreactor, and an eventual connection to pre-vascularized osteogenically differentiated tissues.

P388 Development of novel bioreactor system with pulsatile-pressurization for tissue fabrication

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[Background and Aim] One of the final goals of regenerative medicine is organ fabrication in vitro. To perform this, numerous studies have demonstrated the feasibility of developing tissue perfusion systems in vitro for tissue engineering. We have previously reported successful fabrication of thick rat cardiac tissue with neovascularized structure using cell sheet-based tissue engineering and perfusion culture system. However, long term maintenance of an efficient flow rate has been challenging. Because sustainable long term perfusion and angiogenesis are vital for tissue fabrication, we attempted by creating ex vivo pressurization system to mimic the mechanical stress found in vivo.

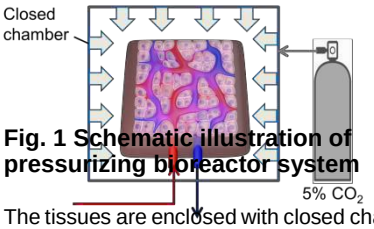

Fig. 1 Schematic illustration of pressurizing bioreactor system

The tissues are enclosed with closed chamber. Pressurization is performed

[Methods] First, we constructed a bioreactor which has a pressure chamber, an infusion pump, an electronic balance connected to an artery and vein, and a gas mixer for pressurizing (Fig.1). Next, we confirmed pressurizing conditions by comparing perfusion ratios. We tested 2 conditions; constant-pressurization (CP) and pulsatile-pressurization (PP) which mimics pressure change from body moving compared with non-pressurization (NP). The tissue used for perfusion was harvested from femoral artery and vein from 8 week-old rat, and connected to the developed bioreactor. To verify the effect of pressurization on the tissue, bioluminescence activity assay and histological analysis of perfused tissue were conducted. In addition, angiography during PP perfusion was performed to observe inner blood flow.

[Results] The perfusion with CP at 10 mmHg did not increase perfusion ratio when compared to NP one. However, in the case of PP which allow imitation such as skeletal muscle pump, the perfusion ratio from 0-10 mmHg was significantly higher than NP group (42 ± 6.8% versus 8.0 ± 5.2% at 14-day, values are means ± SE, $p<0.01$, $n=4$, Fig.2). After 14-day perfusion, the PP group maintained higher bioluminescence indicating better tissue viability and reduction of fibrosis in the tissue than NP. In the flow visualizing experiment, the PP perfusion tissue was observed well flowing in blood vessels both of the artery and vein compare to NP perfusion tissue.

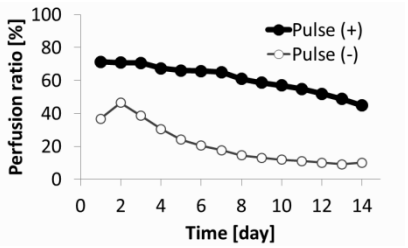

Fig. 2 Perfusion ratio in 14-day pulsatile- or non-pressurization

Representative data of pulsatile-pressurization perfusion constantly yielded higher perfusion ratio than non-

[Conclusion] We have developed a novel bioreactor system which can perform pulsatile pressurization during perfusion which improves perfusion ratio and maintains long term tissue viability.

P389 Study of the effect of collagenous microcarriers on the vasculogenic potential of endothelial cells and their use in perfusion bioreactors

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The development of an efficient vascular network in tissue engineering (TE) is still a challenge, since in most cases the blood vessels formed are not fully functional. In consequence, there is low rate of graft survival after implantation, caused by poor supply of oxygen and nutrients to the cells. This supply is dependent on the vessel structures, mainly formed by endothelial cells (ECs). In their role of building and remodeling blood vessels, ECs have the capability to proliferate, migrate, adhere, sprout and form vessel-like structures. In this study we want to determine the effect of collagenous microcarriers on the aforementioned properties of ECs, by culturing them in dynamic conditions on the collagenous microcarriers. Moreover, colonized microcarriers are used to re-seed the lumen of the biomatrix BioVaSc® placed in a perfusion bioreactor, in order to develop a vascular bed that provides support to any cell type used for differentiation into tissue-like substitutes (e.g. hMSCS for bone substitutes).

The BioVaSc® is a bioartificial vascularized scaffold obtained from a jejunal segment of porcine intestines, that includes a feeding artery and a draining vein. After chemical decellularization, the scaffold obtained is rich in collagen and elastin and with a preserved vascular network. This vascular network is then re-seeded with ECs. Subsequently, colonized microcarriers are seeded on the lumen of the biomatrix. For this purpose, a collagen type I gel is used to keep the microcarriers in place and to provide a 3D environment to the cells. As a result, ECs from the microcarriers can be observed on the biomatrix, specifically in the vessel structures, after 21 days of culture. This results suggest that the collagenous microcarriers can serve as a potential delivery system in defect sites.

Keywords— Collagen, Endothelial Cells, Microcarriers, Vascularization.

P390 Micro-computed tomography techniques applied in the evaluation of polymeric and composite bone scaffolds – in vivo study

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The initiation and development of a fully functional vascular network in bioengineered artificial bone are crucial for reconstruction of large osseous defects. One of the methods of inducing neovascularization in bioactive scaffolds is prefabrication. In our work innovative tissue construct was designed, manufactured and implanted in rats (Fig. 1). The composite scaffolds were fabricated from polycaprolactone, poly lactic-co-glycolic acid and tricalcium phosphate. Advanced micro-computed tomography techniques (micro-CT), including the use of a contrast medium and in situ mechanical tests were used to characterize them. Moreover, the scaffolds were investigated using scanning electron microscope and histology. The influence of degradation process in phosphate buffered saline on the structure and properties of polymeric and composite bone scaffolds was analysed.

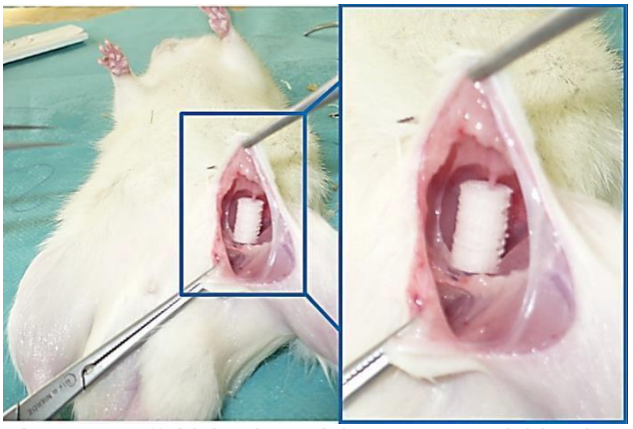

Fig. 1. Scaffold implanted in rat, around blood vessels

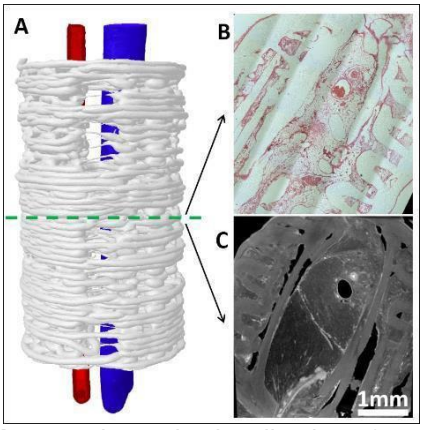

Fig. 2. Schematic visualization of a scaffold with blood vessels (a), histology (b) and micro-CT cross section (c)

Due to the similar X-ray beam attenuation caused by the scaffolds and tissues, suitable contrast agent (Iodine Aflofarm, Poland) was used to reduce the X-ray transmittance of tissues. Scaffolds were immersed in iodine for 12 hours. Then, rinsed with distilled water and scanned. Micro-CT ensures a careful assessment of bone scaffolds structure. In addition to tissue imaging, it allowed an observation of the microstructure of the entire volume of tested materials. Micro-CT images were compared to results obtained by histology (Fig. 2). The manufactured composite scaffolds had the appropriate parameters for the growth tissues and biological compatibility and interact with tissues. Simultaneously composite scaffolds showed significant difference in neovascularization in compare to control group.

P391 Vascularization of human bio-artificial muscles

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Human Bio-Artificial Muscles (BAMs) are tissue-engineered skeletal muscles composed of aligned human myofibers. They are created by casting human myoblasts embedded in a fibrin- or collagen-based extracellular matrix in a custom-made silicon structure containing 2 attachment points. Over the course of one week, by switching to differentiation conditions in the medium, multinucleated myofibers are formed, aligned in the axis of the attachment points. These BAMs are capable of generating contractile force. The current dimensions are 2 cm in length and a thickness limited to ±1mm, due to the passive diffusion limit. Recently, our group has proven that co-cultures of myoblasts and endothelial cells can generate endothelial networks interspersed in between the aligned myofiber structures. It is however not clear in how far BAMs can be vascularized in vivo and if tissue survival is affected by pre-implantation engineered endothelial networks. We present recent work on implantation of BAMs, created with and without endothelial cells in NOD-SCID mice. BAMs were created with human myoblasts and endothelial cells genetically labeled with different fluorescent labels. After implantation for 7-14 days, BAMs were harvested, cleared by 3DISCO and analyzed by confocal microscopy. Endothelial network structure was analyzed in ImageJ with Angiogenesis Analyzer. We demonstrate that host blood vessels are able to vascularize the BAMs, which is needed to maintain long-term survival after implantation.

(P392)

P392 Fabrication of a human transplantable vascular bed using re-endothelialized acellular porcine intestine

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[Background and aim of this study]

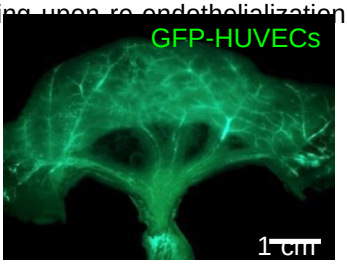
Creating three-dimensional tissues in vitro for the purpose of regenerative therapy is highly desired. Although, engineering 3D tissues in vitro is known to be difficult due to the system that lacks the continuous supply of oxygen and nutrients to the tissues. Our laboratory succeeded in constructing a pulsating myocardial tissue in vitro by layering cardiomyocyte sheets on rat- derived as well as the collagen based vascular bed which induces perfusion in the 3D tissues.[1),2)] The aim of this study is to create a human transplantable vascular bed derived from porcine tissue. To make transplantable tissues, it is necessary to reduce the risk of possible rejection and thrombus formation after transplantation. Therefore, we report decellularization technology and in vitro re-endothelialization method to create the implantable vascular bed for the construction of 3D pulsatile myocardial tissues.

[Method]

An intestinal tract with an arteriovenous circulation loop was selected for the vascular bed. After harvesting a tissue of φ = 20 mm, L = 60 mm, the tissue was then decellularized by sequentially perfusing three kinds of reagents; ultra pure water, sodium deoxycholate, and DNase-I, through the artery and intestinal lumen of the tract. The histological examination using H.E. and DAPI and the evaluation of the amount of DNA were performed. Subsequently, GFP-positive HUVECs were seeded into the decellularized intestinal vasculature and cultured to re-endothelialize the lumina. In order to promote cell adhesion, the lumina was coated with biofunctional proteins. In the final step, it was examined to confirm the effect of the lumina coating upon re-endothelialization by comparing morphological observations.

[Results]

The amount of DNA of the decellularized porcine tract was recorded to be 42.7 ± 6.6 ng/mg, which satisfied the standard level of decellularized tissue. In addition, the results of H.E. and DAPI staining showed that there were no nuclei in the tissue. Moreover, the observed result of the lumina re-endothelialization indicated that the cell adhered to the blood vessel and formed the luminal structure completely by performing the coating in a shorter period compared to non-coating method. The figure shows the top view of the re-endothelialized intestinal tract with GFP-HUVECs. In this study, we developed a promising way to create a human implantable vascular bed using decellularization and re-endothelializing technique.



[Future plan]

We expect that the 3D pulsatile cardiac tissues which are constructed by layering cell sheets on the implantable vascular bed will serve as a major therapeutic application and the prime contributor to the development in the field of organ regeneration.

P393 The effect of platelet rich plasma on the blood vessels volume of CCl4-induced hepatotoxic model

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Platelet rich plasma (PRP) is an autologous source of growth factors including those involved in angiogenesis. Activated PRP has been reported to increase the blood vessel and microvascular density. PRP also has healing impact on the fibrosis in cirrhosis model rats and has beneficial effects on regeneration of liver after hepatectomy. Morphological and structural deformity was reported in microvasculature of cirrhosis liver. The aim of this study was to investigate the effect of PRP administration on the volume of microvasculature and blood vessels of hepatotoxic-induced liver fibrosis.

Rats were fed with CCl4 in corn oil for 10 weeks. The total protein and albumin were checked in peripheral blood samples to confirm hepatotoxicicity. The rats were divided into 4 groups; animals without any intervention, hepatotoxic animal model, hepatotoxic animal model with PRP and hepatotoxic animal model with PRP vehicle. After 1 week, the liver removed, fixed, sectioned by IUR method and stained with H&E. The volume of the vessels was calculated by stereology method. The results were ANOVA.

CCL4 had no impact on the microvasculature volume compared with normal control. PRP led to a significant decrease in the microvasculature of hepatotoxic liver (P=0.003). CCl4 also led to a significant increase in central vein and portal vein volume compared with control (P=0.002, P=0.001). The central vein volume reduced significantly by PRP treatment (P=0.001) but it has no impact on portal vein volume. Hepatic artery volume did not change by treatment of both CCl4 and PRP.

PRP had adverse effects on microvasculature and macrovasculature of hepatotoxic liver. The decrease in blood vasculature volume may result on a reduction in blood flow.

Key words: Microvasculature, Microvasculature, Hepatotocicity, Platelet rich plasma

P394 Reconstruction of rat bladder with human amniotic membrane graft

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Introduction & Objectives: Different autolog, xenolog and synthetic materials were tested for the repair of urinary tract defects and for prevention of anastomotic bowel leakage. Human amniotic membrane (HAM) is assumed to have several unique characteristics allowing its perfect ingrowth in the surrounding tissue. In this animal rat study we first applied HAM for the repair of bladder and bowel defects.

Material & Methods: Following the IDEAL recommendations of surgical innovation preliminary animal study (Stage 0) was conducted to show the feasibility of the method. In a total of 48 sprague male rats a small defect was set at the bladder dome or coecum and repaired by either by a suture or grafting with a multilayer cryopreserved HAM from caesarian section. Bladder volume capacity, adhesions and leakage after grafting were measured. Peri- and early postoperative complications were assessed. Histological and immunohistological analyses were performed to look for the degradation of HAM, graft rejection and the ingrowth of surrounding tissue 7, 21 and 42 days after the implantation.

Results: Four rats died due to sepsis. All other rats survived, had no severe complications and showed no signs of leakage. The bladder capacity did not change over time. In the bladder group no signs of HAM degradation were found and the initially increased inflammation in the HAM group diminished significantly over time ($p<0.05$). The smooth muscle staining increased over time. In the colon group adhesions were more prominent in the HAM group ($p<0.05$) and HAM degraded over time. The initially increased inflammation in the HAM group reduced over time but remained significantly increased ($p<0.05$).

Conclusions: HAM seems to be a suitable and durable graft for the reconstruction of urinary tract. However, HAM was not suitable for bowel augmentation in a rat model due to increased adhesions. Further animal and human studies are necessary to proof the possible indications of HAM.

P396 Let's mesh it ~~be~~tter: Advanced cell based gynaecological care for women with pelvic organ prolapse (pop)

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Pelvic organ prolapse (POP) is a deliberating gynaecological condition that affects 1 in 4 women across all age groups globally. POP leads to incontinence issues that affects quality of life and requires surgical intervention for a large proportion of women. The need for re-operation is high (~29%) because of the inadequate performance of current surgical meshes. Recently, the US Food and Drug Administration have issued public warnings against the adverse effects of marketed surgical meshes. With rise in ageing population, POP represents a significant healthcare burden because of the lack of an optimal long-term solution.

Our team has developed new tissue engineered surgical solutions with improved biocompatibility and long-term efficiency using mesenchymal stem cells isolated from the endometrium layer of the uterus. Our group discovered human endometrial (eMSC) and identified the marker SUSD2+ for magnetic isolation and purifications. We further identified a small molecule-A83-01 that prevents eMSCs from spontaneous differentiation into fibroblasts and undergo replicative senescence, decreasing their purity, survivability and efficacy for upscaling to clinical applications. We show that A83-01, a TGFß receptor inhibitor promoted SUSD2+ eMSC proliferation, and blocked senescence and apoptosis in vitro. There was no change in expression of CD90, a standard MSC marker or CD140b, while CD146 was downregulated. The A83-01 treated eMSC survived under the kidney capsule of mice for a longer duration than the untreated eMSCs as detected by mCherry immunofluorescence, SUSD2 flow cytometry and Alu sequence PCR. Secondly, we combined eMSC cells with biomaterials to develop newer strategies to manage POP using eMSCs. In our immunocompromised mice model, we examined the anti- inflammatory properties of mCherry labelled eMSC delivered on novel polyamide/gelatin (PA+G) mesh. Our results show transduction efficiency of 80% with many M1 macrophages was observed around mesh filaments at 7 and 14 days but the M1/M2 ratio reduced in later time- points. Less IL-1 β was found in eMSC/PA+G explants than PA+G after 7 days but TNF-α and IL-6 were similar for all time-points. In nature, cell behaviour and structural development is supported by the nanoscale arrangement of the extracellular matrix architecture that provides a larger surface area to adsorb proteins and binding sites to cell membrane receptors. In order to improve the efficacy of POP mesh in the tissue microenvironment, we explored the application of biomimetic electrospun nanofibers of Poly Lactic acid and Poly e Caprolactone as surgical construct for POP. Our ongoing studies in rodent and sheep models show the feasibility of human eMSC for POP cell based therapy and its immunomodulatory effect to be used as a tissue engineered with nanostructured meshes. Currently there are no clinical trials of cell based therapies for POP- with or without biomaterials. Our research provides foundation for a novel, one-step safe and effective surgical treatments for POP that will significantly improve women's quality of life, and promote wellbeing.

P397 The use of pooled human platelet lysate for isolation and *ex vivo* expansion of skeletal myoblasts for clinical use

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Introduction: *Ex vivo*-expanded autologous myoblasts are being used in clinical trials for the treatment of various skeletal muscle conditions, including stress urinary incontinence caused by urethral sphincter damage. The use of fetal calf serum (FCS) as a growth media supplement in these procedures still raises various technical and ethical concerns. As an alternative to xenogeneic supplements, human platelet lysate (hPL) has recently gained increasing attention for *ex vivo* expansion of mesenchymal stem cells in clinical studies. However, little is known regarding the ability of hPL to support *ex vivo* expansion of skeletal myogenic precursors.

Aim: The aim of this study was to assess the performance of hPL for the isolation and expansion of human skeletal myoblasts in comparison to FCS.

Materials and methods: Skeletal myoblasts were isolated and expanded in a commercial medium formulation (MyoTonic, Cook Myosite) supplemented with either 10% FCS or 5% pooled hPL (Stemulate, Cook Regentec). Cells were assessed during multiple passage expansion by analysis of cell proliferation, cellular phenotype, and gene expression profiles. The ability of media to preserve the differentiation capacity of the cells was evaluated in cells from early and late passages by immunocytochemistry and qRT-PCR after induction with MyoTonic medium supplemented with 2% horse serum.

Results: Analysis of cell proliferation over five passages revealed that myoblasts cultured in hPL-supplemented medium displayed a significantly higher cumulative cell number over time, due to consistently larger growth rate. Cells grown in hPL media evidenced a lower activation during propagation, as revealed by a significantly lower transcription of the skeletal myogenic factors MYF5 and MYOD. The percentage of CD56 positive cells appeared to decrease over time with passaging irrespective of the media type, suggesting a progressive depletion of myogenic precursors over time. Nevertheless, the cells expanded in hPL evidenced a robust capacity for differentiation at early (P1) and late passages (P5), as demonstrated by the formation of myosin-rich mature myotubes displaying high levels of myogeninactivation.

Conclusions: Our results suggest that the hPL supplemented medium supports isolation and efficient expansion of skeletal myoblasts while preserving their differentiation capacity. In perspective, hPL may represent an efficient alternative to FCS for the ex vivo expansion of myoblasts aimed for cell based therapies.

P398 Sensitivity of different cell types towards paclitaxel

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Introduction: Stenting of the urinary tract has become a common treatment to overcome ureteral strictures or renal obstruction e.g. due to stone formation. However, the overshooting proliferation of epithelial cells in the anastomosis area is major problem. The use of paclitaxel (Ptx) eluting stents for preventing coronary artery restenosis is well accepted. Paclitaxel works by stabilizing microtubules, rendering them nonfunctional. Will et al reported in 2011, that canine ureteral smooth muscle cell proliferation is already inhibited at concentrations of 10 nM Ptx. The aim of this study was to examine whether the application of paclitaxel could reduce the proliferation rate in the human urinary epithelial cells as well as smooth muscle cells at the same range of Ptx concentration.

Materials & Methods: Non-toxic concentrations of Ptx were determined by HPLC measurements. Experiments on non-tumorigenic human urothelial cells (SV-HUC-1, ATCC CRL- 9520) and smooth muscle cells (A-10, ATCC CRL-1476) comprised cell viability assay, analyses of cell cycle, apoptosis and visualization of microtubules via confocal microscopy. Continuous monitoring of the cells metabolism and adhesion state was performed using the Bionas® analyzing system.

Results: The non-cytotoxic concentration of paclitaxel was determined by MTS test. We found a clear dose-dependent inhibition of the cell proliferation at low doses and induction of apoptosis at higher doses. Continuous monitoring of the O_2-value in the medium over 48 h revealed that Ptx immediately inhibits the O_2 consumption by urothelial cells. However, the cell adhesion capacity is at first influenced 12 h later. The comparison with smooth muscle cells showed that these cells react nearly at the same range of concentrations.

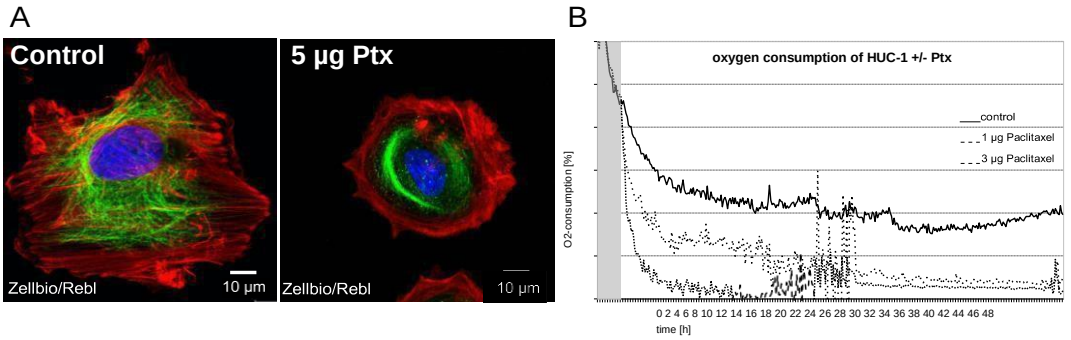

Fig A: Smooth muscle cells (A-10) labelled with tubulin (green), actin (red), DNA (blue). Control (left) or treated with 5µg/cm² Ptx (right) for 24h (LSM 780, Carl Zeiss). Fig B: Continuous monitoring (48h) of O_2-consumption after incubation with different concentrations (1, 3 µg) of Ptx.

Conclusions: The results confirm the assumption that paclitaxel affects the proliferative activity in the urothelial cells. Low doses can induce fast intracellular metabolic changes, where anti-proliferative activity is observed without inducing apoptosis in the cells. The hypothesis that smooth muscle cells would be more sensitive to the cytostatic drug could not be confirmed. We consider Paclitaxel to be a promising candidate for urothelial stent application.

P399 Autophagy is needed during the differentiation of adipose derived stem cells to functional smooth muscle cells for use in bladder engineering

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Introduction:Tissue engineering using smooth muscle cells may provide a treatment option for diseases with smooth muscle pathology such as bladder dysfunction, urinary incontinence, and erectile dysfunction. As autologous smooth muscle cells (SMC) cannot be harvested from organs with end-stage disease there is a need for other cell sources. Adipose derived stem cells (ADSC) can be easily harvested and differentiated into SM tissue. We have shown that autophagy, a conserved lysosomal degradation pathway, is required for cell survival and differentiation of stem cells. ADSC undergoing differentiation to SMC efficiently remodels their cytoskeleton and shape in an energy-consuming process. We investigated functional role of autophagy during differentiation and remodeling of ADSCs to SMC in vitro.

Methods: Rat and human ADSCs were characterized and induced towards SMC using induction medium for 1 to 6 weeks. The changes in gene and protein expression level for SMC markers: calponin, smoothelin, α-SMA, MyH11; and autophagy: LC3, Atg5, Beclin1, were investigated by ICC and WB.

Results:

Upon induction, up-regulation of Atg5-Atg12 and free Atg5 was observed during 4-6 weeks. This was supported by an increase in conversion of cytosolic LC3I to membrane-bound LC3II protein. At the same time the contractile proteins calponin, MyH11 and smoothelin were up regulated during 1-3 weeks and decreased after 5-6 weeks of differentiation detected by WB and ICC. Pharmacological blocking of autophagy by 3-methyladenine (3-MA) during the differentiation abolished the differentiation capacity of stem cells.

Conclusion: Our study demonstrates that autophagy plays an important role in ADSC differentiation to SMC. Influencing autophagy by pharmacological agents might be used to further optimize the induction to SMCs.

P400 Synergistic effects of combining undifferentiated adult stem cells and differentiated cells for the engineering of functional bladder smooth muscle tissue

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INTRODUCTION: Tissue engineering using a combination of cells may offer an approach for functional reconstruction. Adipose derived stem cells (ADSC) are investigated as an alternative cell type to bioengineer contractile bladder tissue when differentiated to smooth muscle cells (SMC). However, it is uncertain whether these cells maintain their phenotype long term invivo. It is our aim to evaluate different combinations of cells to improve the smooth muscle formation, by improving the microenvironment and cell-to-cell interactions

METHODS: Rat ADSCs were characterised and differentiated to SMC (3 weeks) prior to subcutaneous injection into nude mice. Cells were injected in different combinations (ADSC, ADSC + differentiated ADSC, SMC, differentiated ADSC+SMC). Tissue formation was followed by MRI and PKH labelling. Formed tissues were analysed for gene and protein expression by RT-PCR, Western Blot and immunohistochemistry.

RESULTS: In all experimental conditions the PKH-positive cells were detected after 4 weeks, indicating the presence and survival of engineered tissues in vivo. Tissue size differed between the experimental conditions with tissues grown from cells with 3 weeks differentiation+ADSC showing largest constructs with good correlation in histology. Differentiated ADSC combined with ADSC or SMC showed positive upregulation of smooth muscle makers calponin, smoothelin, MYH11 and αSMA similar to bladder derived SMC.

CONCLUSIONS Our research offers key information on survival and functionality of bioengineered smooth muscle tissue grown using differentiated ADSC in combination with differentiated cells. This approach could help to engineering contractile bladder tissue for future clinical application.

P401 Bone marrow stromal cells from elderly patients undergoing hip replacement surgeries: an in vitro model for impaired bone healing

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Introduction: Regeneration of bone defects represents one of the most pressing health problems, especially in the aging population. Patients in need for bone surgery are often elderly people with limited bone regeneration potential. To develop new bone regenerative therapies tailored for these patients, a good in vitro model is needed. Cells from healthy young donors are often used for in vitro studies of bone regeneration [1,2]. However, to better mimic the systemic aging-affected cell phenotype, we isolated and characterized bone marrow stromal cells (BMSCs) from femoral heads of trauma patients that underwent hip replacement surgeries. **Methods:** Collection of bone samples for research studies was conducted under ethical committee approval with the patient consent. Trabecular bone tissue was excised from the femoral heads, minced and washed to collect the bone marrow fraction (n=11, 43-90 years, 6 female, 5 male). Upon washing, the whole marrow tissue was seeded into culture flasks and the adherent cells were culture- expanded for six passages. Control BMSCs (< 30 years, male) were purchased and cultured in the same conditions. Cumulative cell growth was determined by cell counting and the surface antigen expression was evaluated by immunofluorescence and flow cytometry. Osteogenic and adipogenic differentiation were induced in monolayer cultures and evaluated by immunohistochemistry. Accumulation of senescent cells was determined by senescence associated beta-galactosidase assay and metabolic parameters were measured to evaluate the cell fitness (mitochondrial activity, reactive oxygen species, ATP content). **Results and Discussion:** From all samples used, cells were successfully isolated and cultured until passage six. Young control BMSCs had the fastest proliferation. However, a linear correlation between increasing sample age and decrease in cell proliferation was not observed. Most of the samples showed a slow decrease in proliferation over passages. However, a predominant change from fibroblastic to enlarged senescent cell morphology was not noticed. BMSCs were strongly positive for mesenchymal surface markers CD73, CD90 and CD105 and negative for endothelial and hematopoietic markers CD31 and CD45. No significant differences in the expression levels were observed between BMSCs of different ages or genders. BMSCs show a strong decline in the adipogenic differentiation potential with increasing patient ages compared to the young control BMSCs and in most cases a complete loss of the osteogenic differentiation potential, similar to previous reports for iliac crest BMSCs [3]. Furthermore, an increase in the proportion of senescent cells was observed in middle-aged and aged BMSC cultures, as expected [4] and an alteration in metabolic parameters was observed. **Conclusions**: Aging affects regenerative properties of BMSCs, which might contribute to the impaired bone healing of elderly patients. We therefore suggest the use of BMSCs from elderly patients with bone fractures as a more relevant in vitro model for future bone regeneration studies.

P403 Identification of differential GDF6 signalling responses in subpopulations of human bone marrow and adipose-derived stem cells: implications for intervertebral disc regeneration

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Growth differentiation factor 6 (GDF6) has previously been shown to be involved in development and homeostasis of the intervertebral disc (IVD). GDF6 signals through the type I receptors BMPR1A and BMPR1B and the type II receptors BMPR2 and ACVRIIA. Recently, we showed that stimulation of bone marrow- and adipose-derived stem cells (MSCs and ASCs respectively) with GDF6 promotes differentiation to a phenotype similar to that of nucleus pulposus (NP) cells from the central region of the IVD. Furthermore, GDF6 promotes this differentiation better than other members of the TGF-β superfamily. However, patient-matched MSCs from these two sources showed different responses to GDF6, with ASCs differentiating to a phenotype more closely resembling NP cells. Here, we aimed to investigate if GDF6 signalling differed in MSCs and ASCs with a view to optimising differentiation and enhancing the efficacy of future stem cell based therapies.

GDF6 receptor expression was profiled in patient-matched MSCs and ASCs (N=6) using western blotting and immunocytochemical (ICC) analysis. Signal transduction following GDF6 or TGFβ stimulation was investigated through analysis of Smad1/5/9 phosphorylation and alternative non-Smad pathway activation. BMPR2 was blocked using siRNA knockdown and subsequent analysis of smad1/5/9 phosphorylation, NP-marker and chondrogenic gene expression, and aggrecan production. The role of these signalling pathways in the induction of NP-like gene expression in ASCs was investigated through Smad1/5/9, p38 MAPK and ERK1/2 pathway specific blockers. FACS was performed on a cohort of minimally expanded ASCs (P2; N=3) which were sorted into $BMPR2^{hi}$ and $BMPR2^{lo}$ populations.

Western blotting and ICC demonstrated higher expression of the GDF6 type II receptor BMPR2 ($p<0.05$) in ASCs compared to patient-matched MSCs. Correspondingly smad1/5/9 phosphorylation following stimulation with 100 ng ml^{-1} GDF6 was also significantly higher in ASCs compared to MSCs ($p<0.05$). BMPR2 siRNA successfully knocked-down BMPR2 expression and attenuated GDF6 signalling and downstream effects. Through specific pathway blocking, the smad1/5/9 and smad-independent alterative pathways were found to have differing effects on GDF6-mediated effects. Through FACS it was possible to identify and isolate ASC subpopulations that were $BMPR2^{hi}$ and$BMPR2^{lo}$.

The higher BMPR2 expression in ASCs and corresponding enhanced smad1/5/9 phosphorylation in response to GDF6 stimulation indicates an enhanced discogenic potential in ASCs in comparison to MSCs, suggesting that they may be the more suitable candidate for GDF6 mediated cell therapy for IVD regeneration. This corroborates our previous reports and provides a mechanistic explanation for the observed responses. Furthermore, the isolation of an $BMPR2^{hi}$ ASC subpopulation and the importance of BMPR2 in GDF6 signalling as discerned here by siRNA knockdown, could further improve the efficacy of the proposed cell therapy.

P404 Radiological evaluation of bone in animals with experimental osteoporosis after implantation of bioceramic granules

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Within this study Calcium phosphate (CaP) ceramic and Strontium ions containing CaP ceramic are investigated for local treatment of an osteoporotic bone.

Materials and methods

Calcium deficient hydroxyapatite (CDHAp) and Sr-containing CDHAp precursors of the biphasic calcium phosphate (BCP) and Sr-containing BCP (Sr-BCP) granules were prepared through aqueous precipitation. The (Ca+Sr)/P molar ratio of 1.55 were employed in order to obtain granules with HAp/β-TCP phase ratio of 30/70 weigh %. Bioceramic granules were prepared by an extrusion and thermal treatment at 1150 ^{o}C. A total of 20 matured female rabbits in age of 8 months were used in our study. Healthy group consisted of 4 rabbits. 16 rabbits underwent experimentally induced osteoporosis. Afterwards 4 of them had sham surgery in greater trochanter of femur bone, creating a 4 mm wide bone defect. For the remaining rabbits implantation of ceramic granules was performed - HAp/β-TCP (6 rabbits) or Sr-containing HAp/ β- TCP (6 rabbits). After 3 months animals were euthanized. The established osteoporosis and effect of implantation was evaluated with micro-computed tomography using InSyTe FLECT/CT system, the obtained measurements were registered in Hounsfield unites (HU).

Results

Bone optical density was decreased in osteoporosis group (mean 1901 HU), reaching 2812 HU in healthy group ($p<0.005$). Implantation of ceramic granules increased bone optical density of osteoporotic bone reaching 3993 HU after implantation of HAp/β-TCP and 3391 HU after implantation of Sr-containing HAp/β-TCP ($p<0.05$).

Conclusion

HAp/β-TCP and Sr-containing HAp/β-TCP ceramic granules implanted in osteoporotic bone showed local bone tissue remodelling potential by increase of bone optical density.

Acknowledgement

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P405 The impact of donor age on the morphology and functional properties of mesenchymal stem cells from adipose tissue

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Aims: Adipose stem cells (ASCs) belong to the mesenchymal stromal cell category and are widely used for the regenerative medicine purposes [1-3]. It is, however unclear if the ASCs derived from old donors are equally able to regulate tissue repair as the cells collected from young donors. The aim of the study was the comparison of phenotype, proliferative potential, angiogenic potential and differentiation capability into osteo- chondro- and adipogenic lineages of ASCs derived from young or aged rats.

Material and methods: In this study we used WAG inbred rats aged 6 month and 2 years. Adipose tissue was collected from 4 regions: dorsal (white or brown fat), gonadal, renal and inguinal. After isolation by enzymatic digestion, ASCs were in vitro cultured in standard conditions (DMEM + 15% FBS, 37ºC, 5% CO2). Cell numbers, viability, phenotype (CD11b, CD29, CD90, CD34, CD45, CD31), doubling time and proliferation potential were analyzed. Using differentiation medium (LONZA) ASCs were stimulated to osteogenesis, chondrogenesis and adipogenesis and was analized by Real Time PCR and histochemical staining (Oil Red, Alizarin Red). Using growth factor-reduced Matrigel (CORNING) and sprouting assay, the ability to form angiogenic tubes and spheres was evaluated. The numbers of cell passages of ASCs from old and young rats were also compared.

Experiment was approved by the Local Ethical Committee, licence No: 71/215/30/06/2015

Results: Our data show no statistical difference between ASCs from old and young animals. There were no visible differences between DNA content (Picogreen test), enzimatic activity (MTS test), clonogenic potential (CFU-F test) and also doubling time, which could suggest the proliferative potential difference between ASCs from old and young donors. In both group of rats there were no significant differences in the ability of ASCs to differentiate into bone, cartilage or adipose tissue. ASCs from old or young donors showed equal ability to form angiogenic tubes and spheres.

Impact of investigation: Our animal model is equivalent to human donors of 25 or 70 years old. No differences in ASC parameters which are commonly tested for the qualification of cells for clinical purposes may be the proof that old age donor - derived cells are equally useful for regenerative medicine purposes, or that these parameters are inadequate to qualify ASCs for clinical use. The problem will be solved by forther in vivo experiments using ASC from donors of different age.

Acknowledgments: This study was supported by the National Science Centre (UMO-2013/11/B/ST8/03401, project OPUS-6)

P406 Cartilage degeneration affects underlying subchondral bone remodelling

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INTRODUCTION: Osteoarthritis (OA) affects both cartilage and subchondral bone in the joints. With the progression of OA, bone remodelling and bone resorption occur as results of changes in the loading pattern[1], leading to the change of physical environment that supports the overlying cartilage. In this study, we report the distribution of volumetric bone mineral density (vBMD) within osteoarthritic joints and the biomechanical properties of degenerated cartilage.

MATERIALS and METHODS: Human femoral heads were collected during hip replacement operation, and the cartilage was graded using ICRS (Outerbridge) classification[2]. The vBMD distribution in osteoarthritic joint tissues was determined by using a peripheral quantitative CT (pQCT). The samples were indented dynamically in unconfined compression by applying non-destructive cyclic loading to the cartilage, using a Zwick 0.5 machine. The same was applied to the subchondral bone corresponding to each cartilage grade. The samples were then processed for histology examinations.

RESULTS: The examination of retrieved tissue explants from osteoarthritic patients revealed cartilage in different stages of degeneration. The vBMD in the subchondral bone varied with the severity of the overlying cartilage degeneration. Higher vBMD values are observed in the regions that have sever overlying cartilage degeneration. The lowest vBMD values were observed in the regions where the overlying cartilage is morphologically normal. It was shown that the dynamic modulus and stiffness of cartilage and subchondral bone differed significantly across different zones, and were linked to histological changes and ICRS grades. Bone resorption and remodelling were observed in all osteoarthritic joints. The bone surrounding the cysts has a much higher vBMD than that in other regions.

DISCUSSION & CONCLUSIONS: This study showed an association between cartilage degeneration and underlying subchondral remodelling. Cysts, as a result of bone resorption, were observed in all osteoarthritic joints and they were located close to the weight-bearing regions. Taking in to account the relationship between histological, mechanical and macroscopic changes, it is postulated that the cartilage degeneration alters the loading pattern of the joints, leading to bone remodelling and this in turn stimulate the overlying cartilage degeneration.

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P407 The optimal timing of hydrogel injection for treatment of intervertebral disc degeneration: Quantitative analysis based on T1ρ MR imaging

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Background. Currently, numerous methods are employed for treatment of degenerative disc disease (DDD), including physical therapy in the early stages and discectomy in the late stages. Although all these methods might relieve the symptoms of this disease immediately, these therapies do not prevent DDD from progression. Recently, there has been an increasing interest in the study of biological therapy for intervertebral disc degeneration (IDD); however, the optimal timing selected for regeneration of degenerative disc is still unclear, which curtains the understanding of the new therapeutic modalities in clinical practice.

Objectives. To investigate the optimal time of bio-regenerative treatment for intervertebral disc degeneration based on the T1ρ MR imaging.

Methods. Eighteen rhesus monkeys were randomly divided in to the severe, moderate and mild groups, with L5/6 disc degeneration induced by injection of Pingyangmysin into the subchondral bone adjacent to discs. Biocompatible hydrogel was injected into the central part of L5/6 nucleus pulposus of each animal under fluoroscopic guidance at the time points according to the T1ρ values of the nucleus pulposus of L5/6 disc. The treatment effects were investigated by using radiography and MR imaging preoperatively and at 1, 3, 6, 9, 12-month time points postoperatively.

Results. All animals completed the whole protocol. There were no significant differences in disc height index during follow-up period at each group. The T1ρ values of nucleus pulposus at L5/6 increased to 110ms at 1-month time point at all groups. In the minor and moderate groups, the T1ρ values decrease a little bit after 3-month time point, and tended to be stable after 12-month time point. However, in the sever groups, the T1ρ values decreased significantly, with the average values lower than 80 ms.

Conclusion. Our findings demonstrated that the rapid degenerative stage of disc degeneration (T1ρ values range from 95ms to 80ms) was the optimal timing of hydrogel injection therapy for disc degenerative disease, based on T1ρ-MR imaging technique and quantitative analysis.

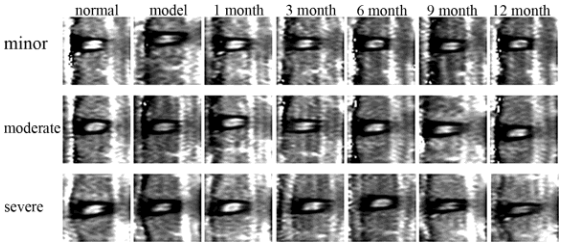

Fig1 The T1ρ-mapping images of L5/6 of monkeys in all the groups at different time points. The images was investigated before the monkey was built to degenerative model; model, the monkey was built to degenerative model; 1,3,6,9,12month, the images was investigated at 1,3,6,9,12month after the model was treated.

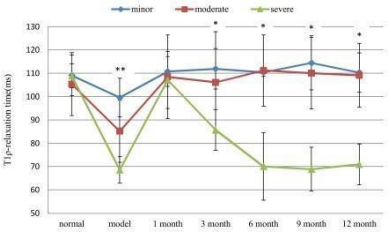

Fig 2 The T1ρ values of nucleus pulposus of L5/6 of monkeys in all the groups at different time points. * P<0.05 **p<0.01.

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P408 MicroRNA-132 in bladder wound healing

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Congenital malformations in urinary bladder are often treated with surgical interventions. In regenerative urogenital medicine, we hypothesize that a deeper understanding of normal bladder wound healing could further enlighten our understanding of factors that are essential for good treatment outcome and eventually lead to new treatment modalities.

MicroRNA (miR) is a group of conservative small non-coding RNAs, crucial for post-transcriptional regulation in most, if not all, biological events including wound healing.

Epithelial cell wound healing within skin and cornea regarding miRs expression has been studied extensively, and miR-132 has been reported as a key regulator promoting keratinocyte migration, suppressing proliferation and inflammation.

The aim of this study was to analyze if miR-132 could be of importance to enhance urothelial cell migration and proliferation for wound closure.

Materials and Methods: Human urothelial cells were isolated from bladder washings or biopsies. To stimulate wounding conditions, cells were cultured until confluence before performing a standardized 2D scratch assay. The expression of miR-132 was analyzed upon wounding after 6h, 12h, 24h using real-time quantitative PCR by the -$\Delta\Delta$Ct with an internal control for U6 and a non-scratch control group (6 replicates within each group).

Results: We found that the area of wound gap was significantly reduced within 24h, miR-132 was up regulated by 1,8 times 6h after wounding of confluent urothelial cells in culture conditions. At 12h and 24 h after wounding, miR-132 was up regulated 1,4 times. Same results were found in primary urothelial cells isolated from bladder washes as well as from bladder biopsies.

Conclusion: Our results indicate that miR-132 was induced after wounding, and demonstrated a similar expression pattern as in normal skin wound healing. This finding strongly supports our hypothesis that miR-132 could be an important factor for promoting urinary bladder wound healing, most probably by enhancing urothelial migration and suggests us to proceed with validations including functional studies.

P409 Upregulation of osteo-related genes and BMP2 secretion in muscle-derived stem cells after mRNA transfection

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Limitations associated with the use of growth factors represent a major hurdle to musculoskeletal regeneration. Growth factors are needed to induce neo-tissue formation for the substitution of necrotic or missing tissue. However, these factors are used in supraphysiological concentrations due to their characteristic short half-life. As a result, several side effects have been reported in patients. Gene therapy has shown clear advantages over protein delivery and may provide a solution. An attractive alternative to DNA-based gene therapy is mRNA delivery. Since mRNA exerts its function in the cytoplasm, limitations related to the transport across the nuclear membrane are overcome. For the therapeutic use of mRNA, this molecule needs to be chemically modified in order to reduce its immunogenicity and to increase its stability. We have recently reported a chemical modification of the mRNA molecule that improves stability and toxicity. The objective of the present study was to further optimize the chemical modification to the mRNA molecule by introducing sets of long-lived UTR sequences. Furthermore, we investigated the development of an osteoinductive transcript-activated matrix by loading collagen sponges with a chemically modified mRNA coding for BMP-2.

Nucleotide modification of the mRNA proved to be very efficient in increasing mRNA stability. The sequences containing hAG-UTR and translation initiator of short 5'UTR (TISU) resulted in elevated metridia luciferase expression in HEK 293 and MC3T3 E1 cells up to 72 hours post- transfection. This expression was significantly higher when compared to the modified mRNA with minimal UTRs and to the pDNA. Similar results were obtained for both UTR transcripts coding for hBMP2. As TISU is considerably shorter, this UTR was chosen. Thus, this modified mRNA molecule was used to develop the osteoinductive transcript-activated matrices. Collagen sponges (5 x 3 mm) were loaded with 1.25 µg hBMP2 modified mRNA lipid complexes and subsequently freeze dried to produce the mRNA-activated matrices. Rat muscle-derived stem cells cultured on hBMP2 modified mRNA/collagen sponges were transfected and able to secrete significant amounts of hBMP2. Furthermore, hBMP2-transfected cells showed higher alkaline phosphatase activity *in vitro* when compared to the noncoding mRNA control. The expression of osteo-related genes like RunX2, ALP and Collagen I was greatly enhanced as result of the hBMP2 mRNA transfection. The developed hBMP2 modified mRNA/collagen sponges also supported better mineralization of seeded muscle stem cells.

Overall, our results indicate the feasibility of chemical modification on the mRNA molecules together with the use of relevant UTRs to increase translation. In addition, the development of osteoinductive transcript-activated matrix may present an interesting therapeutic potential for bone tissue engineering.

P410 Delivery of modified BMP-2 plasmid enhances mesenchymal stem cell osteogenesis and critical size defect repair over unmodified BMP-2 plasmid

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INTRODUCTION: Gene-activated scaffolds developed within our lab have been shown to induce controlled, sustained release of transgenes *in vitro* and *in vivo*. The aim of this study was to use the gene-activated scaffold platform to deliver and compare a series of novel modified plasmid DNA (pDNA) constructs encoding bone morphogenetic protein-2 (BMP-2) and to assess their effects on enhancing MSC osteogenesis and ultimately bone regeneration in a critical sized bone defect.

MATERIALS AND METHODS: Previously optimised chitosan nanoparticles (NPs) were used to deliver a series of pDNA constructs encoding BMP-2, termed pBMP-2, pBMP-2/7 and pBMP-2 Advance, all under the control of the cytomegalovirus (CMV) promoter, as well as pBMP-2 Advance under the control of the elongated factor 1α (EF1α) promoter. BMP-2 protein expression post-transfection was quantified using ELISA. Cellular response was measured *in vitro* by quantifying ALP up-regulation and calcium deposition by MSCs. Cell free gene-activated scaffolds were then implanted into 7 mm calvarial defects (n=8) in male Wistar rats and new bone formation by host cells was assessed using micro-computed tomography (μCT) and histomorphometry.

RESULTS: The chitosan NPs were capable of efficiently delivering the therapeutic pDNA constructs to mesenchymal stem cells (MSCs), transiently up-regulating encoded growth factor expression; in turn significantly enhancing MSC-mediated osteogenesis when compared to untreated controls ($p<0.001$). The pBMP-2 Advance (CMV) induced MSCs to produce approximately 2000 μg of calcium per scaffold, significantly higher ($p<0.001$) than all other groups. Just 28 days post-implantation *in vivo*, the pBMP-2 Advance-activated scaffold induced a significant acceleration in bone tissue formation, significantly higher than the unmodified pBMP-2 gene-activated scaffold, gene-free scaffold and untreated controls ($p<0.001$) as indicated by microCT and histomorphometry (**Fig. 1**).

DISCUSSION: The gene-activated scaffold system described in this study essentially acts as a single-treatment therapeutic factory, inducing host cells to produce BMP-2 protein at physiological levels, resulting in more bone formation at an earlier time-point than previous studies delivering recombinant proteins, without the adverse side effects. Furthermore, the transient protein expression induced by the non-viral vector overcomes concerns associated with gene therapy. This cell-free gene-activated scaffold therefore represents a new 'off-the-shelf' product capable of accelerating bone repair in critical sized defects.

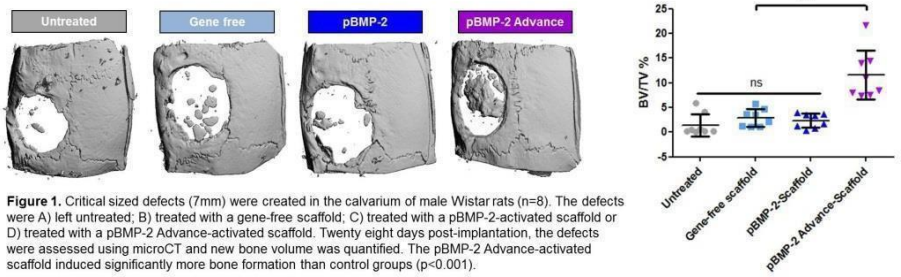

Figure 1. Critical sized defects (7mm) were created in the calvarium of male Wistar rats (n=8). The defects were A) left untreated; B) treated with a gene-free scaffold; C) treated with a pBMP-2-activated scaffold or D) treated with a pBMP-2 Advance-activated scaffold. Twenty eight days post-implantation, the defects were assessed using microCT and new bone volume was quantified. The pBMP-2 Advance-activated scaffold induced significantly more bone formation than control groups ($p<0.001$).

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P411 Generation of integration-free iPSC using mRNA delivery

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Potential risk of insertional mutagenesis in human has prevented the broad clinical applications of induced pluripotent stem cells (iPSCs). Although previous studies revealed that mRNA transfer can generate integration-free iPSCs, this technique may be limited due to mRNA degradation by RNase. Here we introduce an mRNA delivery system employing graphene oxide (GO)-polyethyleneimine (PEI) complexes to efficiently generate integration-free iPSCs. GO-PEI complexes were effectively loaded with mRNAs of reprogramming transcription factors and prevented their degradation by RNase. The iPSCs generated via delivery of GO-PEI/RNA complexes showed all the hallmarks of pluripotent stem cells such as expression of pluripotency genes, epigenetic reprogramming, and differentiation into the three germ layers. Furthermore, both rat and human iPSCs were successfully generated from adult adipose tissue-derived fibroblasts using this method. We suggest that GO-PEI complex-based mRNA delivery system may serve as a new therapeutic approach that elucidates iPSC technology into the clinic settings.

P412 DNA and RNA editing in exon 26 of DYSF gene for gene-cell therapy of LGMD2B and miyoshi myopathy

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Mutations in DYSF gene lead to limb girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy, which are among the most prevalent muscular dystrophies. These diseases cause skeletal muscle degeneration primarily in calf muscles in the lower limbs. There is no effective treatment for these diseases yet. We have chosen spliceosome-mediated RNA trans-splicing (SMaRT) and CRISPR\Cas9 to correct mutation in this gene. SMaRT allows to reprogram RNA splicing process to substitute a corrupt exon to a wild-type via pre-trans-splicing molecules (PTMs). PTMs are complex molecules consisting of 3 domains: binding domain, targeted to an exon of interest; splicing domain, initiating splicing process on the PTM; and a coding domain, a sequence, coding new exon.

We have obtained patient's skin and gingiva fibroblasts with the mutation in an exon 26 of DYSF gene. Corrupt DNA exon in these cells will be substituted to a wild-type by CRISPR\Cas9 gene editing tool and donor DNA. Another part of cells is proceeding DYSF gene transcriptional activation by CRISPR\Cas9 SAM tool for subsequent transduction by AAV8 carrying PTMs. Changes in DYSF gene and DYSF gene expression will be evaluated by PCR, restriction analysis, full transcriptome analysis and new generation sequencing. After DNA and RNA editing performed fibroblasts will be trans-differentiated to myoblasts and myotubes to assess membrane ultrastructural changes by electron microscopy. Such genetically modified myoblasts will have potential for muscle regeneration and gene-cell therapy of LGMD2B.

P414 Long term 3D spheroid culture of Huh-7 cells for production of HCV particles

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Objectives: Hepatitis C virus (HCV) is one of the most prevalent infectious diseases responsible for high morbidity and mortality worldwide, causing acute and chronic hepatitis and hepatocarcinoma. Progress in the field of vaccine development could prevent HCV infection and may sustain the efficiency of new available drug therapies. Three-dimensional hepatic cell cultures may represent physiologically relevant systems to reproduce *in vitro* the entire viral cycle and release HCV particles. The aim of this study was to bioengineer a three-dimensional culture model of one of the rare cell lines permissive to HVC, Huh-7 cells, in order to produce a large number of spheroids, to maintain a long term viability and productivity of HCV particles. However, due to their tumor origin, Huh-7 cells are hyperproliferative which hamper their stability as spheroid when cultivated over few weeks, particularly regarding diameter and viability. Various differentiation agents added in the culture of hepatic cell spheroids were used to stabilize the cell proliferation, improve the expression of differentiation markers while maintaining the HCV particle productivity over long time culture.

Methods: Huh-7 cells were cultivated upon an anti-adhesive support to produce spheroids on orbital shaker. An anti-adhesive substrate was elaborated by coating Petri dishes with Pluronic F108 copolymer to avoid Huh-7 cell adhesion despite the dynamic culture condition. Dimethyl sulfoxide (DMSO) at 1%, used as differentiation agent, was added to the medium at day 0 of culture. The mean diameter sizes and the growth kinetics through DNA quantification were followed during four week-time. Albumin (ELISA method) and alpha anti-trypsin (qRT-PCR) were used as markers of cell differentiation state. During infectious tests with JFH1-HCV (genotype 2a), the production of new HCV particles was followed through RNA viral and infectious level of spheroid culture supernatant measurements.

Results and discussion: Pluronic F108 coating efficiently prevents cells from attaching to the Petri dish plastic. Our data show that Huh-7 cell self-assemble in multicellular aggregates to generate mature spheroids in five days. On the contrary to the control condition, DMSO addition limits the increase of the mean diameter and the size disparity of spheroids during the culture time. Improvements of the cell viability and of differentiated cell status were observed, and most importantly, the spheroids remain highly permissive for HCV infection upon DMSO treatment.

Conclusion: We propose a model of Huh-7 cell spheroid culture presenting many advantages in the objective to be scaled-up in bioreactor: it is a simple, reproducible and low cost method. The DMSO addition stabilizes the morphological characteristics of spheroids while allowing a prolonged production of viral particles. This novel 3D culture model could thus be developed to assess new antiviral strategies.

P415 Leukocyte and Platelet-rich Fibrin in Oro-Dental and Maxillo-Facial Surgery: Evidence from Randomized Controlled Clinical Trials

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Despite significant improvements, in reconstruction techniques and materials, during last decades, the regeneration, restoration and/or repair of oro-dental and maxillo-facial defects remains a challenge. Platelet Concentrates are autologous blood extracts obtained through centrifugation of whole blood samples. The preparation procedure allows the gathering and concentration of platelets and other therapeutic blood constituents (fibrinogen/fibrin, growth factors, leukocytes and circulating cells), in clinically-usable preparations (surgical adjuvants), which may enhance, accelerate and promote tissue (hard and soft) wound healing and regeneration. Despite promising clinical observations, their overall effectiveness remains debated, to date. This is mainly due to: mixed/variable clinical outcomes, limited high-quality evidence-based literature, and poor characterization of end-products (and preparation protocols) used in studies; and - until recently - lack of proper terminology systems to classify these preparations. Indeed, to the best of knowledge, the first "classification" consensus was published in 2009, describing 4 different Platelet Concentrate sub-families, based on variability in biological content (fibrin and cell), properties (gelification) and potential applications: Pure Platelet-Rich Plasma (P-PRP), Leukocyte and Platelet-Rich Plasma (L-PRP), Pure Platelet-Rich Fibrin (P- PRF) and Leukocyte and Platelet-Rich fibrin (L-PRF). Today, it can be safely stated that, in oral and maxillofacial surgery, the L-PRF sub-family is receiving the utmost attention, mainly due to simplicity, user-friendliness, malleability and potential cost-effectiveness, when compared to the PRPs. **Objectives:** Leukocyte and Platelet-Rich Fibrin (L-PRF) is a second generation 3-D autogenous/autologous platelet concentrate (and biomaterial: slowly- and strongly-polymerized fibrin gel; rich in growth factors and lymphocytes) derived via simple and rapid centrifugation of whole venous blood, in the absence of anti-coagulants, bovine thrombin, additives or any gelifying agents. A relatively new "revolutionary" step in modern platelet concentrate-based therapeutics, clinical effectiveness of L-PRF remains highly-debatable, whether due to preparation protocol variability, limited evidence-based scientific and clinical literature and/or inadequate understanding of its bio-components. This critical review provides an update on the application and clinical potential/effectiveness of L-PRF during oral surgery procedures, limited to evidence obtained from available/accessible human Randomized and Controlled Clinical Trials; PubMed (Jan. 2015–Feb. 2016). **Study Selection:** Eligibility criteria included: "Human Randomized Controlled Clinical Trials" and "Use of Choukroun's classic L-PRF preparations only". **Conclusions:** Autologous L-PRF is often associated with early bone formation and maturation; accelerated soft-tissue healing; and reduced post-surgical pain, edema and discomfort. Preparation protocols require revision and standardization. Well-designed RCTs (according to the CONSORT statement) are also needed for validation. Furthermore, a better analysis of rheological properties, bio- components and bioactive function of L-PRF preparations would enhance the cogency, comprehension and therapeutic potential of the reported findings or observations; a step closer towards a new era of "super" oro-dental and maxilla-facial bio- materials / - scaffolds.

Clinical Significance: L-PRF is a simple, malleable and safe biomaterial suitable for use in oral surgery. An innovative tool in Regenerative Dentistry, L-PRF seems a robust and possibly cost-effective biomaterial alternative for oral tissue repair/regeneration.

P417 Metabolic capabilities of a psoriatic skin substitute

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Purpose: Psoriasis is an inflammatory skin disease characterized by the presence of whitish and scaly plaques for up to 90% of the body surface. These plaques result from hyperproliferation and abnormal differentiation of keratinocytes. The absence of healthy or pathological skin substitutes mimicking closely enough their *in vivo* counterparts is a disadvantage for dermopharmaceutical testing. The aim of this study was to determine the metabolic capabilities of a psoriatic skin substitute.

Methods: Skin substitutes were produced according to the self-assembly method and tested regarding their percutaneous absorption toward a topical formulation of tazarotene followed by UPLC analyses. The psoriatic phenotype of these substitutes was confirmed by histological and immunohistochemical analyses.

Results: Histological and immunohistological results confirmed both the healthy and psoriatic phenotypes. Results from percutaneous absorption showed a significant level of drug metabolism when the formulation was applied over 24 h on the skin substitutes. It appeared that our psoriatic skin substitute showed good metabolic capabilities in this precise context.

Conclusion: The absence of tazarotene in the receptor compartment clearly demonstrated the usefulness of our skin substitute for topical drug screening.

P418 Designing 3D cell niches exploiting peptide self-assembly for TERM applications

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In the past two decades significant efforts have been made to develop novel biomaterials for a variety of biomedical applications such as regenerative medicine and cell therapy. One such class of material, which has attracted significant interest, is hydrogels as these soft, highly hydrated materials can be engineered to mimic the cell niche to promote in-vitro and in-vivo tissue regeneration [A Mujeeb et al. Acta Biomat., 2013, 9, 4609; C Diaz et al. J. Tissue Engineer., 2014, 5, 2041731414539344 & 2016, 7, 2041731416649789] or can be used as drug and/or cell in-vivo delivery platforms. A variety of approaches can be used to design hydrogels, one such approach is self-assembly of short peptides. Peptides are of significant interest as they can be synthesised using standard chemical routes and therefore be obtained with high definition and high purity. In addition, being built out of natural amino acids they can be designed to be biocompatible and biodegradable and can be metabolised by the body[A. Markeyet al. J Peptide Sc., 2016 in press].

The formation of hydrogels involves two distinct processes; the self-assembly of the peptides molecules to form thin fibrillar structures and the self-assembly of these fibrils into a 3 dimensional percolated network. Developing a fundamental understanding of these two processes at all length scales is crucial as the properties of the final materials will not only depend on the intrinsic properties of the fibres, but also on how they assemble and ultimately on the properties of the network formed.

We have investigated how network topology affects the mechanical properties of a family of β-sheet forming peptides which design is based on the alternation of hydrophobic and hydrophilic residues. Our results clearly show that in addition to the fibre intrinsic properties, the way these fibres self-assemble themselves to form a 3D percolated network has a significant impact on the final macroscopic properties of the resulting hydrogels. Taking advantage of this new understanding, we have shown how by design, in this specific case introducing a highly interacting hydrophilic amino acid arginine, the fibre-fibre interactions can be manipulated to control the level of fibre lateral association / bundling and therefore to fine tune the mechanical properties of the hydrogels. These highly controlled hydrogels were then used to culture a variety of cells ranging from simple cell lines such as chondrocyte and osteoblasts to stem cells such as embryonic, mesenchyme and induced to try to develop a fundamental understanding of cell material interactions in order to control cell behaviour and/or cell fate in 2D and/or 3D culture.

P421 Micro-computed tomography analysis of tissue engineering scaffolds: How?

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Micro-computed tomography (Micro-CT) is an excellent method to analyze the internal architecture of tissue engineering scaffolds in a 3D and non-destructive fashion. The acquisition parameters have a vital role in the entire analysis process. There is no consensus on the optimal acquisition parameters for tissue engineering scaffolds. There is a wide range of used acquisition parameters in the literature without justification. The aim of the study is to identify the effects of the scanning parameters on the final results (*e.g.* porosity, mean pore size and mean wall thickness) of different polymeric (*e.g.* silk fibroin) and ceramic (hydroxyapatite) scaffolds for application in tissue engineering strategies.

P422 Bioactive glass / fibroin composite scaffolds for the treatment of osteochondral defects

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Due to the aging of the population and some sports related injuries, there are more and more degenerative changes in the joints which reduce the activity of the people. Among the more painful are osteochondral lesions, where due to the severe damage of articular cartilage, underlying subchondral bone is damaged as well. Such osteochondral defects demand treatment of two different tissues. The effective material for osteochondral regeneration should induce bone regeneration on the bone side of the defect and at the same time allow cartilage regeneration on the cartilage side of the defect. Therefore in this study 3D scaffolds combining fibroin and bioactive glass were prepared. Bioactive glass is amorphous osteostimulative inorganic material, clinically proven to be safe and efficient for bone regeneration *in vitro*, *in vivo* [1], whereas silk is a natural biopolymer that has been widely studied in tissue engineering due to its good biocompatibility and mechanical properties such as elasticity and high tensile strength[2].

As bioactive glass is soluble material it does not affect only the bone regeneration but also has a great impact on the biopolymer itself. Ca^{2+} ions released from the bioactive glass change the fibroin secondary structure similarly as in nature *B. mori* silkworm does during the spinning process. We demonstrated using HRTEM analysis how the Ca^{2+} ions released from bioactive glass, result in the reduction of □-sheet domain size that effectively controls important scaffold's properties, such as degradation, mechanical stiffness, acellular bioactivity as well as cell response.

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[2] D. N. Rockwood et al., Materials fabrication from Bombyx mori silk fibroin, Nat. Protocols, 6**,** 2011.

P423 Differential mechanism of bone differentiation on mulberry and non-mulberry silk

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Despite extensive research on bone tissue engineering, osteogenic signaling on silk matrices is still poorly understood. Therefore a major focus of our research group is to understand the key signaling mechanisms that regulate bone differentiation on 3D silk matrices by monitoring the expression and in vitro responses of osteogenic progenitors (Midha et al. 2016a). In this context, recently we reported how textile-based silk braided structures developed from mulberry source Bombyx mori regulate hydroxyapatite crystal formation along the crystallographic c-axis (002), longitudinally parallel to the fibroin fibres; a phenomenon resembling biomineralisation of collagen type I in native bone (Midha et al. 2016b). Further, a comparative analysis between the mulberry and non-mulberry silk in vivo demonstrated complete regeneration of the defect site in rat calvariaea within 6 months, albeit only in the case of non-mulberry Antheraea mylitta porous scaffolds (Sahu et al. 2015). Based on these studies, we conducted an in-depth characterization to explore the differential mechanisms of osteogenic differentiation that regulate varying extents of bone differentiation on the two most studied silk species. In vitro culture with human pre-osteoblasts revealed rapid differentiation of cultured cells into characteristic osteocytic phenotype within 14 days. Surprisingly, this phenomenon was only evident in A.mylitta textile-braids. Detailed molecular investigations including real-time gene expression profiling and whole genome sequence analysis revealed the involvement of Wnt/beta-catenin signaling in stabilizing mature osteocytic phenotype of differentiated human osteoblasts in non-mulberry silk matrices. Also, a series of physical characterizations highlighted that varying biochemical (composition), physical (tensile strength, compression, surface roughness, wettability) and geometric properties of textile braids exert strong influence on differential mechanisms of osteogenesis, hence leading to varying extents of bone formation on the surface of the two silk species. Our recent application in this context extends to us of Next generation sequencing to develop comparative signalling pathways on two types of silk fibroin and establish 3D in vitro model of bone differentiation, which may find potential in therapeutic applications.

P424 Spider silk nerve implant for reconstruction of peripheral nerves: schwann cell migration in 15.0cm artificial nerve grafts

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Introduction: Peripheral nerve defects are a common and debilitating consequence of iatrogenic acquired nerve lesions or severe trauma. Standard surgical approach in reconstructive surgery is the tension free end- to- end suture. If this is not achievable an autologous nerve transplantation is indicated. In particular cases a subsequent treatment with autologous donor materials is not applicable due to limitation of donor nerve tissue. Alternatives to this gold standard technique are the surgical application of artificial nerve grafts. Concern of this study is the spider silk nerve implant, constructed out of spider silk from Nephilla edulis and ovine decellularized vessels.

Aim: Investigation of cell survival, cell migration and distribution of nutritive substances on spider silk fibers through an artificial nerve graft of various length ranging from4.0-15.0cm.

Methods: Schwann cells were isolated, cultured and purified from adult rodent animals. Purification was determined by S-100/Dapi counterstaining. Spider silk was collected from adult spiders of genus Nephilla edulis, sterilized and pulled through decellularized ovine vessels to form inner lining of spider silk nerve grafts. Implants were settled with Schwann cells and viability and proliferation was controlled after 7 and 21 days by viability essays.

Results: Cell enhancement and proliferation increased during observation, cell number developed continuously, and viability was controlled and unobtrusive. There were no obvious influences of construct length on Schwann cell viability.

Conclusion: Results indicating that spider silk nerve implants are an appropriate environment for Schwann cell migration independently to vessel length. That makes further application in vivo on distances >6.0cm interesting for reconstructive surgery.

P425 Development of silk based angle-ply construct for annulus fibrosus tissue engineering

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Degenerative disc disease (DDD), the major cause of law back pain (LBP), occurs due to several factors including injury, age, vascular ingrowth and poor disc nutrition [1]. For the past few years, a number of tissue engineering strategies have been investigated as alternative approaches to repair disc degeneration [2-3]. These efforts rarely satisfy annulus fibrosus (AF) replacements due to its critical architectural organization. However, major challenge remains toward successful AF tissue engineering, mainly because of the tremendous complexity at cellular, biochemical, microstructural, and biomechanical levels. To mimic AF anatomical structure, we fabricated a novel multi-lamellar angle-ply construct (maintaining ~30° angle) with varying composition of mulberry (*B. mori*) and non-mulberry (*Antheraea assamensis* and *Philosamia ricin*i) silk fibroin (SF) that provided differential mechanical properties. The purpose of this study was to investigate the effects of non-mulberry SF on physicochemical properties, biological responses concomitant with alignment and mechanical properties on extracellular matrix secretion. Physical characterizations including FTIR, FESEM, XRD, porosity, swelling, mechanical properties and degradation studies were performed. The blends showed significant changes in physical properties as compared to only mulberry SF based constructs. Concentration dependent increase in compressive modulus (12.37 ± 5.29 kPa to 655.02 ± 56.83 kPa) and decrease in porosity (~65 to ~35 %), inter-lamellar distance (~15 to ~5 µm), swelling rate (~10 to ~4), and degradation rate (~ 92 to ~ 37 % mass remaining) were observed in blends. Constructs were seeded with AF cells for cytocompatibility study. Presence of non-mulberry SF, particularly *A. assamensis* SF in blends supported the increased proliferation of AF cells (~1.5 fold) on day 21. Cellular alignment on lamellar scaffolds was assessed by Hoechst 33342 staining. The construct was further subjected to histological analysis, biochemical assays, and gene expression studies. The biochemical analysis revealed that the blends (particularly, *B. mori* and *A. assamensis*) showing higher mechanical strength, were associated with higher collagen, but lower sGAG (sulphated glycosaminoglycans) deposition. Overall, the developed construct supported cell proliferation and ECM formation resulting in a functional AF tissue like construct, indicating its potential as an alternative strategy in intervertebral disc (IVD) replacement therapy.

Keywords: *Degenerative disc disease; Intervertebral disc; Tissue engineering; Non-mulberry silk; Lamellar construct*

P426 Copper (II)-silk fibroin hybrid flowers with Antibacterial activity and selective cytotoxicity

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Antibacterial activity with selective cytotoxicity is of immense research interest. Taking the advantage of Cu ions, a facile one-pot reaction for the synthesis of organic-inorganic hybrid flower-mimetic silk microsphere is proposed. Silk fibroin molecules act as nucleation site for copper phosphate crystals induce the growth of flower petal like micrometer-sized Cu-silk microspheres. Typical bacterium such as *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) are used to investigate the antibacterial potentiality of the synthesized material. The sensitivity of metallic flower is dependent on interface exhibited by bacterial membrane; Gram positive bacteria reveals less sensitivity due to the presence of thicker peptidoglycan layer compared to Gram negative. In presence of ABTS [2,2'Azino-bis(3- ethylbenzthiazoline-6-sulfonic acid)], metallic flower exerts peroxidase-like activity based on Fenton-like reaction mechanisms that can also cause antibacterial propensity. Contact of high Cu concentration causes bacterial membrane pitting results in enhanced permeability. In contrast, the metallic flower reveals less toxicity to mammalian cells imparting selective toxicity of the synthesized material; indicating promising quality to serve as therapeutic delivery carrier or biocompatible disinfect material coating in medical device.

P428 Silk fibroin nanofibres in cardiac drug delivery

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Introduction: Cardiac regenerative therapy, whereby drugs, growth factors and/or pro-regenerative cells are administered to myocardium, has demonstrated significant potential in post-operative therapy searching novel delivery systems. Silk fibroin (SF) nanofibres have attracted a huge attention recently due to their properties including increased surface reactivity, protein character and high specific surface important for improved drug bioavailability. Those properties make SF-based nanofibres an interesting material for a cardiac post-operative ß- blocker delivery.

Our objective in this study was to develop a nanofibrous SF-based material with a short degradation time for cardiac drug delivery and evaluate its properties relevant for the application site.

Methods: *Bombyx mori* raw cocoons by degum procedure, ionic liquid dissolution and subsequent needle-less electrospinning. The electrospun nanofibres were crosslinked by alcohol dehydration. The SF nanofibres of various crosslinking levels were characterized in terms of morphology (SEM), chemical composition (FTIR), degradation, drug loading capacity and drug release kinetics. Biocompatibility and bioactivity of bare and ß-blocker doped SF nanofibres were evaluated using a cardiac cell model *in vitro* on undifferentiated and differentiated H9C2 rat myoblastic cells.

Results: The degradation kinetics as well as drug loading capacity was revealed to be strongly crosslinking level dependent. The optimal correlation was reached at above 50% crosslinking confirmed via spectroscopic analysis. In contrast to those results the biocompatibility tests showed improved bioactivity of the SF nanofibres with a lower degree of crosslinking due to accelerated degradation and protein mass release. Those results were confirmed in both cases – undifferentiated H9C2 cells and fused myotubes. Affecting their typical protein expression profiles.

Discussion and Conclusions: *Bombyx mori* silk fibroin nanofibres were studied as a potential matrix for a for cardiac drug delivery. SF nanofibres of an optimised degradation profile were confirmed to have a positive impact on heart model *in vitro* represented by myoblast and myotube H9C2 cells culture. Based on results of the study presented, we believe silk fibroin nanofibres are an interesting candidate for a short-term post-operative drug delivery.

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P429 Simulated hyper~~-g~~ravity induces changes in human tendon-derived cells: from cell morphology to gene expression

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Gravity influences physical and biological processes, having an impact on development, as well as homeostasis of living systems. The musculoskeletal system is comprised of several mechano-responsive tissues and altered gravitational forces are known to influence distinct properties, including bone mineral density and skeletal muscle mass. This is particularly relevant in a near-weightlessness (microgravity) environment, which is found during spaceflight and, not less importantly, during bed resting. Over the years, several studies have been conducted under simulated conditions of altered gravity owing to the advances on ground-based facilities, such as bioreactors for microgravity / hypo-gravity (<1g) research and centrifuges for hypergravity (>1g) studies. Interestingly, microgravity-induced alterations are comparable to tissue degeneration caused by disuse and ageing. In turn, exposing musculoskeletal tissues to hypergravity may constitute a way of simulating (over)loading or, eventually, to be used as a measure to rescue cell phenotype after exposure to near-weightlessness conditions. Different studies have focused on bone, cartilage and skeletal muscle, but effects on tendons and ligaments have been underappreciated. Therefore, we evaluated the influence of increasing g-levels (5g, 10g, 15g and 20g) and different hypergravity exposure periods (4 and 16 h) on the behaviour of human tendon-derived cells (hTDCs). For this purpose, hTDCs were exposed to simulated hypergravity conditions using the Large Diameter Centrifuge (LDC) from the European Space Research and Technology Centre (ESTEC, ESA, The Netherlands). Human TDCs cultured under standard conditions (1g, normogravity, Earth gravity force) were used as controls. The effects of hypergravity on the viability of hTDCs, as well as on the expression of tendon related markers at the gene level were evaluated.

Simulated hypergravity resulted in a reduced cell content after 16 h independently of g-level, as determined by DNA quantification. Additionally, the different g-levels studied led to changes in cell and cytoskeleton morphology. Strikingly, a 16-hour period of exposure resulted in alterations of gene expression profiles. Overall, gene expression of tendon-related markers, including collagen types I (*col1a1*) and III (*col3a1*), scleraxis (*scx*), tenomodulin (*tnmd*), decorin (*dcn*) and tenascin (*tnc*), seemed to be increased upon hypergravity stimulation and in comparison to cells cultured under control conditions.

Altogether, these results highlight that altered gravity, particularly simulated hypergravity, has an influence on the phenotype of tendon cells, opening new avenues for research focused on using altered gravity as a model for overloading-induced tendon tissue injury or as measure to rescue the phenotype of degenerated tendon cells.

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P430 Biochemical and biomechanical characterization of a novel xenogenic ACL substitute

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Over 800,000 knee ligament reconstruction surgeries are estimated to be performed each year worldwide. Currently, different types of grafts (i.e., autologous, homologous, synthetic and xenogenous) have been used as good ready to use solution to reconstruct the torn ACL and reduce operating time. All the substitutes which are nowadays available, however, have shown low reliability, in terms of wear, strength, elasticity, long-term durability and biocompatibility, frequently ascribable to current sterilization techniques.

Herein, we hypothesize that inclusion of xenogenous graft in specific package containing a free radical scavenger-enriched hydrogel might help preserving its structural properties during sterilization process and then promoting soft and bone tissue healing once implanted. Our objective was therefore to biochemically and biomechanically characterize an ACL replacement substitute based on xenograft originated from an equine phalanx extensor tendon, processed with a specific enzymatic deantigenation method, sterilized by means of e-beam and preserved in a proprietary hydrogel. The study was performed on three different groups: 1) not processed samples (non-sterile wild type tendons, WT); 2) samples treated via osmotic process, sterilized and packaged into a PBS solution (sterile processed tendons, ST); 3) samples processed and packaged inside a specific Vitamin C-enriched PEO/HPMC-based protectant hydrogel (CPH) and terminally sterilized through beta-rays (hydrogel-protected sterile tendons, HST).

It has been demonstrated that bio-chemical properties after osmotic treatment were preserved: respect to non-treated WT samples, more than 50% of structural polysaccharides were conserved, as demonstrated by GAGs residual concentration; the degree of decellularization was evaluated through measurement of DNA residual content that was under the limit of 50ng/mg. Moreover, the immunoblotting experiment demonstrated that α-Gal epitope was absent in treated osmotic samples. Despite osmotic treatment seems to negatively influence mechanical response of ST samples - UTS (Ultimate Tensile Strength) and E (Elastic modulus) reduction of about 50% and 30%, respect to WT samples – the introduction of CPH allowed to even double both mechanical parameters of WT samples. Lastly, preliminary *in vitro* tests with human tenocytes also confirmed a synergistic effect of osmotic treatment and CPH on biological response.

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P431 Comparing two common production methods for platelet-rich plasma and the boosting effect for human anterior cruciate ligamentocytes

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Introduction: Platelet rich plasma (PRP) has been widely promoted to accelerate wound healing but its efficacy is still debated [1]. Recently, it was applied in sport-related injuries such as anterior cruciate ligament (ACL) reconstruction. PRP has several distinct advantages: it is autologous and biocompatible, making it inherently safe to use although different PRP formulations yield different counts of thrombocytes and leucocytes. PRP is especially interesting for ACL injuries since the ACL has poor self-healing capacities leading to constant failure of primary repair attempts [2]. The purpose of this study was to examine the effects of different PRP formulations: with leucocytes (L-PRP) and pure PRP (P-PRP) on human ACL-derived ligamentocytes (LCs) *in vitro*.

Material and Methods**:** LCs were isolated from patients undergoing ACL reconstruction surgery with ethical approval. 3D cell cultures composed of LCs and collagen patches were exposed to five experimental groups (n=5): 2.5% L-PRP, 2.5% P-PRP, 20% L-PRP, HG-DMEM and HG-DMEM + 10% FCS. Cell proliferation, cell phenotype on an mRNA transcript level, and extracellular matrix production were evaluated. PRISM software (version 6.0e, GraphPad, La Jolla, US) was used to analyse the data with 2-way analysis of variance (ANOVA) comparing differences between groups. Values are given means ± SD. A p-value < 0.05 was considered statistically relevant.

Results: .In both PRP groups thrombocytes were increased four-fold compared to whole blood. Leucocytes were higher, as expected, in L-PRP than in P- PRP and whole blood. DNA content and metabolic cell activity increased significantly in all groups on day 21 compared to day 7, except in the negative control. No changes in ECM production were detected. 2.5% L-PRP induced predominantly MMP3 (Figure 1) whereas 20% L-PRP expressed MMP13 on day 21.

Discussion: Soluble factors in very low concentrations of 0.05x PRP stimulate cell proliferation and mitochondrial activity with and without leucocytes similar to 0.8 x L-PRP concentrations. Additionally, MMP expression depends on leucocyte count with enhancement of MMP 3 in low and MMP13 gene expression in high leucocyte concentration. Zhou *et al*. [3] showed similar to our study in rabbit tendon stem/progenitor cells treated with 0.3x PRP with and without leucocytes induce both cell proliferation, additionally L-PRP also promoted the expression of catabolic marker genes and their respective proteins.

P432 Triggering the activation of activin A type II receptor in human adipose stem cells towards tenogenic commitment using mechanomagnetic stimulation

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Tendon injuries are a considerable problem worldwide and increases with aging. Available treatments fail to fully restore tendon's functionality and stem cell therapies could be an alternative approach to stimulate tendon regeneration and homeostasis. Mechanosensitive membrane receptors can be stimulated by the presence of magnetic nanoparticles (MNPs) through magnetic remotely actuation, activating stem cells towards controlled cellular responses [1]. Activins are members of the TGF-β superfamily which participate in regulation of several biological processes. Upon activation, the phosphorylation of Smad2/3 is induced allowing translocation of the complex to the nucleus, regulating the transcription of scleraxis and tenomodulin genes driving differentiation.

In this work, we propose to target the Activin receptor type IIA (ActRIIA) using MNPs and triggering the downstream Smad2/3 pathway and signalling cascade. Herein, the ActIIRA activation is externally controlled through magnetic actuation with a vertical oscillating magnetic bioreactor (MICA Biosystems Ltd).

Adipose derived stem cells (hASCs) are widely available stem cells with clinical potential for tendon tissue engineering strategies [2, 3] and therefore were selected in this work. hASCs (100,000 cells/well) were incubated with 250nm carboxyl functionalised MNPs (Micromod) previously coated with anti-ActRIIA antibody (Abcam), anti-Rabbit-IgG antibody (Abcam) or RGD tri-peptide (Sigma) by carbodiimide activation. After a 30min labelling period with conjugated MNPs, hASCs were incubated for 2, 10, or 30min in basal αMEM medium under magnetic stimulated (1Hz) and non-stimulated conditions. hASCs cultured without MNPs in basal medium supplemented with Activin A (R&D systems, 10 and 20ng/mL) were considered positive controls of ActRIIA activation. The MNPs conjugated only with secondary IgG antibody or RGD constitute the negative controls of the ActIIRA activation. After each incubation period, cells were analysed by enzyme-linked immunosorbent assay (ELISA) for phospho-Smad2/3 (Cell Signaling Technology) proteins detection.

The detection of phospho-Smad2/3 proteins was significantly more intense after 10 minutes in hASCs under magnetic stimulation, which highlights a more efficient ActRIIA activation. Results also show a significant elevation in response to the control group labelled with secondary IgG or RGD particles. These outcomes suggest that ActRIIA is a mechanosensitive receptor that can be remotely activated upon magnetic stimulation. Ongoing studies are focused on the possible effects of remote magnetic ActRIIA activation on hASCs tenogenic differentiation. In conclusion, the remote activation of MNPs tagged hASCs may have potential for controlling stem cell differentiation resulting in successful cell therapies for tendon regeneration.

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[1] M. Rotherham, A.J. El Haj, PloS one (2015); [2] A.I. Goncalves, et al, PloS one (2013); [3] A.I. Gonçalves, et al, Advanced Healthcare Materials (2016).

P433 Development of an extracellular matrix scaffold for the treatment of tendon injuries

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Introduction

Chronic and acute injuries of the tendon are frequent afflictions, with important impact on work and everyday life. Materials with sufficient biomechanical properties are desired when facing extended injuries and when donor tendons are not available from the patient. Commercially available extracellular matrices (ECM) exist to treat injured tendons, but they originate from other tissues (small intestinal submucosa, dermis, etc.) and present biomechanical properties weaker than normal tendon. Our aim is to develop a tendon substitute starting with equine tendon to better simulate original characteristics.

Methods

Horse superficial digital flexor tendon (SDFT) from accredited food industry with traceable sources was used as starting material to prepare an ECM scaffold. Prototype pieces of 100 x 10 x 1.2 mm were decellularized with Triton 1%, rinsed with water, sterilized with ethanol and finally rinsed again with culture medium. Quality of decellularization and preservation of structure were evaluated histologically and with DNA dosage. Biomechanical properties were evaluated with a tension assay. Finally, biocompatibility was evaluated by seeding human Fetal Progenitor Tenocytes (hFPTs) and histological evaluation at days 3 and 21.

Results and Discussion

The treatment resulted in excellent decellularization of the matrix with no visible cells (HE and DAPI stain) and DNA content was reduced to 1.3 ng per mg of dry tissue. The Sirius red stain highlighted the good preservation of native tissue. Biomechanically, the Young's modulus was 116.4 (SD 45.8) MPa which is lower than native tendon, but higher than what is usually found with commercial ECM (1). The biocompatibility with hFPTs was excellent as the cells had attached to the surface at day 3 and migrated deeply within the developed scaffold at day 21. These results are promising, but prototype samples with relatively low thickness (1.2 mm) had to be used to be able to evaluate higher number of samples from one tendon. The technique should now be scaled up to allow the preparation of thicker scaffolds (3-5 mm thickness).

Conclusion and Impact of Investigation

It was possible to develop a scaffold with interesting biomechanical properties and good biocompatibility with tenocytes (hFPTs). The development of larger scaffolds could lead to a useful solution for treatment of tendon injuries.

P434 Can collagen matrix composition drive a viscous profibrotic cycle in chronic tendinopathy?

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INTRODUCTION:

The exact mechanisms by which loss of homeostatic cell-matrix interactions drive tendon disease (Tendinopathy) is yet to be uncovered. Histopathological investigations have shown that the molecular composition of tendon microenvironment in chronic tendinopathy has many features typical of dysfunctional fibrotic scarring (↑↑collagen III, ↑↑fibronectin fragments and ↑↑proteoglycans). However, little is known whether this aberrant milieu can be viewed as causal in the pathoaetiology of tendinopathy. We hypothesized that fibrosis in chronic tendinopathy is mainly matrix-driven phenomena and that collagen III might play a significant role in initiating a pro-fibrotic feedback loop.

METHODS:

Hybrid collagen I/III hydrogels of increasing collagen type III concentration (0, 10, 30%) were seeded with primary rat tail tenocytes at $5x10^5$ cells ml^{-1} collagen. Mechanical tension was applied to the constructs via two PDMS cantilevers. Gene and protein expression was assessed for markers of matrix production, matrix remodelling and fibrosis.

RESULTS & DISCUSSION:

Fibrosis is characterized by excessive accumulation of collagens, fibronectin and proteoglycans which were all upregulated in the tendinopathic-like conditions (10% and 30% collagen III). The qPCR data reveals that the expression of the matrix remodelling enzymes, MMP-2, MMP-9 and MMP-13, are increased relative to controls. MMP-2 was markedly increased (up to 4-folds) at early time points (day 1) while MMP-13 spiked at day 7. Differences in the gene expression for markers of fibrotic dedifferentiation were also observed proportional to the increase in collagen III.

The results of this study demonstrate that collagen I/III hybrid matrices are able to recapitulate some key aspects of the pro-fibrotic healing response in chronic tendinopathy. Several studies showed that MMPs 2, 9 and 13 are peaked markedly in tendionopathy. MMP-9 and MMP-13 orchestrate the degradation of collagens and are highly expressed in ruptured tendons, while MMP-2, among other proteases, is known to regulate the remodelling of scar tissue.

P435 Identification of miRNA with a putative role in tendogenic formation of mesenchymal stem cells

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In comparison to other mesenchymal lineages the biology of tenogenic differentiation is barely understood. Therefore physiological tendon regeneration remains a part of musculoskeletal therapy research. Specifically easy and efficient protocols are required that might enable tendon cell and tissue differentiation based on adult (stem) cell sources. Previous studies have shown that overexpression of the growth factor BMP2 and a constitutively active transcription factor, Smad8 L+MH2, mediates tendon differentiation *in vitro* and the formation of tendon-like tissue *in vivo* in both the murine mesenchymal C3H10T½ progenitor cell line and human mesenchymal stem cells. Transcriptome analyses revealed four genes encoding secreted factors that were notably upregulated during BMP2/Smad8 L+MH2- tenogenic differentiation as compared to BMP2-overexpression resulting in osteogenic differentiation. However gain-of-function analyses in mesenchymal stem cells did not reveal obvious functions in tenogenic differentiation.

Therefore we reasoned that the biological effects observed in both cell lines might be caused by more complex biological processes. In line with this argument we performed a microarray screen for miRNAs. During this screening, we identified 11 promising miRNA candidates which show up- or down-regulation in the tenogenic cell line C3H10T½ BMP2/Smad8 L+MH2 in comparison to C3H10T½ BMP2 cells (Figure 1). Currently these miRNAs are characterized individually with respect to their influence on tenogenic differentiation in both the murine C3H10T½ cells and human mesenchymal stem cells. The data obtained will be presented in the meeting.

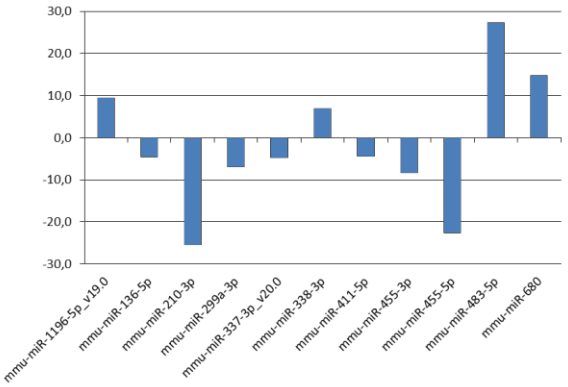

Figure 1: 11 regulated miRNAs. C3H10T½ BMP2/Smad8 L+MH2 cells and C3H10T½ BMP2 cells were cultured under tenogenic differentiation conditions for up to 7 days and total RNA was isolated at day 0 and day 7 of differentiation. Data represent miRNA levels of C3H10T½ BMP2/Smad8 L+MH2/ C3H10T½ BMP2 normalized to day 0.

P436 Ciprofloxacin and uremic toxins affect human tenocytes in vitro

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Background: Quinolones such as ciprofloxacin are commonly used, synthetic antibiotics for the treatment of a variety of infections. Tendinopathy, including tendon rupture, is a rare but serious complication of quinolone therapy. Risk factors associated with quinolone-induced tendon disorders include chronic kidney disease, renal transplantation, and hemodialysis. The exact pathophysiological mechanisms by which quinolones and uremic toxins could contribute to tendinopathies are still unclear. Knowledge of shared mechanisms might improve tendon repair strategies in hemodialysis patients.

Aims: The present study explored the effects of the uremic toxins phenylacetic acid and quinolinic acid, alone and in combination with ciprofloxacin on human tenocytes *in vitro*.

Methods: Primary human tenocytes derived from hamstring tendons were treated with ciprofloxacin, and the uremic toxins phenylacetic acid, and quinolinic acid alone or in combination at different concentrations and time courses. Cells were investigated for metabolic activity, vitality, gene and protein expression of the dominant extracellular matrix (ECM) protein type I collagen, cell-matrix receptor beta1 integrin, proinflammatory interleukin-1(IL-1)beta and the ECM degrading enzyme MMP1.

Results: Ciprofloxacin, when administered at higher concentrations, suppressed tenocyte viability after short-term exposure and, in addition, at therapeutic concentrations after prolonged exposure. Phenylacetic acid reduced tenocyte viability only after prolonged exposure to very high doses and when combined with ciprofloxacin. A small increase of cell death was detected in tenocyte cultures treated with phenylacetic acid and its combination with ciprofloxacin. Quinolinic acid alone hardly developed any cytotoxic effect. Combinations of ciprofloxacin with phenylacetic acid or ciprofloxacin with quinolinic acid did not cause greater cytotoxicity than incubation with ciprofloxacin alone. On the contrary, gene expression of the pro-inflammatory cytokine IL-1beta was reduced by ciprofloxacin after 72h. In contrast, phenylacetic acid and quinolinic acid up- regulated interleukin-1beta mRNA levels. Protein levels of type I collagen, decreased in response to high ciprofloxacin doses, whereas phenylacetic acid and quinolinic acid – even in combination with ciprofloxacin – did not affect collagen type I synthesis significantly. MMP-1 mRNA levels were increased by ciprofloxacin. This effect became more pronounced in the sense of a synergism following exposure to a combination of ciprofloxacin and phenylacetic acid. Quinolinic acid and its combination with ciprofloxacin did not influence MMP-1 mRNA levelssignificantly.

Conclusion: Ciprofloxacin was more tenotoxic than the uremic toxins phenylacetic acid and quinolinic acid. Whether long term exposure of phenylacetic acid and quinolinic acid are involved in the development of tendinopathies requires further investigation.

P437 Bevacizumab reduces angiogenesis in a rat tendon defect model

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Healthy tendon is poorly vascularized and has a low cell density. Hypervascularization is observed in tendons after injury and is a hallmark of tendinopathy. Degenerative processes such as ectopic mineralization, fatty infiltration and nerve ingrowth are closely associated to neoangiogenesis. So far it remains unclear, whether hypervascularization is causing these effects or whether it is only a side effect.

Here, we hypothesize that Bevacizumab (Beva), a humanized monoclonal antibody reducing VEGF- activity, is capable to reduce angiogenesis in a rat Achilles tendon defect model and to affect tendon healing.

A full Achilles tendon defect was generated in female Lewis rats.At 4 and 11 days post-surgery, 125µg of Beva were injected into the defect. The repair tissue was analysed by immunohistochemistry 14d and 28d after surgery using: the rat endothelial cell marker RECA 1, the macrophage marker CD68 and the proliferation marker KI67, while Beva was detected with an antibodyagainst human IgG.

Beva was detectable 4 days after injection, thus is not immediately cleared by macrophages. Immunohistochemistry with RECA1 revealed a reduced (-58.5%; $p<0.05$) vascular density 28 d post-surgery in the Beva treated animals compared to the vehicle controls. Also, the number of CD68+ macrophages is reduced by 52.6% at day 14 and by 49.5% ($p<0.05$) 28d after surgery, as is the percentage of proliferating, Ki67+ cells at d28

With this work we show that Bevacizumab is capable to reduce angiogenesis in tendon defects and to affect inflammatory processes.

P439 Immunogenicity of glutaraldehyde-fixed porcine heart valves in patients after implantation up to 51 months is induced by both antigenic tissue proteins and the αGal epitope

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Background: Glutaraldehyde-fixed porcine heart valves (ga-pV) are one of the most frequently used substitutes for insufficient aortic and pulmonal heart valves which however degenerate after 10-15 years. Yet, xeno-immunogenicity of ga-pV in humans including identification of immunogens still needs to be investigated. We here determined the immunogenicity of ga-pV in patients with respect to antibody formation and identity of immunogens.

Methods: Levels of tissue-specific and anti-αGal-antibodies were determined retrospectively in patients who received ga-pV for 51 months (n=4), 25 months (n=6) or 5 months (n=4) and compared to age-matched untreated subjects (n=10). Immunogenic proteins were investigated by Westernblot approaches and mass spectrometry.

Results: Tissue-specific antibodies in patients were elevated after 5 (1.73-fold) and 25 (1.46-fold, both $p<0.0001$) but not after 51 months whereas anti-Gal antibodies were induced 4.75-fold and 3.66-fold after 5 and 25 months (both $p<0.0001$) and still were significantly elevated after 51 months (2.85-fold, $p<0.05$). Westernblots of porcine valve extracts with and without enzymatic deglycosylation revealed strong specific staining at ≈65 and ≈140 kDa by patient sera in either group which were identified by 2D Westernblots and mass spectrometry as serum albumin and collagen 6A1.

Conclusion: Immune response towards ga-pV is induced by the porcine proteins albumin and collagen 6A1 as well as αGal epitopes, which seemed to be more sustained. Since collagen is a structural extracellular matrix protein these findings most likely also apply to decellularized porcine tissue which have been shown to display a weaker but still present residual immunogenicity.

P440 The number of M2-macrophages do not correlate with fibrosis in peritoneal adhesion formation: an animal study

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Introduction

Peritoneal adhesions remain a relevant clinical problem after surgery. To prevent the formation of adhesions several barrier materials are currently in clinical use. However, the optimal material has not yet been found. In this context macrophages play an active role in the pathophysiology of regeneration and fibrosis. Today two main subpopulations of macrophages are known, M1-macrophages and M2-macrophages. Because macrophages are involved in the regulation of the synthesis of extracellular matrix components, it is of interest to understand more fully the cascade of adhesion formation and fibrosis with respect to the macrophage subpopulations. In the present study, a potential correlation between the number of M2 macrophages and the extent of fibrosis after using different barrier materials for adhesion prevention was analysed.

Materials and Methods

42 Wistar rats were studied in this experiment. They were randomly divided into 6 groups according to the treatment of the barrier material: Adept® (n=7), Intercoat® (n=7), Spraygel® (n=7), Seprafilm® (n=7), SupraSeal® (n=7). The barriers were implanted after standardized peritoneal damage. As a control group 7 animals remained untreated. After 14 days the treated areas were explanted and histological, histochemical and immunhistological analyses were performed regarding the extent of inflammation (H & E staining) and that of fibrosis (EVG-staining). M2-macrophages were identified immunohistochemically using antibodies against CD163.

Results

In each group different amounts of infiltrating M2-macrophages could be detected: The highest number of M2-macrophages was found within the Spraygel®-group, followed by the Supraseal®-group, the Intercoat®-group, the Seprafilm®-group, the control group and finally the Adept®-group. The Intercoat®-group, the Seprafilm®-group and the Supraseal®-group showed a narrow band of fibrosis. The control group, the Adept®-group and the Spraygel®-group demonstrated a moderate band of fibrosis. No statistical correlation could be found between the number of M2-macrophages and the extent of fibrosis.

Discussion

Our results revealed no correlation between the extent of fibrosis and the amount of M2-macrophages. Since M2-macrophages play a regulatory role within wound healing and regeneration, not only the number of infiltrating cells seemed to be relevant for the pathomechanisms of fibrosis. Thus, the number of M2-macrophages alone seemed not be relevant. In further studies the interplay of M2-macrophages and other cells of the regenerative cascade need to be analysed in more detail. Furthermore, the microenvironment of fibrosis and its cellular and humoral composition should be analysed not only at one check point but over time in order to understand better the effect of time on these regulatory processes.

P441 Harnessing bacterial antigens for bone regeneration

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Background: during the inflammatory phase of normal bone regeneration, certain innate and adaptive immune cell subsets have a key regulatory role in osteoblast differentiation and neovascularization. Also in a pathological context, inflammatory reactions are sometimes linked to new bone formation. Although the uncontrolled response to a bacterial infection has detrimental effects on bone tissue, new bone formation can occur during bone infections under milder inflammatory conditions.

Hypothesis: the complex tissue response evoked by bacterial antigens may induce unique pathways involved in osteogenesis when applied under controlled conditions.

Methods: different strains of bacteria with unique cell wall antigens were inactivated by gamma-irradiation. In a tibia model in rabbits, we aimed to answer how the local response to these antigens affected osteogenesis in comparison to an uncontrolled bone infection. Changes in the bone were quantified by micro-CT after 28 and 56 days. Furthermore, in an ectopic location, we addressed how these bacterial antigens modulate bone formation when incorporated into clinically relevant bone tissue engineered constructs. The bone volume in the constructs after 8 weeks was quantified by histomorphometry.

Results: the inoculation of inactivated bacteria in the tibial medullary cavity induced new periosteal bone formation similar to that observed during an implant infection. Osteolytic bone changes were also observed, however these were less profound compared to those found for virulent bacterial strains. Since the presence of inactivated bacteria generally resulted in a transient bone volume increase, bone anabolic pathways may predominate in this process. The incorporation of bacterial antigens did not change the bone induction by biphasic calcium phosphate scaffolds. However, in these scaffolds, the bacterial antigens drastically affected new bone formation in synergy with the osteoinductive growth factor bone morphogenetic protein-2. A milder inflammatory response was stimulatory for bone formation, while an exaggerated response was inhibitory to the process. This response could be successfully controlled by applying the appropriate dosing regime within the bone substitutes.

Conclusion: the combined application of osteogenic and immunomodulatory agents promotes bone formation

P442 The role of inflammation in epimorphic regeneration

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Epimorphic regeneration involves de-differentiation of terminally differentiated cells back to precursor cells which resume active cell proliferation and later re-differentiation. Our previous study using the zebrafish *reg6* mutation showed that histamine could rescue the blood vessel branching defects in *reg6* (Huang et al., 2008), indicating the involvement of inflammation in blood vessel regeneration. Here we further explore the role of inflammation in overall regeneration. First, we used the TG(*mpo*:GFP) transgenic zebrafish which express GFP in neutrophils to track the neutrophil migration during regeneration. We found that neutrophils appeared in regenerating fin before 36 hours post amputation (hpa) and disappeared by 60 hpa, indicating that neutrophils are involved in the early regeneration. However, when we made a second amputation on 2- or 3- dpa regenerating fins, neutrophils did not reappear at all. Interestingly, if the second amputation was made on 7- or 9-dpa regenerating fins, neutrophils did reappear, albeit in smaller number than in the first amputation. These results suggest a model that neutrophils probably induce inflammation for the regeneration at the early time and leave but the inflammation continues. In consistent with this model we found that cox-2 expression continued to go up from 3-9 dpa, which did not seem to correlate with the MAPK activity. Finally, a cox-2 specific inhibitor NS398 was found to inhibit regeneration. Surprisingly, NS398 increased the cox-2 gene expression which correlated with the increased MAPK activity. Together, our results suggest that inflammation is required to maintain regeneration and the intricate relationship between cox-2 and inflammation in epimorphic regeneration.

P444 Porous gelatin/alginate/fucoidan scaffold for anti-inflammatory activity in BV2 cells

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Microglia, which is the immune cells of the central nervous system. Overexpression of inflammatory mediators by microglia can induce of several neurological diseases. In this study, we have developed a method to create porous scaffolds by added fucoidan into sodium alginate/gelatin following sodium alginate/gelatin 1%/fucoidan (SaG1Fu), sodium alginate/gelatin 2%/fucoidan (SaG2Fu), sodium alginate/gelatin 4%/fucoidan (SaG4Fu). Gelatine was extraction at 75 ^{0}C from *Paralichthys olivaceus* skin. For mechanical characterization, such as *in vitro* degradation, stress/strain, swelling test, and pore size were measured. The biocompatibility was evaluated by assessing the adhesion and proliferation of BV2 microglia cells on the SaGFus porous scaffolds using scanning electron microscopy and lactate dehydrogenase assay, respectively. Moreover, we studied the neuroinflammatory effects of SaGFus on BV2 microglia cells. Gelatine yield is calculated on dry weight of clean skins. The yield of gelatin extraction from the *Paralichthys olivaceus* skin was 14.32 g/100 g. The effect of gelatine and fucoidan content on the various properties of the scaffold is investigated and the results showed that mechanical properties increased porosity and swelling ratio to the increase in the gelatine and fucoidan adding, while the *in vitro* biodegradability decreased. The average SaGFus diameter attained by fabrication of SaGFus about 100 µm with porosity about 78.30 %. Cell culture tests, carried out using gelatine 2.0% and 4.0%, showed a good cell proliferation more than 60 – 80% of sodium alginate alone. Following stimulation with 0.5 µg/mL LPS, microglia cultured in 3D SaGFus decrease their expression of nitric oxide, prostaglandin E2, and reactive oxygen species. SaG2Fu and SaG4Fu also inhibited the activation and translocation of p65 NF-κB protein levels. These results provide insights into the diverse biological effects and open new opportunity for the applications of SaGFus in neuroscience.

P445 Macrophages priming towards an alternative activation profile by allogeneic adipose tissue-derived stem cells

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Recent evidences have shown solid interactions between mesenchymal stem cells (MSCs) and macrophages (Mφs) at the injury site. Adult MSCs have been investigated as tools for the treatment of graft-versus-host disease, to prevent transplant rejection and to suppress the progression of chronic diseases. MSCs are also involved in modulating the inflammatory process and consequent tissue repair. Their action has been reported in many immune system cells, particularly Mφs, B and T lymphocytes, and neutrophils. In addition, there is data showing alternative activation of Mφs after priming with MSCs in transwell systems.

In this work we hypothesised that MSCs are able to prime allogeneic Mφs towards an alternative activation profile (M2) in different cell culture conditions.

Human adipose tissue-derived mesenchymal stem cells (hASCs) were directly cultured with allogeneic human peripheral blood-derived macrophages (hPBMφs) in different cell ratios as well as in a transwell system, for the relevant time periods. The phenotype of hPBMφs after priming included the detection of cell surface markers (HLA-DR, CD86, C163. CD206), cytokine release and gene expression (IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α) for the characterization on classical (M1) or alternative (M2) activation profiles.

The results showed that hASCs induce a significant downregulation of the M1-markers CD86 and HLA-DR as well as an upregulation of M2-marker CD163 on macrophages, which was similar in both co-culture and transwell system. The evaluation of the transcription of cytokines revealed a diminished detection of inflammatory mediators, such as IL-1beta, IL-6, IL-12p40 and TNF-alpha, when cells were cultured in the indirect method. These results indicate a role of hASCs secreted factors in M1 phenotypic modification. Importantly, the observed effect was not dependent on the cell ratio but become more significant over time.

With the obtained results was possible to confirm the hypothesis that allogeneic Mφs can be "educated" towards an alternative activation profile (M2 – anti-inflammatory) which, ultimately, may aid the resolution of the inflammatory process at identified exacerbated inflammation sites.

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P446 Antibody-based tumor clearance and tissue regeneration characters of a Poly(L-lactide) electrospun membrane incorporated with anti-CD40 antibody

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Antibody-based cancer immune therapy has attracted a lot of research interests in recent years, however it is greatly limited due to the easily body distribution of antibodies and burst release. In addition, after the clearance of the tissue, the healthy tissue regeneration is another challenge for cancer treatment. Herein, we have developed a specific Poly(L-lactide)(PLLA) platform using mouse anti human CD40 antibody (anti-CD40) incorporated to PLLA scaffold by PDA motif (PLLA-PDA-Anti-CD40). The successful incorporation of the Anti-CD40 on the scaffold was proven by immunofluorescent staining. The PLLA-PDA-anti-CD40 has a clear effect on tumor cells by locally released anti-CD40. The extract of the PLLA-PDA-anti-CD40 induced lymphoma Daudi cell apoptosis directly, evidenced by cell proliferation detection, cell cycle analysis and semi-quantitative PCR. Meanwhile, the released CD40 antibodies activated dendritic cells (DC) in vitro, confirmed expressions of CD11c, CD83, MHC-II on cells and secretions of IL-12 and IFN- γ, which have been well proven to initiate the adaptive immune response against tumor cells indirectly. More interestingly, after anti-CD40 released, the scaffold switched its role and acted as 3-D supporting material for inducing the proliferation of fibroblast MC3T3-E1cells, being suggestive of further promoting the regeneration of healthy tissue in sute. The developed delivery system of PLLA-PDA-Anti-CD40 opens new avenue for duo-functional tumor treatment and healthy tissue regeneration.

P447 Cellular interplay of cancer associated fibroblasts in endometrial tumor tissue

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Introduction: Endometrial cancer causes the abnormal cell growth that have the ability to invade or spread to other parts of the body. It is known that endometrial cancer associated fibroblasts (eCAFs) promote degree of the malignancy. However, it remains unclear that the cellular interplay between eCAFs and non eCAFs in endometrial tumor tissue. Here we compare that functional properties of endometrial tumor tissue models derived from co-culture with different fibroblast types and investigated the molecular mechanism for the observed differences.

Materials and Methods: Endometrial tissue models were fabricated by co-culturing human endometrial cancer cells (ECs) with human umbilical vein endothelial cells (HUVECs) and two types of human fibroblasts (eCAFs or healthy human uteri fibroblasts (HUFs)). eCAFs were differentiated from ECs through TNF-□-induced epithelial mesenchymal transition.

Results: Tumor tissue models obtained from eCAFs or HUFs showed numerous malignancies. To investigate the underlying mechanisms in the presence of eCAFs, ECs and HUVECs were cultured in DMEM containing conditioned medium of eCAFs. Soluble factors from eCAFs markedly promoted cancer cell proliferation and construction of closing point number of blood vessels (Fig.1-2.). To investigate the molecular mechanisms, we performed a comprehensive genetic analysis between each type of fibroblast using microarrays. Several candidate factors were identified.

Discussion and Conclusions: These data indicate eCAFs-derived soluble factors improve ECs cell proliferation and angiogenesis. eCAFs might be a key inducer of endometrial cancer malignancy.

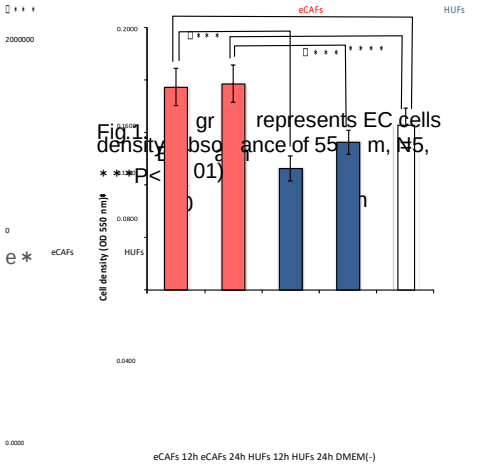

Fig.1. gr represents EC cells density bso ance of 55 m, N5, * *P< 01)

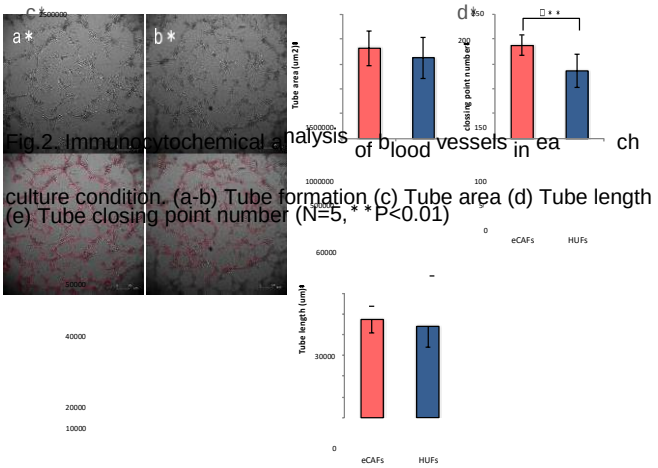

Fig.2. Immunocytochemical analysis of blood vessels in each culture condition. (a-b) Tube formation (c) Tube area (d) Tube length (e) Tube closing point number (N=5, * *P<0.01)

P451 Polyurethanes: a promising platform for engineering in vitro healthy and pathological tissue models

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Tissue engineering (TE) combines engineering and life sciences tools to design biomimetic constructs mimicking the biological, structural, surface and mechanical properties of a tissue. These matrices can find application in regenerative medicine, or in pharmaceutical, diagnostic and pure research to test newly developed drugs/therapeutics, investigate cell function, and study the mechanisms involved in disease onset and progression. The design of bioengineered constructs able to finely replicate both healthy and pathological tissues, to be used as valuable in vitro models requires compliance to strict requirements, since each single tissue has different features. In particular, the three-dimensional (3D) structure (scaffold), the cells and chemical/physical cues are the main constituents of these complex systems. For what concerns the design of the 3D matrix, attention must be focused on the selection of the best scaffold-forming material and fabrication technology, i.e. those that allow a fine replication of the physico-chemical properties of the investigated tissue. In this context, polyurethanes (PUs) are an interesting and valuable alternative as their high chemical versatility results in the possibility to synthesize a wide array of polymers with finely modulated physico-chemical properties and suitability to different fabrication technologies, either conventional (e.g. thermally induced phase separation -TIPS-, salt leaching) or advanced (e.g. additive manufacturing (AM) techniques). In this contribution, a new platform of biodegradable and biocompatible PUs (thermoplastic poly(ε-caprolactone)-based PUs and amphiphilic water-soluble Poloxamer-based PUs) has been designed and thoroughly characterized, and their suitability to both conventional (TIPS and electrospinning) and AM techniques has been demonstrated. For instance, in order to design in vitro models of the myocardium at different levels of ageing, the composition of an elastomeric-like PU and a PU-poly(ε-caprolactone) blend (PU/PCL: 50%/50%) was properly tuned to replicate the differences in mechanical properties between young and aged myocardial tissue. Scaffolds were produced by TIPS to obtain an oriented fiber texture similar to the native cardiac tissue; moreover, scaffold surfaces were functionalized with fibronectin to mimic the composition of cardiac extracellular matrix. The designed models showed mechanical properties and morphology similar to those of native tissues and maintained long-term cardiomyocyte viability, showing spontaneously beating cells. PU chemical versatility can thus be exploited to modulate their final properties to a large extent, allowing their application in several branches of the TE field, as reparative/regenerative constructs or engineered tissue models offering new options for a better understanding of the progress of uncurable diseases, such as cancer, at early stages, and the development of new treatments, in accordance to the 3Rs principle.

P452 Three-dimensional *in vitro* neural stem-endothelial cells co-culture model: The recapitulation of neuroblastoma microenvironment for long term drug testing

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Neuroblastoma is one of the most common yet fatal paediatric cancers. However, limited drug treatments are currently available on the market. Drug testing with biopsy specimen provides a personalised response, but the specimen itself can only be maintained for less than one week in culture. Hence, any long term effects or recurrence are not being observed, impeding drug discovery investigations. We describe the development of a cancer model that establishes a neuroblastoma-like microenvironment, which facilitates the evaluation of cell response to drugs and extends the viability of tissue biopsies.

Our model comprised neural stem cells (NSCs) and human umbilical vein endothelial cells (HUVECs) co-cultured in laminin-rich hydrogel matrix. The experiment was carried out within 25 µL micro-wells made of agarose gel, structured inside a 24-well plate. Optimisation experiments were performed to identify the choice of hydrogel, the hydrogel stiffness as well as the co-culture cell seeding and media ratios. The hydrogel fragmentation model and the interactions of co-cultures were further explored by immunostaining and image analyses, which enabled us to maximise the cell viability, proliferative potential and cell morphologies between both cell types. Finally, the 3-D NSC-HUVEC co-culture model was developed and evaluated for expressions of cellular markers at different time points up to 14 days. In parallel, perfusion culture of the model was performed inside a modified TissueFlex® bioreactor as well.

A 0.80% fragmented hyaluronan hydrogel (HyStem) was found to be the optimal NSC-HUVEC co-culture model. Hydrogel fragmentation allowed the formation of concentrated laminin and cellular networks along the gel granule boundaries with neurite extensions observed. Besides, immunostaining with TuJ1, nestin and CD31 suggested an optimal NSC-HUVEC seeding density ratio of 3:1, cultured in 100% endothelial growth medium. Compared to monocultures, the significant number of proliferating neurons and their cellular areas were observed after co-cultured with HUVECs. Incorporating the results into a 3-D model, it was maintained up to at least 14 days. The viability of the model was further extended beyond one month when cultured inside our perfused TissueFlex® bioreactor.

Optimisation of the hydrogel choice, structure as well as cell seeding densities and medium constituents is necessary to achieve 3-D recapitulation of cell-cell, cell-ECM interactions, in order to create neuroblastoma-like microenvironment. Our 3-D hydrogel perfusion platform has implications for increasing the predictive power of drug investigation and can be applied further to the culture of biopsy specimens; closing the gap between cell culture and physiological tissue investigations. This work also provides opportunities to develop more efficient high throughput drug screening as well as personalised medicine in cancer therapeutics.

P453 Polyesterurethanes engineered theranostic nanoparticles for cancer diagnosis and treatment

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Efficient cancer treatment requires novel multifunctional approaches combining concomitant delivery of different drugs and/or biomolecules, imaging agents; and targeting ability.

To achieve this goal, several innovative nano-tools have been developed. Among them, polymer nanoparticles (nps) have gained great attention for their high loading efficiency, versatility, ease of surface modification and passive and active cancer targeting ability.

This work will discuss the preparation of multifunctional hybrid nps for concomitant loading of multiple drugs and imaging agents and their in vitro validation on traditional cell cultures as well as on 3D cancer models.

Nps have been prepared by a nano-precipitation/self-assembly method in order to provide two different compartments: (i) a lipid shell for long circulation and ready conjugation with targeting ligands or imaging agents; and (ii) a polymer core of multi-block polyurethanes (PURs) of different hydrophilic/hydrophobic balance.

Two drugs with different water solubility (e.g. Docetaxel and Doxorubicin hydrochloride) have been successfully co-encapsulated in the PUR core together with MRI contrast agents (iron oxide nps) to obtain a theranostic nanosystem. Moreover, the surface compartment has been modified with Cyanine5.5-labelled lipids for concomitant fluorescence/photoacustic imaging.

Our results showed that the polyurethane core was able to entrap three different agents at the same time with high loading efficiency, comparable with the results of other authors based on traditional polyesters (such as PLGA) as a single-agent loading platform.

In vitro tests on glioblastoma U87 cell line evidenced a dose and time dependent cytotoxicity of drug-loaded nps, with enhanced efficacy for the combined treatment for all investigated polyurethanes. The nanoplatforms also showed good selectivity as T2 contrast agents on agar phantoms with a high r2/r1 ratio.

Cyanine 5.5-labelled nps functioned as optimal imaging probes under fluorescent microscopy, as confirmed by preliminary in vivo tests on nude mice.

In conclusion, the high versatility of PUR nps can be successfully exploited to maximize the loading of drugs and imaging agents with different physical properties to contemporary provide (i) a multi-agent chemotherapy with synergistic effects and (ii) an advanced theranostic nanosystems with a huge potential in cancer treatment. More in general, this work contributes to create a multifunctional platform that integrates nps with engineered artificial microenvironments exploiting the technology of tailor-made, biomimetic multiblock polyesterurethanes providing new advanced tools for cancer diagnosis and treatment.

P454 Combining the versatility of polyurethane chemistry and additive manufacturing technologies in the design of engineered *in vitro* models

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Although animal models have strongly contributed to both our understanding of human biology and the development of modern medicine, they often show limits in the reproduction of specific human conditions and turned out inefficient predictors in drug screening. In this context, tissue engineering (TE) approaches have been proposed for the design and development of alternative *in vitro* models that would replace to a certain extent animal models, in accordance to the 3Rs principles. These models are promising tools in research practice as a new platform for drug screening, new therapy testing or the investigation of the mechanisms underpinning pathology onset and progression, as well as for regenerative medicine applications in the treatment of damaged tissues or organs.

In this work, bioengineered scaffolds have been designed by exploiting the wide versatility of both polyurethane (PU) chemistry and additive manufacturing (AM) technologies to accurately develop functional tissue/organ models. PUs are block copolymers which chemical versatility can be exploited to design a wide variety of degradable biomaterials (thermoplastic PUs and amphiphilic PUs, which aqueous solutions are thermo- and/or photo-sensitive) showing different physico-chemical properties, depending on the selected building blocks. On the other hand, Bio- fabrication technologies based on the principles of AM are emerging as promising tools for the fabrication of highly controlled and engineered complex three-dimensional (3D) scaffolds. AM's high technological versatility allows a fine modulation of the architecture of the resulting scaffolds and the processing of a wide variety of materials, thus overcoming the typical drawbacks of conventional scaffold fabrication techniques.

In particular, thermoplastic PUs based on poly(ε-caprolactone) and hydrogels based on an amphiphilic polyurethane were processed through different AM technologies (e.g. fused deposition modelling, 3D bioprinting, stereolithography) in various multi-layered structures. In order to obtain a functional tissue/organ, mesenchymal stem cells (MSCs) were cultivated due to their ability to differentiate in different cell types. As stem cell self-renewal and differentiation can be guided by structural, mechanical and biochemical cues, the previously mentioned high chemical and technological versatility of PUs and AM techniques, respectively, can be exploited to design smart biomaterials and scaffolds to properly stimulate cells they interface with. To this aim, multicomponent and multifunctional 3D substrates based on both thermoplastic and amphiphilic PUs were designed, thus further increasing the versatility of this approach, leading to an additional and fine modulation of the physico-chemical and biological properties of the resulting constructs. In this way, the architecture, composition, mechanical and surface properties of the obtained scaffolds can be properly optimized to meet the requirements to bioengineer functional tissue/organ models for application in the repair and regeneration of a target soft tissue or for a "patient-specific" testing of drugs and therapies, that could finally answer to the need of new strategies to treat incurable diseases, such as cancer and degenerative pathologies.

P456 Polyphenols and bioactive glass: a smart couple against cancer

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Polyphenols are natural molecules with selective cytotoxic activity against cancer cells and, on the opposite, stimulating activity on healthy ones [1,2]. Poor bioavailability and stability are actually their main drawbacks for their application in the medical field but there are key points for the improvement of their effectiveness. Surface functionalization is a promising strategy to combine the properties of biomaterials with those of polyphenols in order to guarantee an adequate amount of active molecules at the desired site. Bioactive glasses are materials of choice for bone contact applications due to their ability to chemically bind to bone and stimulate its regeneration. In this research work a silica based bioactive glass (CEL2) [3] was used as substrate and gallic acid (GA), as model molecule and natural extracts of green tea leaves (TPH) and red grape skins (GPH) as natural polyphenols for the surface functionalization. The grafting procedure was optimized in previous works of the authors [4-6]. The presence and activity of the molecules on the surface was verified by means of the Folin & Ciocalteu test, XPS and fluorescence microscopy. Direct and indirect cytotoxicity, cells morphology, RONS, apoptosis and DNA damage were performed using a human osteosarcoma cell line (U2OS) and human foetal pre-osteoblasts (hFOB) as representative for tumor and healthy cells, respectively. The direct cytotoxicity tests showed a not significant reduction of healthy cells viability ($p>0.05$, 20% for GA and 10% for TPH), while a significantly reduction of the viability for the tumoral ones ($p<0.05$, 60% for GA and 50% for THP) was noticed after 7 days. Conversely, the viability reduction of both cell lines is negligible in the indirect assay, in accordance with the minimum release of the biomolecules. The presence of the polyphenols was effective in protecting hFOB cells from inflammation after 24 and 72 hours in comparison with tumorigenic U2OS cells where inflammation due to RONS was not lowered. Fluorescent analysis revealed that U2OS cells linked into typical tumoral aggregates on bare CEL2 while resulted as dispersed on CEL2+TPH. Finally, a permanent DNA damage related to apoptosis has been registered for U2OS cells cultivated onto CEL2+TPH by means of 53BP1 and Cyclin B1 staining. These results demonstrated that it is possible to graft polyphenols onto the surface of bioactive glasses preserving their antitumoral activity allowing delivery by local application to cells and tissues. Surface functionalization with natural polyphenols is a promising way to obtain smart surfaces for implant in critical situations (e.g. bone substitution after tumor resection). Moreover, the molecules considered in this research can be obtained as by-products of the agri-food industry production chain, thus opening the opportunity for a sustainable use of resources.

P457 A hydrogel-based platform for the ex vivo activation and expansion of T cells

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Adoptive T cell therapy is gaining traction in becoming a mainstream anticancer strategy due to recent advances. The procedure generally involves the ex vivo activation and expansion of tumour-specific T cells, before transfusion into the patient. Critical to the success of the therapy is the manufacture of large quantities of ex vivo activated T cells. To this end, antibody-coated synthetic materials for mimicking the in vivo interactions between T cells and antigen-presenting cells (APCs) have been developed. So far, the development of such artificial APC surrogates has focused primarily on the identity of T cell-stimulating ligands immobilised on the synthetic substrates. Yet, studies in T cell mechanobiology have revealed that physical parameters, such as culture substrate stiffness, are critical regulators of T cell activation. To address this, we aim to engineer an ex vivo culture platform for fine-tuning T cell behaviour and expanding the repertoire of modifiable immunomodulatory parameters, by incorporating both chemical and physical cues.

Polyacrylamide hydrogels of different stiffness were used as stiffness-tunable substrates, which were surface-immobilised with T cell-activating proteins. Immunofluorescence was used to detect stimulatory ligands (such as anti-CD3) conjugated to the gel surface. Confocal microscopy showed that antibodies were consistently confined to a thin layer on the surface of all hydrogel samples. Gel compositions were also optimised such that no difference in fluorescence intensity was observed across gels of different stiffness.

In summary, the findings suggest that polyacrylamide hydrogels are capable of acting as a T cell-activating culture substrate and a versatile tool to independently investigate the optimal stiffness range and ligand density for ex vivo activation.

P458 A new gold nanoparticle-based nanosystem for the delivery of anticancer drugs

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The use of nanotechnology in medicine and more specifically drug delivery, is set to spread rapidly. Currently many substances are under investigation for drug delivery and more specifically for cancer therapy. Interestingly pharmaceutical sciences are using nanoparticles to reduce toxicity and side effects of drugs and to improve their efficacy.

Gold nanoparticles (AuNPs) exhibit physical properties that make them suitable for several biomedical applications. In this study, a new synthetic route leading to stable colloidal AuNPs with diameters in the range 4 - 20 nm was investigated (Fig.1). The colloidal stability was monitored with x-ray diffraction, ultraviolet-visible spectroscopy and dynamic light scattering. AuNPs proved stable at least for 8 months. Susequentially, AuNPs were conjugated with Herceptin, a chemotherapic agent used to treat breast cancer and their efficacy was evaluated *in vitro.* In particular, two breast cancer cell lines were used (SK-BR-3 and MCF-7) and compared with fibroblast-like cell line (NIH3T3). AuNPs functionalized with Herceptin showed increased efficacy and specificity for target cells in comparison with free drug. Noteworthy, the AuNP chemical modification procedure is a “green” one since it is carried out in water using biocompatible stabilizing agents.

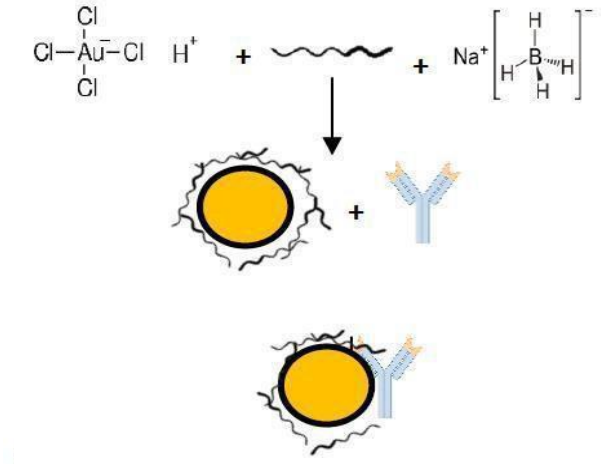

Fig.1 – Scheme of AuNPs synthesis and functionalization

P459 A goat liver extracellular matrix-based tumor-mimicking environment for mechanobiological study of cervical cancer progression

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Modern day cancer research is more focused on the investigation of in vivo mechanical cues and associated mechanotransduction than just the molecular origin and biochemical aspects. A growing body of literature suggests that when cells proliferate uncontrollably in a solid tumor within a confined space, it not only creates well-studied oxidative stress but also compressive stresses on the developing tumor. These forces can act as a cue for altering gene expression, cancer cell proliferation, autophagy, apoptosis, invasiveness, stromal cell function, extracellular matrix synthesis and coordinated migration of cancer cells. The role of compression-induced autophagy on cellular migration still remains an enigma in most cancers and currently believed to be a key to the early steps of cancer progression. Liver happens to be the second most preferable niche for metastatic cervical cancer. In this study, we used different concentrations of an indigenously prepared Caprine(Goat) Liver Extracellular Matrix(ECM) of varying elastic moduli (starting from 50

Pa to about 250 Pa) to embed 1000 cell-spheroids of HeLa grown by hanging-drop method and studied the invasion of cells into the matrix, in presence and absence of a known autophagy inhibitor Wortmannin. Interestingly, we found that while there was no difference in migration of cells from spheroids on a cell culture plate in presence or absence of the inhibitor, the migrational behavior of cells with or without inhibitor treatment, changed inside gels of varying strengths. Below 100 Pa, it was observed that inhibition of autophagy reduced cellular invasion compared to control. Close to 150 Pa, no invasion was observed in control spheroids but invasion was noted to occur after 96 hrs from autophagy-inhibited spheroids. No invasion was observed above 150 Pa in any of the experimental conditions which may have been due to the inability of the cells to invade the matrix above this stiffness level. The results thus hint at a plausible involvement of compressive force-induced signaling modulation of HeLa cell invasion in a growing tumor microenvironment and needs to be exploited for identification of underlying mechanotransduction pathways that could serve as molecular targets. Our model, encompassing the use of a compatible ECM for HeLa, thus provides a good in vitro tumor-mimicking environment to identify and understand the contribution of mechanical cues in progression of cervical cancer.

Keywords: HeLa, spheroids, Caprine Liver Extracellular Matrix, compression, autophagy, invasion.

P460 Exploring the role of PCL electrospun matrices on in vitro cell response to chemotherapeutic drug therapies

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In the last decade, several studies have been performed to define in vitro tissue models to reproduce main extracellular matrix functionalities, by controlling morphology, biochemistry and molecular transport for an optimal administration of biochemical signals (i.e., enzymes, DNA fragments, drugs) to address cells behaviour in different ways. One of most promising approaches involves the use of engineered scaffolds as ECM-like platforms able to provide main physical and/or morphological cues required to mimic those exerted by healthy tissue microenvironment or tumour niche. Recent studies demonstrated that micro/nanotextured platforms fabricated via electrospinning may be successfully used as preclinical model to investigate action mechanism of chemotherapeutic drugs in vivo [1].

Here, we investigate how Poly-ε-caprolactone (PCL) electrospun scaffolds may influence the behavior of hMSC or tumor cells - i.e., HEPG2 from human hepatocarcinoma - under the administration of different chemotherapeutic drugs (i.e, 5-azacytidine, doxorubicin) to validate their use as in vitro model for tissue regeneration and cancer therapy. Morphological fibre features at micro and nanometric scale – fiber diameters equal to (3.22 ± 0.42) μm and surface roughness of (17.84 ± 4.43) nm – concur reproducing a peculiar organization on collagen fibrils of native ECM. Moreover, hydrophobic character of PCL electrospun fibres corroborate the capability of chemotherapic drugs to slow down proliferation rate of cells, thus promoting selected differentiation mechanisms (i.e., YIP-1B expression increase as myotube maturation index) in the case of 5-azacytidine administration on hMSC, or inhibiting in vitro growth – in the case of doxorubicin administration on Hep G2 cells.

Hence, we conclude that this study may constitute a starting step forward the development of new in vitro models for soft tissues, having the potential to be routinely adopted as instructive platform for high-throughput evaluation of innovative therapeutics use of healthy or cancer tissue diseases.

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P463 Chitosan-based hydrogels as biological inks for fabrication of cellularized 3D-complex structure mimicking living organs

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Introduction

Bioprinting is a promising technique that enables the simultaneous deposition of multiple types of cells and biomaterials in order to create complex 3D tissue constructs. Chitosan is a natural polymer derived from alkaline deacetylation of chitin, one of the most abundant polysaccharides which can be found in crab and shrimp shells. Chitosan-based hydrogels are broadly used in tissue engineering as they contain several features of natural ECM components and allow cell encapsulation in a highly hydrated, mechanically supportive 3D environment.

Materials and Methods

Medical grade Chitosan (CS, Mw 200-400kDa, Kraeber) and β-Glycerophosphate disodium salt (β-GP, Santa Cruz) solutions were separately prepared by dissolving CS M.G. in 0.2 M acetic acid and β-GP in bidistilled water. Both solutions were conditioned to 4 °C for 20 minutes, then the β-GP solution was added drop-by-drop into CS solution to obtain a final CS/β-GP solution. Complete physico-chemical, rheological and biological characterization of CS/β-GP hydrogel were performed. Furthermore, the printability of cellularized CS/ β-GP hydrogel was assessed. Proof-of concept results were obtained printing CS/β-GP through a home-made bioprinter.

Results

The developed CS/β-GP hydrogel remained in a liquid state at room temperature while phase-transition was recorded upon an increase in temperature to approximately 37 °C. Rheological analysis confirmed the gelation mechanisms at a molecular level.

Neuroblastoma cell line (SH-SY5Y), mesenchymal stem cells (MSCs) and neuronal precursors (NPs) were loaded into CS/β-GP hydrogel and a good cell survival was detected after 3 days.

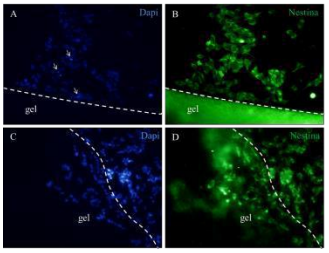

Fig.1 SH-SY5Y cells seeded near the gel (A,B) and inside the gel (C,D).

Conclusion

These results suggest the potential use of chitosan-based hydrogels as a bioink for 3D printing applications allowing the fabrication of customizable patient-specific, tissue-engineered constructs or pathological models of the central nervous systems.

P464 Layer-by-layer printing of Hep3B human hepatoma cell line for cancer cell migration in 3D

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One of the common characteristics of aggressive cancers is the ability to rapidly invade and metastasize in other tissues. It is thus important to study what causes cancer cells to migrate for an accurate assessment of migration behaviour. Traditional migration assays including wound healing analysis focus on cell movement in two dimensions (2D), but a three dimensional (3D) system would ultimately be a better mimic for the tissue microenvironments. “Microarray 3D bioprinting” technology on a microwell chip has been explored to create miniaturized tumor-like spheroid structures in two hydrogel layers and rapidly study cancer cell migration. To investigate the effects of various chemoattractants on cancer cell migration in high throughput, several extracellular matrices and hepatic growth factors were mixed with photocrosslinkable hydrogels including oxy-methacrylated alginate (OMA), methacrylated collagen, and methacrylated heparin, and printed at the bottom of the microwell chip. Hep3B human hepatoma cells in the photocrosslinkable hydrogels have been printed on the top and cultured for several weeks to better mimic tumor microenvironments. The photocrosslinkable hydrogels were polymerized *via* near-ultraviolet light in the presence of photoinitiators. Our goal is to demonstrate rapid creation of tumor-like structures *via* microarray 3D bioprinting and develop a high-throughput, 3D cancer cell migration assay. To achieve this goal, layer-by-layer cell culture conditions were optimized in the hydrogels by varying light exposure time, photoinitiator concentration, hydrogel concentration, and cell seeding density. Cancer cell migration in 3D was determined by acquiring images of Hep3B cells in the top layer migrated toward the bottom layer containing chemoattractants and analyzing in-focus and out-of-focus images of Hep3B cells using ImageJ (Fourier transform analysis for detecting image blurriness). Conclusively, we found that our approach can be used as a powerful tool to rapidly analyze cancer cell migration in 3D and potentially provide better mimic of tumor tissues *in vivo*.

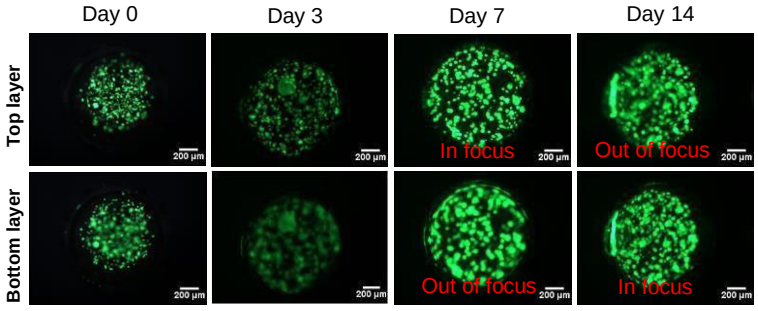

Figure 1. Images of Hep3B cells taken from top and bottom layers over time. The top layer contained Hep3B cells in 2 w/v % OMA and the bottom layer contained 1.5 mg/mL Matrigel in 2 w/v % OMA. The green dots represent live He3B cells. Scale bar = 200 µm.

P465 A phasor approach analysis of multiphoton fluorescence lifetime imaging microscopy measurements to probe the metabolic activity of three-dimensional in vitro disease models

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Fluorescence lifetime imaging microscopy (FLIM) is a useful approach to obtain information regarding the endogenous fluorophores present in biological samples. In this study, we aimed to identify if FLIM is a suitable method to non-invasively detect a shift in cellular metabolic activity towards glycolysis or oxidative phosphorylation utilizing 3D Caco-2 models of colorectal carcinoma that were treated with potassium cyanide or hydrogen peroxide. In addition, epidermal growth factor (EGF) was used to induce a biologically relevant change in cell metabolic behaviour.

Autofluorescence, attributed to nicotinamide adenine dinucleotide (NADH), was induced by two-photon laser excitation using a femtosecond laser at an excitation wavelength of 760 nm. The autofluorescence lifetime decay was analysed using a standard multi-exponential decay approach as well as a novel custom-written code to perform phasor analysis.

While both methods allowed the detection of a statistically significant shift of metabolic activity in 3D Caco-2 models towards glycolysis when treated with potassium cyanide, and oxidative phosphorylation when treated with hydrogen peroxide; using a phasor approach, fewer initial assumptions were required to quantify the lifetimes of contributing fluorophores when compared to multi-exponential decay fitting. Treating 3D Caco-2 cysts with EGF resulted in a predicted increase in glucose consumption, lactate production, and ATP content. FLIM analyses of these cultures revealed a significant shift in the contribution of protein-bound NADH towards free NADH, which indicates an increase in metabolic activity towards glycolysis. This demonstrates potential of FLIM to interpret the metabolic changes in 3D in vitro models using a standard multiexponential decay approach and the customised phasor approach developed in this study which could significantly impact the rapid diagnosis of cultures in vitro.

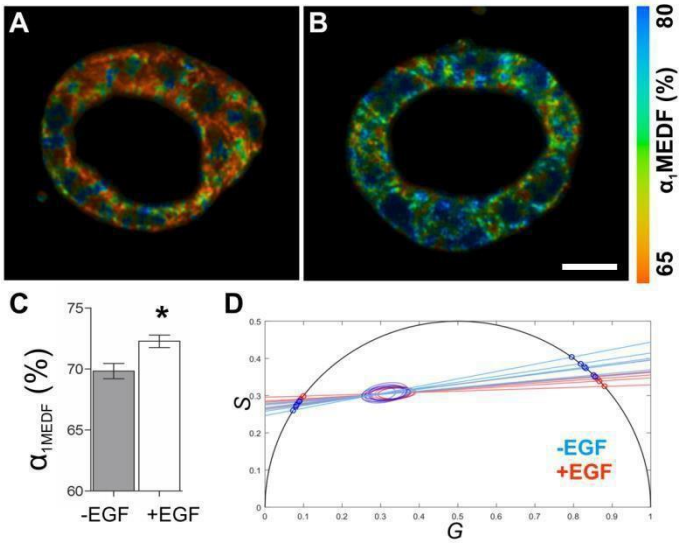

Figure: (**A-B**) False color-coded images of (**A**) untreated and (**B**) EGF-treated 3D Caco-2 luminal cyst models indicating the range of α_{1MEDF} from 65% (red) to 80% (blue). (**C**) Treatment of 3D Caco-2 models with EGF induces an increase in the short fluorescence lifetime contribution α_{1MEDF} (n=4, * indicates p=0.0213). (**D**) Phasor plot of untreated and EGF-treated 3D Caco-2 models, showing 95% confidence ellipses and fitted linear functions on the universal circle.

P466 metastatic melanoma human 3d models for in vitro evaluation of targeted drugs efficiency

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Targeted therapy molecules have been approved for the treatment of BRAF-mutated metastatic melanoma. Nevertheless, these molecules can cause serious side effects to patients and are efficient on 50 to 60 % of them. Indeed, melanoma cell sensitivity to targeted therapy molecules is dependent on tumor microenvironment (cell-cell and cell-extracellular matrix interactions). To better unravel factors modulating cell sensitivity to B-Raf inhibitor such as Vemurafenib, we have developed and compared several melanoma models: from metastatic melanoma cells cultured as monolayer (2D) to a co-culture in a 3D dermal equivalent.

Cell response was studied on different melanoma cell lines such as SK-MEL-28 (mutant B-Raf (V600E), sensitive to Vemurafenib), SK-MEL-3 (mutant B-Raf (V600E), resistant to Vemurafenib), SK-MEL-2 (wild-type B-Raf and mutant N-Ras (Q61R)) and a primary culture of dermal human fibroblasts (HDFn). Assays have initially been performed on a monolayer cell culture (2D), then a second time on a 3D dermal equivalent (dermal human fibroblasts embedded in a collagen gel). All cell lines were treated with Vemurafenib (a B-Raf inhibitor) for 48 hours at various concentrations. Cell sensitivity to treatment was assessed under various aspects: Cell proliferation (cell counting, EdU incorporation, MTS assay), MAPK signaling pathway analysis (Western-Blotting), Apoptosis (TUNEL), Cytokine release (IL-6, IL-1α, HGF, TGF-β, TNF-α) upon Vemurafenib treatment (ELISA) and histology for 3D models.

In 2D configuration, the inhibitory effect of Vemurafenib on cell proliferation was confirmed on SK-MEL-28 cells (IC50=0.5 µM), and not on SK-MEL-3 cell line. No apoptotic signal was detected in SK-MEL-28-treated cells, suggesting a cytostatic effect of the Vemurafenib rather than a cytotoxic one. The inhibition of SK-MEL-28 cell proliferation upon treatment was correlated with a strong expression decrease of phosphorylated proteins involved in the MAPK pathway (ERK, MEK and AKT/PKB). Vemurafenib (from 5 µM to 10 µM) also slowed down HDFn proliferation, whatever cell culture configuration (monolayer or 3D dermal equivalent). SK-MEL-28 cells cultured in the dermal equivalent were still sensitive to high Vemurafenib concentrations. To better characterize all cell population impacts (melanoma cells, dermal fibroblasts) on Vemurafenib efficacy, cytokine release is being studied in 2D and 3D models.

We have successfully developed and validated a relevant 3D model, mimicking cutaneous metastatic melanoma and tumor microenvironment. This 3D melanoma model will become more complex by adding a third cell population, keratinocytes, allowing us to characterize the epidermis influence on the melanoma cell sensitivity to Vemurafenib. In the long run, the establishment of more relevant 3D melanoma models with patients' cells might be useful for personalized therapy development.

The authors would like to thank the Picardie region and the European Regional Development Fund (ERDF) 2014/2020 for the funding of this work and Oise committee of "La ligue contre le cancer".

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Keywords— 3D human skin model, melanoma, tissue engineering, vemurafenib efficiency.

P467 Evaluation of tumor malignant mechanism of pancreatic cancer associated fibroblasts

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Introduction: Pancreatic cancer associated fibroblasts (pCAFs) are one of the largest components of tumor tissue, it promotes degree of tumor malignancy. However, it is not fully understood that the cellular interactions for tumor tissue environments in terms of the pCAFs properties. In this study, we evaluated the tumor malignant mechanism of pCAFs by assessing cancer cell growth, angiogenesis and cancer invasion.

Materials and Methods: Human pancreatic cancer cells and human umbilical vein endothelial cells were incubated in conditioned medium of pCAFs or Pancreatic stellate cell (PSCs). pCAFs were differentiated from Panc-1 through TNF-□induced epithelial mesenchymal transition.

Results: When cancer cell growth rate in each cell culture condition were evaluated using MTT assay, pCAFs, but not PSCs, promoted cancer cell proliferation (Fig.1. and Tab.1.). Immunocytochemical analysis revealed that there was no significant difference in angiogenesis between pCAFs and PSCs (Fig.2.). And invasion assay also revealed that there was no significant difference. To elucidate the molecular mechanisms of cancer cell growth in pCAFs, the microarray analysis was performed and we identified candidate soluble factors that were significantly upregulated in pCAFs compared with PSCs.

Discussion and Conclusions: These findings indicate that pCAFs-derived soluble factor mostly have relations to pancreatic cancer cell proliferation. The effects of these factors in pCAFs remains to be determined.

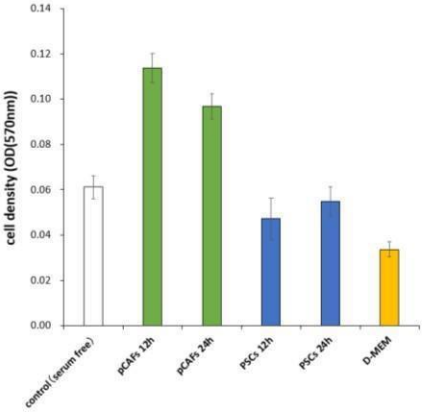

Fig.1. MTT assay in each culture condition medium (N = 6)

	control	PSCs 12h	PSCs 24h	D-MEM
pCAFs 12h	**	**	**	**
pCAFs 24h	**	**	N.S	**

Tab.1. Significant difference of cancer cell growth (** p < 0.01)

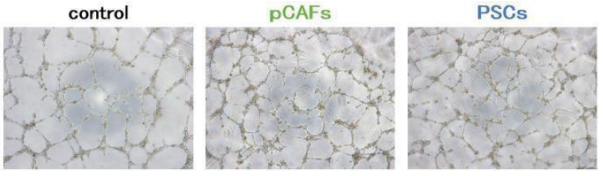

Fig.2. Construction of blood vessels in each culture condition (N = 5)

Figure2: appearance of construction of blood vessels by HUVEC in each condition medium.

P470 3D bioengineered systems for tissue engineering cancer research

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The University of Manchester, Manchester, UK

In the context of tissue engineering and regenerative medicine, the pivotal role of the extracellular microenvironment, cell-matrix and cell-cell interactions in the signalling and regulation of pathophysiological states has been extensively studied. This work addresses challenges in cancer research, conceiving a more relevant *in vitro* model enabling to study the cross-talk between the primary tumour and the pre-metastatic niche site. In this study we report the use of manufacturing techniques to fabricate 3D cancer *in vitro* models by means of alginate based hydrogel-spheres and porous cryogels. In particular, the use of the encapsulating technique enables a precise control over the number of embedded cells per bead and bead size, both of them fundamental for a precise modelling of cancer signalling phenomena. By increasing their levels of complexity, these tumor in vitro models will enable us to follow not only the dynamics and characteristics of the primary tumour, but also the effects imposed to the distal metastatic site, e.g. the presence of cell-released factors (soluble factors and extracellular vesicles), controlled material properties (mechanical and diffusion properties), physiological relevance (co- culture of cancer with stromal cells, mimicking the connection between the primary tumour compartment and the pre-metastatic site). Here, emphasis is given to a relevant cancer- associated surface receptor (CD44 and its variants), characterised as a function of the cell culture methods i.e. 2D monolayer, encapsulation and porous 3D culture, co-culture. This approach will enable to develop and test targeted formulations for the delivery of drugs such as small nucleic acids to the tumours, but also to identify new targets to the stromal components to prevent the spreading of aggressive/metastatic cancers

P471 Tailored design multifunctional and programmable pH-sensitive drug delivery nanocarrier based on self-assembling polypeptides for breast cancer therapy

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The purpose of this study is to develop the biodegradable nanoparticles composed of natural polypeptides and calcium phosphate (CaP) with sequential pH-responsivity to tumor microenvironments for active targeted drug delivery. Two different amphiphilic copolymers, poly(ethylene glycol)$_{3400}$-aconityl linkage-poly(L-glutamic acid)$_{15}$-poly(L-histidine)$_{10}$-poly(L-leucine)$_{10}$ and LyP1-poly(ethylene glycol)$_{1100}$-poly(L-glutamic acid)$_{15}$-poly(L-histidine)$_{10}$-poly(L-leucine)$_{10}$, were exploited to self-assemble into micelles in aqueous phase. The bio-stable nanoparticles provide three distinct functional domains: the anionic PGlu shell for CaP mineralization, the protonation of PHis segment for facilitating anticancer drug release at a target site, and the hydrophobic core of PLeu for encapsulation of anticancer drugs. Furthermore, the hydrated PEG outer corona is sued for prolonging circulation time, while the active targeting ligand, LyP-1, is served to bind to breast cancer cells and lymphatic endothelial cells in tumor for the reduction rate of metastasis. From the release profile, mineralized DOX-loaded nanoparticles (M-DOX NPs) efficiently prevent the drug leakage at physiological pH value and facilitate the encapsulated drug release at acidic condition when compared to DOX-loaded nanoparticles (DOX NPs). *In vitro* intracellular uptake experiments demonstrate that M-DOX NPs with LyP-1 targeting ligand effectively accumulated in MDA-MB-231 breast cancer cells through receptor mediated endocytosis. The inhibition effect on cell proliferation also enhances with time, illustrating the prominent anti-tumor efficacy. Moreover, the *in vitro* metastatic inhibition model shows the profound inhibition effect of inhibitory nanoparticles. In brief, this self-assembling peptide-based drug delivery nanocarrier with multifunctionality and programmable pH-sensitivity is of great promise and potential for anti-cancer therapy.

P472 Application of tissue engineering strategies for generating of an in vitro and in vivo model for understanding breast cancer progression and angiogenesis

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Normal and tumor breast tissue constitute a multitude of different cell types such as epithelial cells, fibroblasts, adipose-derived stem cells (ADSC), endothelial cells (EC) and immune cells, which can be affected by normal lactation, age and the stage and tumor type. The development of in vitro or in vivo tissue culture models in order to accomplish a better understanding of tumor progression and further development of innovative therapeutic strategies is still a great challenge.

In the current study different cell types were isolated from normal and tumor mammary tissue. For characterization of the different cell types a comprehensive analysis was performed including real-time PCR, immunofluorescence, DNA fingerprinting and functional analysis (e.g. cell differentiation, angiogenesis assays). For functional analysis co-culture experiments were performed and cells were seeded on different scaffolds. In ongoing experiments the well-known tissue engineering arteriovenous (AV) in vivo loop model is currently being evaluated for its suitability as a breast cancer angiogenesis model. An artery and vein are anastomosed to a loop vessel which is transferred into a closed implantation chamber together with breast cancer cells embedded in a hydrogel/scaffold.

Five different cell types including ADSC, epithelial cells (EPI), mesenchymal (MES), EC and endothelial progenitor cells (EPC) were successfully isolated from breast (tumor) tissue and blood, respectively. Gene and protein expression of single cell types differed significantly. E. g. MEC showed high expression for EPCAM, CD49f, CDH1 and KRTs; MES for e.g. Vimentin, CD10, ACTA2 and MMP9; and ADSC for e.g. CD105, CD90, CDH2 and CDH11 [1]. EPC and EC were positive for typical angiogenic markers (CD31, vWF, CD24, KDR) and showed typical angiogenic functional properties (tube formation, acLDL-DiI uptake, sprouting). Initiating 3D cell cultures, breast MES could properly adhere and grow on 20-fiber layer quadratic scaffolds and ADSCs and MDA-MB 231 on 10-layer quadratic and dodecahedron scaffolds (Fig. 1A). Using the AV loop model (Fig. 1B) ongoing vascularization was visualized within the closed implantation chamber similar to a “living bioreactor” using histological and 3D imaging methods.

Combining different cell types isolated from primary mammary (cancer) tissue and scaffolds/hydrogels in the AV loop, it will be possible to establish a patient-derived tumor xenograft model. The establishment of such a standardized tumor / vessel network in an isolated environment would provide significant benefits for characterization of the cellular network and tumor neovascularization. In the future this model could possibly be implemented for preclinical testing of novel anti-tumorigenic and especially anti-angiogenic drugs.

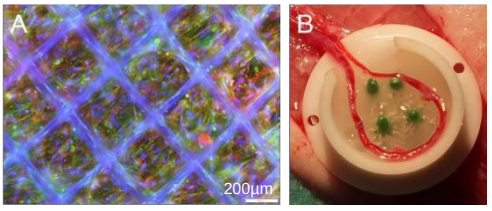

Fig. 1: (A) Scaffold with ADSC, (B) AV loop.

P473 Evaluation of the chemical and biomechanical viscoelastic properties of decellularised tracheal scaffolds

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Tracheal tissue engineering using decellularised extracellular matrix (ECM) scaffolds was one of the first fields to be clinically introduced to patients with phase I/II clinical trials underway in the UK. A key requirement in functional tissue engineering is to characterise the biomechanical properties of tissue-engineered constructs relative to native tissues. This will provide baseline assessment of the initial functional state of the decellularised tissue. In the present study we explored the effect of decellularisation on the biochemical and mechanical viscoelastic properties of porcine tracheal cartilage. Viscoelasticiy describes the biphasic nature of cartilage attributed to its solid like composition of elastin and collagen fibrils and water trapped by glycosaminoglycans. This unique property helps prevent failure of cartilage under repetitive loading. Porcine tracheae were decellularised by vacuum assisted chemical enzymatic approach. Cellularity and key ECM components of the scaffolds were evaluated. And total DNA, glycosaminoglycan and collagen content were quantified. Dynamic mechanical analysis (DMA) frequency sweep test was used to assess the viscoelastic properties of tracheal cartilage. The results showed that decellularisation process removed most of the cellular contents with a significant reduction in total DNA content. There was no significant difference in the level of collagen and glycosaminoglycan content and the scaffolds retained most of the key extracellular matrix components. No significant difference observed in the viscoelastic storage modulus, loss modulus, complex modulus and tan delta. In conclusion, these results suggest that vacuum assisted chemical enzymatic decellularisation appeared to retain the critical structural and mechanical viscoelastic properties of tracheal scaffolds.

P474 A multilayered palate substitute generated by tissue engineering. An in vivo evaluation

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Background: Cleft palate is a common congenital malformation characterized by the lack of palate bone and oral mucosa. Novel biofabrication methods allow the generation of complex multilayered structures consisting of different bioengineered tissue types. In the present study, we generate a complete substitute of the rabbit palate and we evaluated the usefulness of this substitute in an in vivo model of palate damage.

Methods: Primary cell cultures of oral mucosa epithelium and fibroblasts were established from rabbit oral mucosa biopsies, and adipose tissue-derived mesenchymal stem cells (ADSC) were isolated from adipose tissue biopsies and cultured in laboratory. Then, a bioengineered bone substitute was generated by using fibrin-agarose biomaterials with ADSC immersed within with osteogenic differentiation medium, and a substitute of the oral mucosa was constructed with the same biomaterial with fibroblasts within and a stratified layer of epithelial keratinocytes on top. Both structures were fused by plastic compression and autologously grafted in vivo in young laboratory rabbits. Histological and histochemical analyses were used to evaluate biointegration, cell function and cell differentiation in the multilayered palate substitute.

Results: Our biofabrication method allowed us to generate a single multilayered structure consisting of a bone substitute with an oral mucosa substitute on top. In vivo implantation in laboratory rabbits showed proper integration in the host bone and mucosa, and the grafted tissue had high levels of cell differentiation, epithelial stratification and maturation. Histochemical analysis of the grafted oral mucosa epithelium showed that the expression of cytokeratins 4, 13, 5 and 19 were high, although lower than control tissues. Analysis of the oral mucosa stroma and bone showed positive expression of several components of the extracellular matrix, including proteoglycans, elastic and collagen fibers, mineralization deposits and osteocalcin, with collagen signal being comparable to controls. Implantation of the multilayered palate substitute was able to prevent the palate growth alterations found in control animals with unrepaired palate defects.

Discussion and conclusions: These results suggest that the multilayered 3D tissue construct we generated is able to integrate in the host tissue and contribute to normal palate development. Tissue maturation and differentiation were adequate, although full differentiation was not attained in vivo. Therefore, generation of a full-thickness multilayered palate substitute is achievable, and tissues that become partially differentiated upon in vivo grafting were able to improve palate growth and development.

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P476 Development of a perfused organotypic kidney model using decellularized rat kidneys

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Decellularized extracellular matrix (ECM) scaffolds of whole kidneys hold great potential for whole organ tissue engineering. These native scaffolds retain the organ specific ECM-composition, ultrastructure and 3D-architecture. Thereby, the microenvironment to support attachment, organization and maturation of tissue specific or stem cells is provided.

The aim of this study was to identify a decellularization protocol that preserves the kidney ECM as good as possible while efficiently removing cellular components and to re-endothelialize that scaffold using a standardized perfusion system, as a first step towards full recellularization.

Rat kidneys were decellularized with the detergents sodium dodecyl sulfate/TritonX-100 (SDS/TX-100) and sodium deoxycholate (SDC) at different temperatures. The scaffolds were compared with regard to structure, cell removal and composition.

Moreover, we tested these decellularized ECM scaffolds by recellularization with human umbilical vein endothelial cells and hiPSC-derived endothelial cells in 3D-perfusion culture. Pressure, pH, O2-level were monitored and controlled constantly. Glucose, Lactate and Lactate dehydrogenase were measured for metabolic monitoring. Notably, the cells were more viable on SDS/TX-100-decellularized scaffolds than on SDC-decellularized scaffolds. Histology and MRI revealed cells were evenly distributed in the scaffold, lining vessels throughout the kidney, including glomerular capillaries.

Our data show that decellularized kidneys provide a suitable platform for stem cell based renal tissue engineering and are the basis for further investigations of the potency of these scaffolds to promote and direct terminal differentiation of hiPSC-derived renal precursorcells.

P478 Surface modification of polystyrene for cell adhesion and spreading due to active oxygen exposure

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Polystyrene (PS) dish is widely used in the fields of tissue engineering and regenerative medicine. It is necessary to modify hydrophilic surface because of the poor cell adhesion. Plasma irradiation is one of famous surface modification techniques to improve cell adhesion. However, the PS dish is sterilized by gamma ray or electron beam irradiation or ethylene oxide exposure after the plasma treatment and wrapped into a sterilization bag. Active oxygen spices (AOS) has strong oxidation abilities and it is easy to generate from oxygen due to ultraviolet-light (UV) irradiation. We aimed to develop the PS simultaneous treatment, *i.e.*, sterilization and surface modification for enhancement of cell adhesion and spreading in the sterilization bag by using the AOS. We have already reported on the sterilization and the surface modification effects of the AOS by using the sterilization(1). In this study, we investigated optimal surface modification condition for cell adhesion and spreading by using the AOS.

The AOS exposure was carried out in a chamber equipped with a low-pressure mercury UV lamp to generate the AOS. The PS dish wrapped into sterilization bag was equipped into the chamber. After evacuating the chamber with a rotary pomp, oxygen gas was introduced until atmospheric pressure. AOS was generated by the UV light irradiation, and the AOS was exposed to the PS dish via the sterilization bag. After the exposure, chemical composition, surface roughness and wettability were analysed by x-ray photoelectron spectroscopy (XPS), scanning probe microscope (SPM) and water contact angles, respectively. And also osteoblast-like cells (MC3T3-E1) were cultured on the PS dish before and after AOS exposure. After the incubation at 37°C for 24 h in a 5% carbon dioxide incubator, cell adhesion and spreading characteristics were evaluated by microscopic observation.

The oxygen content of the PS surface modified by the AOS treatment increased with increase of the exposure time and decreased with increase of the distance between the UV lamp and PS dish. Peak intensity which represents C-C and C-H bonds was decreased whereas represent O-C=O, C=O and C-O are also increased. The contact angle of the PS dish modified by the AOS decreased with increase of the exposure time and decreased with increase of the distance between the UV lamp and PS dish. It is considered that elemental composition, *i.e.*, polar group, affects the contact angle. Although threadlike shape cells can be observed at the untreated PS dish, wide spread shape cells can be observed at the PS dish modified by the AOS treatment. Especially, PS dish modified by the AOS, whose oxygen concentration is around 10 atomic% provides better cell adhesion and spreading characteristics compared to commercially available tissue culture dish. From these results, it was demonstrated that the AOS treatment is one of effective methods to enhance the homogeneity of the cell spreading.

P479 Reconstruction of vascular networks in perfusion culture using a decellularized liver scaffold

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BACKGROUND & AIMS: Decellularization and recellularization techniques have been studied to reconstruct a whole liver in the field of liver tissue engineering. Decellularization is a technique which removes cells from an entire organ and preserves an extracellular matrix (ECM) architecture of an original tissue. Since ECM architectures around vascular structures are maintained in decellularized organs, a recellularized organ can be surgically connected to the recipient's vascular networks after transplantation. In addition, cells can be cultured in microenvironments which is similar to the condition in vivo. Using the decellularized liver, relatively large blood vessels, such as interlobular portal veins and central veins, can be reconstructed. However, reconstruction of capillaries is still a big challenge, which is important for preventing from haemorrhage and thrombosis. In this study, we investigated the reconstruction process of hierarchical vascular networks including capillaries in a decellularized liver. In particular, the effect perfusion culture on angiogenesis in a decellularized liver was investigated.

METHODS: Rat livers were decellularized and the decellularized livers were partially dissected to have only quadrate lobe and right lobe for reducing the number of cells required for recellularization. Green fluorescent protein expressing human umbilical vein endothelial cells (GFP-HUVECs) were injected through the portal vein of the decellularized liver for the reconstruction of vascular networks. The recellularized livers were cultured in static or flow conditions because it is known that HUVECs can be stimulated by fluid shear stress and show functional response. The flow rate perfused through the portal vein was calculated to be 4.7 ml/min for applying 1 Pa shear stress to the cells lining 50-μm diameter blood vessels in a decellularized liver. The recellularized livers were observed using a microscope and phase- contrast and fluorescence images were taken until day 14 for monitoring the process of vascular network reconstruction.

RESULTS & DISCUSSION: First, we analysed the size of reconstructed vascular networks in static condition. The results revealed that the minimum diameter of reconstructed blood vessels was 20 μm, suggesting that the liver-specific capillaries, which are called sinusoids, were not reconstructed in static condition. We then tried flow conditions to induce angiogenesis in decellularized livers. The perfusion culture resulted in the formation of vascular sprouts on day 7. The diameter of the vascular sprouts was 10 μm. Furthermore, these vascular sprouts were maintained in perfusion culture at least until day 14 while such vascular sprouts were not observed in static condition. These results suggest that perfusion culture promoted reconstruction of vascular networks including sinusoids in a decellularized liver. Further investigations will be necessary to anastomose interlobular portal veins and central veins through sinusoids.

P484 Suspension culture of hiPSC aggregates under plastic fluid condition

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The human induced pluripotent stem cells (iPSCs) are attractive cell source for regenerative medicine as well as drug screening, and the as establishment of mass production process is urgent necessity of the day. The requirement for large-scale suspension culture system mainly includes two factors; low shear stress due to high sensitivity of iPSCs and high mass transfer due to supplying low solubility oxygen in medium. These requirements leads to the adequate mixing in suspension culture.

In iPSC suspension culture, the cells make agglomation after seeding, and aggregates expand in size owing to cell proliferation, causing easy precipitation which makes coalescence between aggregates. On the other hand, for the oxygen supply, the air bubbles introduced in the fluid are easy to rise and escape from the fluid. These phenomena of aggregate precipitation and bubble rising causes growth depression, and adequate dispersion of aggregates and bubbles, which means the floating in the fluid, in suspension culture is required by means of minimum mixing which causes the shear stress to depress the growth.

In the present study, to satisfy contrary requirements, the Bingham fluid of culture medium was applied to establish a novel culture system which consists of non-mixing vessel with air-sparger to prevent the aggregate precipitation and bubble rising. The expansion culture of iPSCs was successfully performed in the system under low shear stress and sufficient oxygen supply, compared to that in the culture using Newtonian fluid of conventional medium with mixing and air bubbling, or in culture using Bingham fluid without air bubbling.

P485 Effect of the CCL5 releasing fibrin gel for intervertebral disc regeneration

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Background: Release of chemotactic factors in response to tissue damage has been described for different musculoskeletal tissues, including the intervertebral disc (IVD). Pro-inflammatory chemokines CCL5 and CXCL6 are released by induced degenerative discs, and CCL5 has been associated with discogenic back pain. However, whether CCL5 has a positive or negative impact on degenerative disc recovery is not clear.

Objective: To evaluate the effect of CCL5 releasing fibrin gel for regeneration of intervertebral discs in vitro and in organ culture.

Methods: Chemotactic and chemokinetic effects of CCL5 at concentrations of 0, 25, 50, 100 ng/mL on bovine annulus fibrosus cells were tested using a transwell assay; SDF1 was used as control. The migrated cell number on the bottom chamber was calculated by Calcein AM staining 17 hours after the cells were plated in the top chamber. Fibrin gel containing different doses of CCL5 (0 ng, 50 ng, 500 ng, 2500 ng) was used to treat an ex vivo bovine caudal disc puncture model cultured under dynamic loading conditions. The disc height and dynamic modulus were recorded at different time points. After 14 days loading, the samples were collected for histological examination. The number of the cells migrated into the gel was counted in the Lactate Dehydrogenase stained sections.

Results: In the chemotaxis test, the number of migrated cells increased with a dose dependent manner of CCL5 ($P < 0.05$), but not with that of SDF1. In the chemokinesis test, the number of the migrated cells did not grow with increasing concentrations of CCL5 (Fig. 1). In the ex vivo study, the disc dynamic modulus decreased markedly after disc puncture and recovered back after 14 days loading. The disc dynamic modulus of the 2500 ng CCL5 group was higher than that of the 0 ng group ($P < 0.05$). The number of cells homed into the gel of the 2500 ng CCL5 group was higher than that of other groups (Fig. 2).

Discussion and conclusion: Fibrin gel combined with high dose of CCL5 could recruit endogenous disc cells for tissue repair. Furthermore, CCL5 might recover the disc dynamic modulus after disc puncture. Although the chemotactic effect of CCL5 on annulus fibrosus cells was verified, the cells homed in the disc should be defined in further studies. CCL5 may have a promising future for intervertebral disc regeneration.

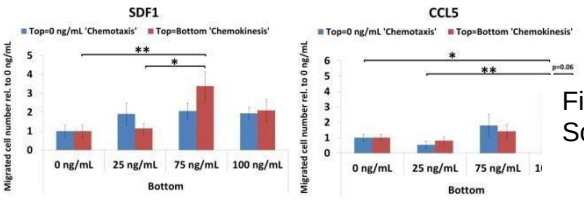

Fig 1 chemotaxis and chemokinesis of CCL5 and SDF1. Mean±SEM, n=3, *p<0.05, **p<0.01

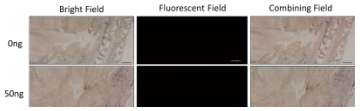

Fig 2 Lactate Dehydrogenase staining. Scale bar = 1000μm

P486 Clinical application of patient-specific 3D-printed biodegradable scaffolds for maxillary reconstruction

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An autologous bone grafting is the most widely used method in the traditional maxillofacial bone reconstruction surgeries. The autologous bone grafting requires harvesting of bone from unaffected area, and not from the defect area, which inevitably causes donor site morbidity and the amount that can be harvested is limited as well. Recently, biodegradable polymer-based porous 3D scaffolds fabricated by 3D printing technology had been studied and applied extensively in the fields of tissue engineering and regenerative medicine. This is due to a porous 3D architecture with fully interconnected pores promoting the formation of 3D tissues.

Polycaprolactone (PCL), which is approved by FDA for the clinical use, was used as a 3D printing material. In this study, three patients with severe bone defect on their face were treated with 3D printed PCL scaffolds for maxillary reconstruction. All patients received the operation of maxillectomy due to facial cancer, as a result of that, they gained severe facial deformity. To resolve the deformity, patient-specific 3D printed scaffolds were implanted to the patients. Design of the scaffold was determined to fit complex defect based on data from the computed tomography. After transplantation, changes in the implanted area were followed up for 4 to 24 months. The followed-up data showed that the 3D-printed PCL scaffolds effectively filled the patient defect. The best result in appearance was the change in eye level because postoperative support was focused on the maxilla and orbital floor. During the follow-up period, the difference in eye height was significantly improved, and it was confirmed in quantitative analysis of computed tomography. Furthermore, quantitative analysis revealed that the density of newly formed tissue was maintained by the process of tissue ingrowth into pore of scaffolds. This study showed the possibility of patient-specific facial reconstruction using 3D printing technology and tissue engineering based on the medical image data (e.g. CT) of the patient. However, since there was a limitation to regenerate real bone using PCL based scaffold, implantation of PCL blended with tri-calcium phosphate will be additionally carried out in the next study.

P487 A 3D thermo-responsive co-electrospun fibrous scaffold for extended culture and enzyme-free passage of quiescent primary human corneal stromal stem cells

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In vivo, corneal stromal cells are known as keratocytes, a quiescent, dendritic cell, responsible for maintaining the extracellular matrix. During 2D *in vitro* culture, keratocytes differentiate into an undesirable fibroblastic repair phenotype which in the eye would lead to scarring and blindness. Our previous work has shown that 3D environments can promote the reversion of activated corneal stromal cells to a quiescent keratocyte phenotype. However, difficulties in using enzymatic digestion to extract cells from 3D culture has led to the development of thermo- responsive 3D scaffolds for longer-term culture and passaging. Primary human corneal stromal stem cells (hCSSCs) were extracted from the limbal of the cornea, and then seeded on thermo- responsive electrospun fibres or on 2D culture flasks. Six thermal and enzymatic passages on scaffolds and flasks respectively were performed. Cell viability (Almar Blue assay) were conducted to measure the difference in cell populations while changing the culture temperature. The effect of extended passaging and 3D culture on hCSSCs was assessed by RT-qPCR and immunocytochemistry. Scaffolds were able to support cell adhesion, proliferation and detachment. Importantly, hCSSCs were not affected by the thermo-responsive polymer as cells were viable and proliferated in a similar manner to those cultured on control. Furthermore, culture of the cells on the 3D scaffolds promoted the quiescent keratocyte phenotype, with increased expression of the keratocyte markers, *CD34* and *ALDH3A1*, and decreased expression of the myofibroblast marker, *ACTA2*, when compared to 2D culture flasks (Fig.1). Immunocytochemistry (Fig.2) resulted with a positive CD34, and negative α-SMA stain at all passages, on 3D scaffolds in comparison with the 2D culture. In summary, thermo-responsive 3D scaffolds allowed the detachment of hCSSCs without enzymes, and promoted a quiescent keratocyte phenotype over multiple passages. This culture system has the potential to provide high numbers of a desirable cell phenotype for regeneration of the ocular surface in cases of disease or trauma.

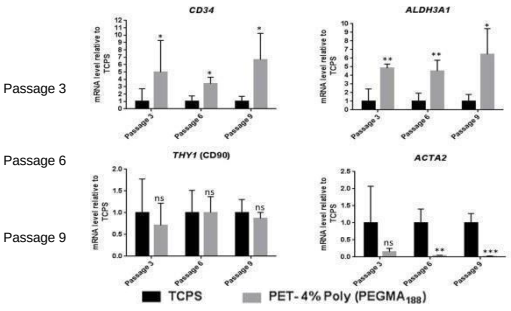

Fig. 1: *Comparative effects of culture environment TCPS and PET- Poly (PEGMA188) scaffolds on gene expression of hCSSCs at different*

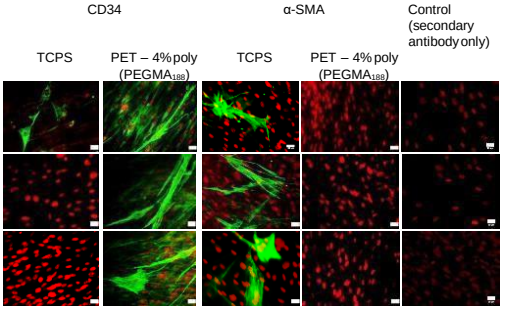

Fig. 2: *The effects of culture environment on keratocyte and myofibroblast markers of hCSSCs.*

P488 Evaluation of 3D hepatic tissue models for bioprinting

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Introduction: Drug-induced liver injury is the leading cause of acute liver failure and post-market drug withdrawals. *In vivo* animal studies cannot be totally translated to humans; therefore, there's huge demand of novel *in vitro* human models. 3D culture conditions would increase models longevity while bioprinting technology is expected to improve their functionality by the *in vivo*-like cell spatial patterning.

Aim: The present project aims at the establishment and characterization of printed liver tissue-like models as co-culture of human cells such as hepatocytes, stellate and endothelial cells in order to reproduce a functional liver sinusoid.

3D equivalents were printed to assess hepatic cells printability (printing test), while high-density hepatocytes models were manually produced and characterized in order to simulate *in vivo* cellular density conditions and define experimental parameters for bioprinting process.

Materials and methods:

- Printing test: HepG2 cells were mixed $6*10^6$ cells/ml with bioink, PEG-based ink produced in house. Matrigel solution was added to further improve cell viability during the printing process. Cell mixture was printed by direct dispensing in a spiral pattern and polymerized at 365nm wavelength. Models were analysed up to 7 days for cell proliferation, viability and morphology.

- High-density 3D models: High-density HepG2 discs and drop models were manually produced mixing 2:1 HepG2 "paste" with ink (supplemented with matrigel). Models were polymerized by exposure to 365nm wavelength for few seconds and cultivated up to 28 days. The equivalents were analysed for cell viability and albumin secretion as well as processed for histological analyses (cell proliferation, tissue-like intercellular tight junctions and lipid storage investigation).

Results and discussion:

- Bioprinting as well as ink is suitable for HepG2 viability and proliferation up to 7 days (15.84 ± 1.46 fold change increase day 7 vs day1). Printed HepG2 are homogenously distributed and round-shaped, forming agglomerates that increase in size over time.

- HepG2-ink-matrigel equivalents are long-term stable characterized by high and constant cell viability up to 28 days. The models resemble native liver with respect to the high cell density and due to the ink supplementation can easily be printed into tissue-like models with exact cell patterning and defined structure. Models are currently under investigation for tissue functionality and morphology and will be subsequently printed.

Conclusions: Bioprinting shows high potential for the manufacture of high-density liver tissue-like models. The printing of different cell types (hepatocytes, stellate and endothelial cells) will allow producing organotypical 3D liver equivalents.

P489 Melt electrospun written scaffolds as in-vitro platforms for cell culture: the effect of scaffold pore architecture

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INTRODUCTION

Melt Electrospinning Writing (MEW) is a recently developed technology demonstrated for the first time in 2011 [1], combining elements of solvent electrospinning and 3D printing. This approach marries the utilisation of heating elements and linear slides to melt and control deposition of a polymer, with a high voltage source to stretch and elongate the material into micron diameter fibres. It is thus an extremely powerful tool which is unique in its ability to generate ECM like fibres and control their orientation in three dimensions. While recent studies have explored the feasibility of MEW constructs as cell culture platform/tissue engineering scaffolds, including fibroblast [2] and human Mesenchymal Stem Cell (hMSC) culture [3], the effect of architecture for specific applications has yet to be determined. Therefore, the aim of this project is to develop *in-vitro* cell culture platforms using MEW scaffolds, and to study the effect of scaffold architecture on cell behaviour.

MATERIALS AND METHODS

A MEW apparatus was designed and built, with an aluminium plate mounted on two slides to allow for x-z translations, while a syringe pump was mounted on a third slide to allow for movement in the y-direction. A custom air heating chamber is attached to the syringe pump, and connected to an inline air heater and PID controller. The effect of system variables on fibre diameter and deposition characteristics were quantified and PCL scaffolds were fabricated with a voltage of 12.7kV on the needle at a distance of 20mm from the print plate, flow rate of 5µl/hr and temperature of 80°C. Four scaffold groups were fabricated, with three of these being characterised by an apparent pore size of 50µm, an actual pore size of 300µm, and alignment angle between consecutive layers of 90°, 45° and 10°. The fourth scaffold group consisted of 15µm diameter fibres deposited randomly.

RESULTS

A MEW apparatus was successfully designed and built, with linear slides in the x-z plane having a resolution of 12.7µm and maximum translation speed of 10.16cm/s, while the slide in the y-direction has a resolution of 1.6µm. Four scaffold groups were successfully fabricated via the controlled deposition of melt electrospun fibres with morphology being validated via Scanning Electron Microscopy (SEM). The creation of 300µm pores with a 50µm apparent pore size was verified, with minimal sagging of fibres across voids meaning that the required pore shape is maintained. Cells were seeded to scaffolds and studied in terms of morphology, infiltration, viability and proliferation.

DISCUSSION

MEW has the ability to create micron scale fibres and control their deposition in three dimensions, which is very difficult to achieve with any other manufacturing technique. Aligned scaffolds, which have previously been shown to be beneficial for osteogenic differentiation and bone regeneration[4], have been manufactured using MEW while also maintaining porosity, which is critical for cell infiltration and proliferation. Initial cell seeding studies have demonstrated successful infiltration of the scaffold regardless of alignment after 24 hours. Future work will involve using these constructs to study the effect of scaffold morphology on MSC lineage commitment, and optimising the architecture for the creation of scaffolds with enhanced osteogenic potential.

We would like to acknowledge the European Research Council (n°336884) and Science Foundation Ireland (n°SFI/13/ERC/L2864) for funding.

P492 Combined thermal and fluid flow simulation for the optimization of a 3D-culture bioreactor

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Motivation: In-vitro cultivation of chondrocytes allows the generation of regenerative cartilage tissue. As described by the literature damaged cartilage tissue lacks the ability to regenerate itself autonomously. One approach of in vitro cultivation is to automated bioreactors. Within the scope of this work, a small-scale incubator was developed that can be placed inside different measurement or loading systems: A hermetically sealed cultivation chamber - that serves as the bioreactor - is heated to 37°C (+0.5/-2.0°C) by a heat plate located underneath for easier handling. In order to guarantee a homogenous temperature distribution within the selected temperature tolerances, an insulation chamber is therefore necessary to reduce the vertical temperature gradient caused by the unilateral heating. To avoid long and expensive field tests a combined thermal and fluidic mathematical model of the system was created ("Conjugate Convective Heat Transfer Model") and verified. The simulation results were then used for the optimization of the insulation material and the wall thickness to not exceed the temperaturetolerances.

Materials and Methods: The small-scale incubator was formulated as a heat transfer problem consisting of heat conduction and natural convection, depicted in a heat transfer model. This model comprises all the significant solid components (heat plate, reaction chamber, insulated enclosure) and the occurring fluid flows (liquid cell culture medium, saturated air, dry air) that are modelled under the application of the Boussinesq-Approximation.

Results and Conclusion: According to the simulation results, the device is able to reduce the temperature gradient to 1.7°C in the cultivation chamber using the material constants of 40 mm thick Polyurethane (PUR) plates to all sides (Fig. 1). The final validation experiment results in a measured temperature gradient of 2.1±0.4°C along the vertical axis through the cultivation chamber and a maximum deviation of 0.7±0.1°C between simulated and measured values in the saturated vapour atmosphere. We conclude that the created heat transfer-model can be used for various generic models in further studies.

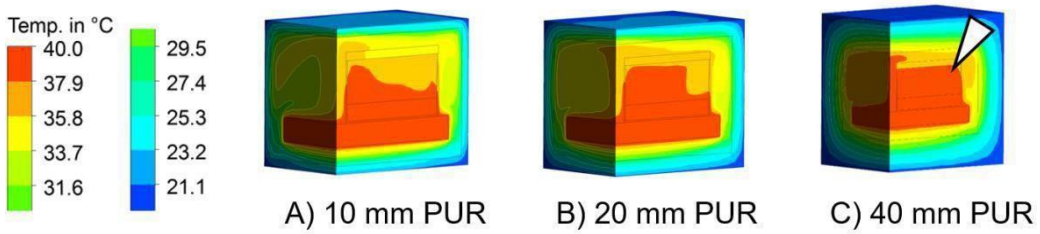

Figure 1: Simulation results for three PUR-insulation enclosures (A to C). Result C) shows a fully homogeneous temperature distribution in the inner bioreactor (arrow).

P494 3d printing of an elastic and biodegradable trachea made by water borne polyurethane for tissue engineering and regeneration

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A tissue engineering trachea with structural similarity to native trachea was fabricated by three-dimensional printing (3DP) technique. Two kinds of water-borne biodegradable polyurethanes (PUs) with different physico-chemical properties were used as 3DP inks in this study. The complicated structure of 3DP tracheal scaffolds was designed to mimic the structure of native rabbit trachea. Because of the organic solvent-free process, bioactive factors or small molecular drug (Y27632) could be conveniently incorporated in the water-based 3DP ink to promote the chondrogenesis of mesenchymal stem cells (MSCs). 3DP tracheal scaffolds containing Y27632 were printed from PU inks in a low temperature condition, and the compression moduli of these 3DP tracheal scaffolds were 0.30 ± 0.02, 0.66 ± 0.03, and 0.78 ± 0.05 MPa, respectively, under the static force 0.1, 0.5, and 0.8 N. The airflow test also demonstrated the gas tightness of 3DP tracheal scaffold at a positive or negative air pressure. In addition, MSCs cultured on the 3DP tracheal scaffolds displayed the chondrogenic potential and secreted glycosaminoglycans and collagen after 17 days of culture. The MSC-embedded 3DP tracheal scaffolds implanted in nude mice showed compression modulus similar to native rabbit trachea. MSCs in 3DP tracheal scaffolds were also differentiated into chondrocytes after six weeks. We considered that the 3DP platform and the fabrication process may be used to produce customized tissue engineering trachea for future clinical applications.

P495 Biocompatibility of polycaprolactone fabricated by three dimensional printing in nasal reconstruction: a experimental study in rabbits

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Objectives

Polycaprolactone (PCL) is an FDA-approved synthetic biodegradable polymer and is easily fabricated into three-dimensional (3D) structures. In this study, the 3D-printed PCL implant for nasal augmentation was further evaluated for its suitability for nasal surgeries such as septoplasty and rhinoplasty.

Methods

Ten New Zealand white rabbits were included and divided into study and sham groups (7 and 3, respectively). A lateral incision was made on the nasal dorsum and a pocket formed in the subperichondrial plane between the upper lateral cartilage and nasal septum. PCL was fabricated based on 3D printing technology into a 0.8×0.8-cm sized rectangular shape for use as a nasal implant. The material was inserted as a septal extension graft and sutured with alar cartilage for nasal reshaping. The implants were harvested 4, 8, and 12 weeks after implantation and evaluated by gross morphological assessment and histological examination.

Results

The initial shape of the implant was unchanged in all cases, and no definitive post-operative complications were seen over the 3-month period. Gross morphological evaluation confirmed that implants remained in their initial location without migration or extrusion. Histologic evaluations showed that the implant architectures were maintained with excellent fibrovascular ingrowth and minimal inflammatory reactions.

Conclusion

PCL can be used for nasal reconstruction such as nasal augmentation**.** PCL is easy to work with and will avoid the increased operative time and morbidity associated with autograft harvesting. Therefore, PCL implants designed by 3D printing can serve as clinically biocompatible materials in craniofacial reconstruction in the future.

P497 Optimizing Process Parameters for 3D Melt Electrospinning Printing

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INTRODUCTION: Melt electrospinning direct writing gives a great opportunity to create scaffolds with fibers of around few microns. Obtaining small fiber diameters for cell growth and proliferation in tissue engineering (TE) and biomedical applications is crucial [1]. There are many process parameters affecting fiber diameter in three-dimensional (3D) melt electrospinning direct writing (MEW). By optimizing them, a scaffold with desired diameter can be obtained. In this study, the effects of feed rate, distance, pressure, temperature and applying voltage on fiber diameter were investigated. After all, a desired 3D scaffold in terms of shape and fiber diameter was printed based on experimental data.

METHODS: MEW head has been connected to 3-axis CNC machine with 250 µm nozzle. Fixed parameters are as follows: 1000 mm/min for feed rate, 10 mm for tip to collector distance, 3 bar for pressure, 80 ℃ for temperature, and 7.5 kV for voltage. In each experiment, only one parameter has been changed, while others were kept constant. For the material, PCL (CAPA 6400, Mw: 37,000 g/mol) has been used. The material viscosity at different temperatures was studied by rheometer. Afterwards, a 4-layer scaffold was printed with feed rate of 2000 mm/min and fixed parameters for the rest to obtain 50 µm fiber diameter. All the fiber diameters were investigated using a SEM.

RESULTS & DISCUSSION: The effect of feed rate, distance, pressure, temperature, and voltage on fiber diameter were shown in Figure 1(a-e). In the lowest feed rate, whipping appears since feed rate and material flow rate are not matching well. By increasing the feed rate, the fiber is stretched and thus, get thinner. The material flow will be increased by raising the pressure hence, the fiber enlarges. The material's viscosity and accordingly its flow are directly affected by temperature, so the feed rate is not enough to match with the deposited material and as a result, whipping will appear. When voltage increases, the fiber diameter gets smaller, since the electric stress on the droplet makes it thinner.

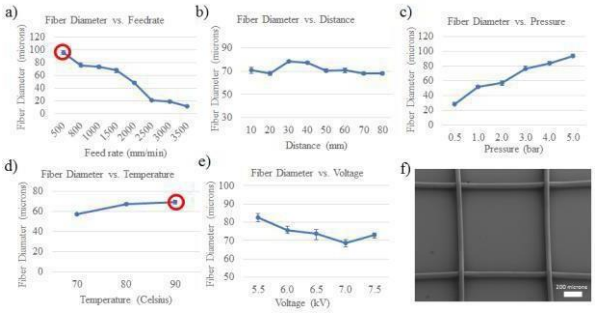

Figure 8:(a-e) Fiber diameter vs. feed rate, distance, pressure, temperature, and voltage, respectively. (f)SEM image of 4-layer scaffold.

CONCLUSIONS: As presented in Fig. 1f, the optimized 3D scaffold was printed successfully with the controlled fiber diameter of 50 µm and with 4 layers as height.

ACKNOWLEDGEMENTS: This study is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) grant number 213M687.

P498 3D printing PCL/PPSu polymeric scaffoldsfor wound healing applications

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INTRODUCTION: Wound healing especially for chronic wounds is one of the major challenges in skin tissue engineering (TE). Several studies have been researched to develop best strategy for wound healing. Finding a method as well as an appropriate material expediting the healing process is of great importance. One such promising and effective method is three dimensional (3D) bioprinting which enables to produce a highly controlled porous structure to resemble the extracellular matrix (ECM) [1]. A 3D printable, biodegradable, non-toxic material with good mechanical properties is also essential to provide a convenient environment for cells to attach, proliferate and migrate [2]. The objective of this research is to develop a 3D bioprinting method for polycaprolactone/polypropylene succinate (PCL/PPSu) copolymer and in-vitro assessment for wound healing applications.

METHODS: A copolymer of PCL/PPSu was synthesized and characterized chemically and thermally by ^{1}H- and ^{13}C-NMR, FTIR and DSC, respectively. The enzymatic degradation of the samples was examined with *Pseudomonas stutzeri* lipase. A 3D scaffold with desired pattern and selected porosity fabricated by a custom-made 3D printer. Microstructure of the scaffold was examined by scanning electron microscopy (SEM). To investigate the cell viability, human skin fibroblast (HSF) cells were cultured on the scaffolds for 1, 3, 7, and 10 days and were compared with control sample.

RESULTS: FTIR and NMR results demonstrated that copolymer has specific peaks of PCL and PPSu after successful polymerization. DSC was used to determine the crystallinity of the copolymer and the suitable temperature for the melt extrusion of compound, and a low processing temperature of 55^{o}C was chosen for the 3D printing process. The enzymatic degradation rate of PCL/PPSu was studied by change in surface morphology of samples after 10 days of incubation. Microstructural observations with SEM indicated that the scaffold had well-ordered, interconnected porous network. MTT assay represented that after 10 days of incubation, printed scaffold had low toxicity against HSF cells.

DISCUSSION & CONCLUSION: The synthesized copolymer demonstrated good chemical, physical, and biocompatibility properties for wound healing purposes. The 3D printed porous scaffold showed a well-ordered microstructure which indicates the compatibility of synthesized copolymer with the 3D printing process requirements in terms of physical properties of the material. The results of this study showed the potentials of the synthesized copolymer as a great candidate material especially with low processing temperature for use in 3D bioprinting and TE applications.

ACKNOWLEDGEMENTS: This study is supported by The Scientific and Technological Research Council of Turkey (TUBITAK) grant number 213M686.

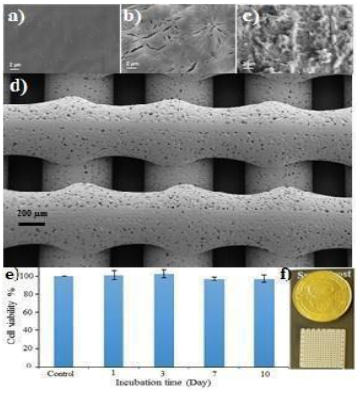

Figure 9: (a-c) SEM images of degradation time for control, day 1 and day 10 respectively, and (d) SEM image of printed scaffold, e) Cell viability assessment vs. time f) image of printed scaffold

P500 Formulation and optimization of a VEGF peptide based bioink for skin reconstruction

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With emerging 3D printing technology in the 21st century, tissue engineering and regenerative medicine fields found a great potential of this technology. 3D printing enables printing of complicated 3D tissue scaffolds in efficient mode. And already various bio printers are on market for tissue construction. Current challenge in 3D printing in biological fields, however, lie in lack of proper printing materials, so called bioinks. Therefore, development of 3D printable materials or bioinks for successful 3D printing of biological substitute is on high demand. Our study aimed to formulate and optimize a bioink having proper rheological, chemical and biological properties. Especially we investigated its applicability to print skin patch. A newly formulated hydrogel is based on gelatin-VEGF peptide complex hydrogel which is designed to enhance angiogenesis and wound healing. The peptide sequence, hydrogel-peptide ratio, stability and release kinetics were investigated for optimal cell viability, angiogenesis and skin regeneration. We thoroughly characterized the formulation *in vitro* for its 3D printability using INVIVO (ROKIT), resolution, temperature condition, mechanical stability, cell biocompatibility and degradability. The newly formulated peptide-bioink has a great potential as a 3D printing material for pattering 3D structure for soft tissue reconstruction since they are easy to print, gelling, not toxic, stable, and cost effective.

P501 Development of porous nanofibrous 3D scaffold by emulsion electrospinning for skin tissue regeneration

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Electrospun nanofibrous scaffold has long been used as skin substitutes for their structural resemblance to the dermal extracellular matrix. However, compact structure and limited porosity restricted cellular infiltration in nanofibrous mats. In this research article, we report the development of a highly porous, self-assembled, nano/microfibrous 3D scaffold with core-shell structure using the emulsion electrospinning technique. Fabricated polycaprolactone/chitosan electrospun scaffold had fiber diameter ranging from ~ 100 nm to ~ 5 µm with nano-patterned ornamentation on fiber surfaces. Further, the scaffold was fluffy and highly porous with a height of ~ 1.1 cm and an average pore size of ~ 20.59 µm. Incorporation of chitosan conferred the scaffold with reduced hydrophobicity, better water uptake capacity and controlled biodegradation. Presence of nano/microfibers with high interconnected porosity promoted efficient cellular attachment, infiltration and proliferation. The scaffold supported extracellular matrix protein expression and stratified epithelialization *in vitro*. Effective integration and attachment of scaffold with wound margins of a full thickness excision wound created in a rat model, and accelerated healing within three weeks endorsed the scaffold as a promising material for skin tissue regeneration.

P502 Difference of 3D printed artificial trachea according to additive manufacturing process

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The purpose of the current study is to characterize the polycaprolactone (PCL)-three dimensionally printed tracheal scaffolds and determine the difference in the additive manufacturing method.

Standard 3D trachea model for FDM was generated by using commercial CAD software (CADian3D®,version 2015, Intelli Korea, South Korea) at a size of 18mm of inner diameter, 22mm of outer diameter, 2mm wall thickness, and 25mm of height that mimics the structure of a native trachea. This standard 3D trachea model was applied to heating barrel bottom up (HBBU) FDM scaffold and 4-axis FDM scaffold. The scaffold morphology, mechanical properties (tensile, compressive strength), porosity, and cytotoxicity of these scaffolds were evaluated. Furthermore, scaffolds were implanted on a 10×10-mm artificial tracheal defect in four rabbits. Four and eight weeks after the operation, the reconstructed sites were evaluated through bronchoscopic, radiologic, and histologic examinations.

Mechanical and physical properties showed differences in two types of scaffolds. 4-axis tracheal scaffold showed more superior mechanical and physiological properties compared to that of HBBU scaffold. However, these scaffolds showed similar cell proliferation. Animal implantation examination of the each scaffold showed successful reconstruction of trachea. Mechanical and biological properties of scaffolds determine the successful replacement of tracheal defect. We concluded that the types of additive manufacturing method could affect mechanical and physical properties of the scaffold. Consideration of additive manufacturing method is one of the important factor for success of three-dimensionally printed artificial trachea scaffold and 4-axis method is more suitable technique for generating tracheal scaffold.

P503 Development of a 3D limbal epithelial crypt using a two photon polymerization system

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Aims. The aim of this study is to develop a biocompatible scaffold mimicking the structure of the native limbal stem cell niche and to evaluate the impact of its architecture on limbal epithelial stem cell differentiation. Limbal niches, called Limbal Epithelial Crypts (LECs) are located between the cornea and conjunctiva and are responsible for cornea epithelium regeneration. Among other factors that can affect limbal stem cell differentiation, such as biochemical signals, the role of a 3D physically protective environment has not been fully investigated yet.

Materials and Method. Crypts were micro-fabricated by photopolymerized gelatin-based hydrogels using a Two-Photon Polymerization system (2PP). Gelatin methacrylate (GelMa, 15% w/v) and GelMa-Polyethylene glycol diacrylate (GelMa-PEGda) were mixed with a cyclic benzylidene ketone-based photoinitiator (P2CK, 1) before the printing process. Volumetric degradation rate was studied by a combination of time lapse and confocal microscope techniques. To assess cell viability and proliferation, human corneal epithelial cells (hCECs) were seeded inside the scaffold and LIVE™ assay was performed over 7 days. To study cell differentiation along the z-axis, limbal explants were obtained from human cadaveric donor corneas from the Veneto Eye Bank Foundation, with written consent from the next of kin. Primary limbal stem cells were seeded inside the scaffold. Cell differentiation was monitored by using confocal microscope in z-stack mode. CK14 and CK3/12 were used as limbal stem cell and cornea markers, respectively.

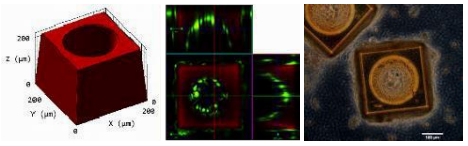

Fig.1: (left) CAD model of LEC; (middle) confocal images of hCEC cells stained with live staining after 7 days; (right) primary limbal cells repopulating the niche.

Results. Scaffolds mimicking the dimensions of the native stem cell niches were developed, following measurements taken from histology sections (2), and reproduced by a CAD model (Fig.1 left). The use of GelMa guaranteed the presence of cell adhesion sites. The addition of PEGda limited susceptibility to enzymatic degradation, without affecting hCECs viability (Fig.1 middle). Explant outgrowth colonised the scaffold, repopulating the niche. Primary limbal cells seeded inside the scaffold proliferated (Fig.1 right) and showed marker zonation along the z-stack. Further investigation into factors influencing cell differentiation is ongoing.

Conclusion. Biocompatible scaffolds mimicking the LEC micro-dimensions, in which primary limbal cells were able to repopulate the crypt, were produced. The 2PP system offers the ability to control cell migration and the differentiation process through altering scaffold geometry and chemistry. Our aim is to translate this technology into a viable clinical treatment for limbal stem cell deficiency.

P504 Controlling tissue morphogenesis in vitro: fabrication of full thickness human skin equivalent completely formed of cell-assembled ECM

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In this work we developed a new full thickness human skin equivalent (3D-HSE), completely formed by cell-assembled extracellular matrix (ECM). 3D-HSE mimics both structure and functions of the *in vivo* counterpart. We propose such model as the unique engineered skin able to replicate tissue morphogenetic process *in vitro*, allowing studies concerning ECM-related phenomena. The market of *in vitro* testing platforms moves many moneys *per* year so several companies (e.g. Mattek Corporation, L'Oreal and Henkel) have invested in developing new HSE to meet the European restrictions on the animal experimentation (Decree 76/768 / EEC, and then EU Cosmetic Regulation 1223/2009). The current HSE models are obtained by culturing keratinocytes on an engineered dermis formed of fibroblast entrapped in exogenous collagen matrices. Such models are extensively used as *in vitro* testing platform to test the safety of chemicals, pharmaceutics and cosmetics. Nevertheless, regardless of the final scope of the compounds, the current HSE are able to provide biological information restricted to the cell behaviour. In our work, we developed a method of production of 3D-HSE in order to control the morphogenesis of the dermal tissue. In this innovative approach, human dermal fibroblasts and porous micro-carriers were mixed in a dynamic culture system in order to obtain micro-tissue precursors (µTPs).The porous microcarriers has been optimized in order to induce the synthesis of new ECM components. The µTPs are moulded in a maturation chamber (perfusion bioreactor), and were able to assemble *via* ECM-ECM contact leading to the formation of a 3D human dermis equivalent completely formed of cell-assembled collagen, elastin and hyaluronic acid. Human keratinocytes seeded on such dermal surface, can differentiate in a complete stratified epithelium reproducing all features of native human skin. Biochemical, mechanical and histological analysis of structural and functional molecules typical of the human skin were performed up to 9 weeks of culture of our HSE by analysing the deposition and the assembly of collagen I, α-elastin and GAGs contents (Fig.1).

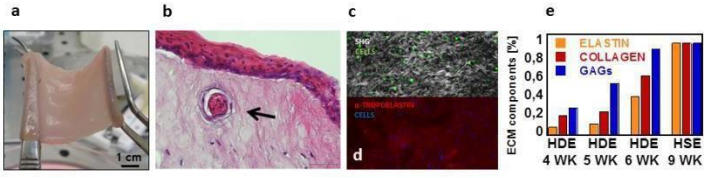

Fig. 1. a) large tissue (5 cm x 5cm); b) H&E of full-thickness human skin equivalent sections with a hair follicle-like structure into the dermis (arrow); c) collagen network of ECM: SHG signal (grey) and cells (green) ; d) immunofluorescence for α-tropoelastin (red) and cells (blu); e) quantification of ECM protein content during the time: collagen, elastin, GAGs amounts.

These results were compared with the native counterpart (human skin biopsy).

We demonstrated that fibroblast in such configuration were continuously engaged in the turnover of their own ECM. This makes our skin model a somewhat unique system capable to underwent ECM-related damage: ECM remodelling after UVA exposure, wrinkle formation, variation of both hydration and mechanical properties. Finally we standardized the production process of 3D-HSE in order to scale-up the fabrication of innovative human skin substitutes.

P506 Contact guidance for cardiac tissue engineering using 3D bioprinted gelatin patterned hydrogel

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Cardiovascular diseases are the major cause of death worldwide. Despite the current medical advances, physicians still find it difficult to tackle the mortality related to heart failure, the prime cause of death among cardiovascular diseases. Mimicking myocardium architecture and attaining its biomechanical properties remain the most challenging aspects in cardiac tissue engineering. Here, we developed 3D bioprinted patterned gelatin hydrogel to promote human mesenchymal stem cell (hMSC) myocardial differentiation as well as to maintain native cardiomyocytes (CMs) contractile functionality. Firstly, we studied the effect of patterned hydrogel on hMSC alignment, elongation and differentiation. Notably, cells displayed well defined F-actin anisotropy and elongated morphology on patterned hydrogel. Further, stem cell myocardial lineage commitment was affirmed using mature cardiac markers. Fluorescence-activated cell sorting (FACS) analysis confirmed a significant increase in patterned cell population committed to myocardial tissue lineage. Moreover, CMs were found to be more aligned and demonstrated synchronized beating on patterned hydrogel. Overall, our study proved that 3D bioprinted patterned gelatin scaffold induces stem cell myocardial differentiation as well as supports CMs growth and contractility.

P507 Hybrid electrospun silk fibroin and polyurethane vascular graft: a semi-degradable vascular access alternative for haemodialysis

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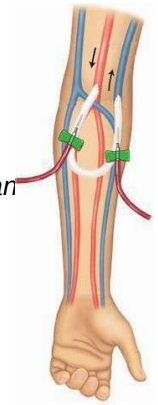

Fig. 1

Current vascular access approaches for medium- to long-term haemodialysis are limited to synthetic vascular grafts (VG, Fig.1) and native arteriovenous fistulae (AVF)[1], but none of them represents the definitive solution to the clinical need. We envision an optimal, ground-breaking alternative that overcomes the limitations of both, i.e. an off-the-shelf semi-degradable graft, that allows early cannulation, and possesses tuneable mechanical properties (VG-like), while providing higher hemocompatibility and patency rates, and enabling tissue regeneration and remodelling (AVF-like). To this purpose, we propose herein the combination of bioactive and degradable silk fibroin with a commercial medical-grade polycarbonate-urethane. Prototype grafts were manufactured by subsequently electrospinning various solutions, aiming to obtain pure fibroin inner and outer layers, with intermediate layers made by a blend of both materials. The grafts, about 200mm in length and 7mm in diameter, were characterized in terms of morphological, physicochemical and mechanical properties.

Scanning electron microscopy images revealed a fibrous morphology of the inner and outer surfaces, with fibre diameter of 0.71±0.05µm and 1.00±0.16µm, respectively.

Infrared spectroscopy (Fig. 2) and differential scanning calorimetry confirmed that both fibroin (SF) and polyurethane (PU) retained, within the blend, their individual functional groups and calorimetric profiles. Circumferential tensile testing, in accordance with ISO 7198, showed a tensile strength of 1.72±0.27N/mm, with a deviation in line with the samples' wall thickness of 312±40µm. Recorded elongation at break was 497±34%. Moreover, hands-on bench trials with vascular surgeons showed easy handling and suturability, together with good leakage retention after repeated puncturing. These preliminary assessments confirmed the potential of our hybrid graft as a novel and promising semi-degradable alternative for vascular accesses, that could combine the extensively demonstrated hemocompatibility and remodelling capability of fibroin[2,3] with the elasticity of polyurethanes. Current work concerns the full characterization of the grafts according to ISO 7198, and *in vitro* biocompatibility tests according to ISO10993.

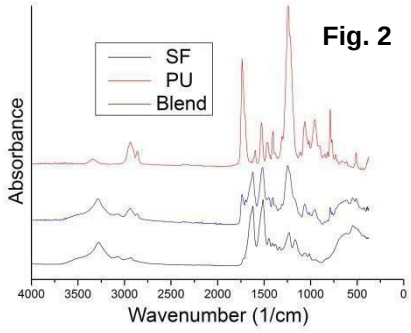

Fig. 2

P508 3D bio-printed artificial skin model for in vitro test

Joo-Yun Won[1], Heejin Kim[1], Min kyoung Kim[1], Geunseon Ahn[1], Kyung-Hyun Min[1], Won-Soo Yun[1,2], Jin-Hyung Shim[2]

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The artificial skin requirements are increased very much due to banned animal testing on cosmetics area. The extracellular matrix (ECM) of mammalian tissues has been studied as a scaffold to reconstruction of numerous tissues. Here, we reconstructed the full thickness artificial skin (composed of dermis and matured epidermis) via three-dimensional (3D) bio-printing technology with skin bioink that was developed by porcine skin decellularized extracellular matrix (dECM) process. To prepare skin bioink, porcine dermis was treated with enzyme and nonionic detergent on the stirrer. After decellularization and freeze drying, the amount of cellular material remaining include DNA, collagen and GAG, was measured by each specific method within the dECM. DNA was dramatically decreased after process, whereas collagen contents were not significantly influenced. Freeze-dried skin dECM was digested with pepsin in acetic acid at room temperature for dECM hydrogel preparation and their properties were confirmed by scanning electron microscopy(SEM), rheological assay and established skin layers. SEM image of skin bioink surface showed porous structure and fiber architecture similar to skin dermis and the storage (G') and loss modulus(G") of skin bioink were maintained until 4weeks. To establish the dermis layer, resuspended human dermal fibroblasts (HDFs) were mixed with the pH-adjusted skin dECM pre-gel and cultured after printing. On day 7, the HDFs exhibited fibroblast-like morphology in the printed constructs, especially, proliferation was increased. These results show that skin dECM has printable properties and provide a suitable environment to cell proliferation as a bio-ink. After dermis printing, keratinocytes were seeded by inkjet bio-printing and induced differentiation, and then, level of epidermis reconstruction was evaluated by H&E and immunofluorescence assay. The length of epidermis was increased at day 12 compare with day 3 as well as differentiation markers.

The present study, we reconstructed full thickness human skin by 3D cell printing with skin specific bioink and discovered the skin dECM has printable properties and provide a suitable environment to cell proliferation as a bio-ink. Moreover, this 3D printed full thickness human skin was exactly realized the skin structure with specific marker expression, confirming its appropriateness as in vitro skin model.

P512 Local antibiotic delivery with thermoresponsive hyaluronan hydrogel successfully treats chronic intramedullary nail-related infection in a single stage revision

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AO research institute, Davos, Switzerland

Introduction. Bacterial infection after trauma and orthopaedic surgery is a severe complication that is difficult to cure. In most cases, the infected implant has to be removed to cure the infection. Biohydrogels are ideal delivery systems for controlled local antibiotic delivery in complex surgical sites as they can cover implant surfaces, and reach small spaces. In this study we evaluated a gentamicin-loaded thermoresponsive hyaluronan hydrogel (THH), which is a fluid at room temperature and gelates at body temperature, in the treatment of a chronic implant related bone infection in a large animal model. **Experimental. Preparation:** THH was prepared by covalently grafting poly N-isopropilacrylamide to hyaluronan. Reconstitution with 1% gentamicin sulphate (GS) was performed under sterile conditions. A cadaver trial was performed to assess the THH distribution within the intramedullary (IM) tibia cavity by imaging a radiopaque gel (KI + $BaSO_4$) with microCT. **Initial surgery:** Swiss alpine sheep (*n*=12) received a intramedullary (IM) nail in the left tibia and were inoculated with 10^6 colony forming units (CFU) of a clinical *Staphylococcus aureus* strain directly into the IM canal. **Revision surgery:** After 8 weeks, a chronic infection developed and the colonised nails were removed and the IM canal was debrided. Six sheep received 25 ml of GS-THH prior to implantation of the replacement nail. The sheep (treated or controls without THH) received systemic antibiotics for 2 weeks (amoxicillin & clavulanic acid s.c.). An ultrafiltration probe was placed in the IM cavity to collect extracellular fluid and determine the local GS concentration for 10 days. **Euthanasia**: the animals were euthanized 2 weeks after cessation of antibiotic treatment to prevent false negatives. Hardware, bone marrow, and tissue samples were harvested for quantitative bacteriology. **Results:** The cadaver trial revealed a uniform gel distribution in the IM cavity. At the 8-week revision surgery, all sheep displayed radiographic signs of infection and the samples taken were culture positive with *S. aureus*. At euthanasia, the sheep receiving systemic antibiotic therapy only were still infected, as confirmed by 5/5 IM nails, 5/5 bone marrow, and 8/25 tissue samples being culture positive for *S. aureus*. For the treatment group receiving GS/THH, *S. aureus* was detected in 0/6 IM nails, 1/6 bone marrow, and 1/30 tissue samples. One control sheep was excluded due to the presence of an anaerobic cultured superinfection. Local GS concentrations peaked 1 day post revision surgery at 1341±963 µg/ml and decreased to 113±73 µg/ml on day 4 and 42±44 µg/ml on day 7. **Discussion:** The sheep developed a chronic, systemic antibiotic resistant device-related infection. The model is clinically relevant as it is on a human scale, and recapitulates the challenge of treating chronic bone infections. After administration of local GS with THH as delivery vehicle during the revision surgery, the infection was cleared in most of the specimens harvested from the treatment group. In contrast, the infection was still present after euthanasia in the systemic antibiotics-only group. **Conclusion:** A human-sized model of device-related infection was successfully established. Owing to the local GS delivery by a THH in combination with systemic antibiotics, the infection rate was significantly reduced.

P514 Interpenetrated polymer network hydrogel for guided periodontal regeneration

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[1]LIOAD, Nantes, France, [2]CERM, Liege, Belgium

Periodontitis is an inflammatory disease resulting from the presence of oral bacteria biofilm in periodontal tissue, which destroys the tooth-supporting attachment apparatus. Untreated inflammation can spread to the gum tissue and lead, ultimately, to the loosening of the supporting tooth bone, with the risk that the tooth eventually falls.

Guided Tissue Regeneration is a technique based on a barrier membrane designed to prevent colonization of the wound space by epithelial cells from soft tissues. Indeed, these cells, with a faster migration and proliferation rate compared to those of bone and periodontal ligament ones, could interfere with the regeneration process. While solid polymer membranes have been used in the past, a photo-crosslinking technology, that would easily fill the periodontal space, would be particularly fit for a GTR application.

In this project, we selected chitosan, a polysaccharide derived from natural chitin, for its antibacterial activity and fast biodegradation. We developed an injectable photo-crosslinkable hydrogel based on Methacrylated Carboxymethyl Chitosan (CMCS) that can be applied as a viscous solution and cured in situ using a photoinitiator system of riboflavin and triethanolamine. A visible light lamp (λ 420-480 nm), already used in dentistry, was prefered over a UV lamp. In order to improve the mechanical properties of the chitosan network, we formulated an Interpenetrating Polymer Network (IPN), system in which 2 independent networks are formed and provide a synergic combination of their properties. Here, we selected a silanized hydroxypropylmethyl cellulose (Si-HPMC) polymer, able to form a 3D network through the condensation of silanoates groups at physiological pH [1].

The chemical grafting of Methacrylated Carboxymethyl Chitosan was characterized by ^{1}H-NMR and Infrared Spectroscopy. The gel point of the solution was determined by rheology and remained compatible with a clinical application. The rheological tests of CMCS/Si-HPMC indicated an increase of the measured moduli as compared to CMCS hydrogels, confirming the formation of a 2nd network based on the cellulose derivative. Mechanical assay under compression showed an augmentation of the Young Moduli.

In this work the chemical synthesis, characterization and formulation of a new IPN was performed. These preliminary results are quite promising for the development of novel IPN systems for Guided Periodontal Regeneration. In the next future, cytocompatibility assays will be performed with polymer extracts as well as IPN hydrogels using human epithelialcells.

P515 Interpenetrated polymer network hydrogel for biomedical applications

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In the recent years, multicomponent hydrogels, such as semi-Interpenetrating Polymer Networks (semi-IPNs) and Interpenetrating Polymer Networks (IPNs) have emerged as innovative biomaterials in drug delivery and tissue engineering. These networks remarkably differ from their macromolecular constituents, due to a synergic combination of favourable properties of each polymer network.

Here, our work describes the development of an IPN that could be injected as a semi-IPN, facilitating the filling process, and photo-crosslinked in situ. To realize this scaffold, we selected polysaccharides polymers, which represent a class of macromolecules of particular interest because they are usually abundant, available from renewable sources and have a large variety of compositions and properties that allow appropriately tailored chemical modifications.

We previoulsy reported a silanized hydroxypropylmethyl cellulose (Si-HPMC), able to form a 3D network through the condensation of silanoates groups at physiological pH [1]. After solubilization in aqueous basic conditions, Si-HPMC condensation is induced by mixing with with a buffer hence decreasing the pH to 7.4. Although easy to perform, this cross-linking, with a gel point of about 30 minutes, is not well adapted to clinician needs. In addition, a photosensible methacrylated dextran was synthetized, as confirmed by ^{1}H NMR, with 10% or 20% grafted groups [2].

Two IPN formulation of Si-HPMC and MA-Dextran were prepared; both solutions were placed in a mould with a photoinitiator (I2959) and irradiated with a UV lamp (λ 365) for 10 minutes. Formation of a scaffold was visually identified immediately.

The injectability of both formulations before cross-linking was studied using a syringe with needle and was found to be comparable to the injectability of distilled water. In presence of the photoinitiator, elastic and viscous moduli were recorded under UV irradiation using a rheometer (frequency 1Hz, strain 1%). Gel point for both formulations was about 60 seconds of exposition. The resulting crosslinked IPN hydrogels were analyzed and we showed a synergism of their mechanical properties, which were different from those of the individual polymers. Finally, NIH-3T3 cells were encapsulated in the IPN hydrogel containing MA-Dextran 20%. After 1, 3 and 6 days of culture, cell viability was demonstrated using an MTS assay

In conclusion, IPNs hydrogels based on Si-HPMC and MA-Dextran, that can be injected as a solution and cross-linked in situ, constitute attractive materials for biomedical applications due to their tailorable mechanical characteristics and cytocompatibility.

P516 Cell proliferation on genipin crosslinked chitosan alginate microcarriers

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The high surface area to volume ratio of microcarriers offers solution to large scale cell expansion as many cell therapies require high cell dosage. Microcarriers also allow adherent cells to be cultured in suspension bioreactors to allow for better control of cell growth conditions. It can hence be lower cost and less labor intensive compared to traditional 2D culture methods. Although several types of commercial microcarriers are available, they possess several drawbacks which include, microcarrier sizes being limited to commercial availability, limited biocompatibility, low seeding efficiency for stem cells and poor cell recovery.

This work focuses on developing a microcarrier based on genipin crosslinked chitosan alginate beads for cell expansion. Alginate beads were produced by electrospraying, which involves passing alginate solution through a nozzle and breaking apart the alginate jet into droplets which then fall into a $CaCl_2$ bath, where they gel into microbeads. This process of making microbeads is mild and does not require addition of strong acids, organic solvents or maintenance of temperature. By controlling the electric field, bead diameter could be controlled. Hence, bead size could be customized based on the amount of cells required or the type of bioreactor used. Since alginate does not contain any cell adhesion proteins as well as being polyanionic, cells do not attach onto the beads. The beads were hence coated with chitosan and crosslinked in genipin. Genipin is fluorescence after crosslinking with amine groups of the chitosan, and whether crosslinking is successful could be characterized by the fluorescence. Furthermore, the degree of crosslinking was estimated and compared by the fluorescence reading of the genipin and the most optimal crosslinking conditions were chosen, aiming for highest crosslinking degree achieved in the shortest experimental time.

Human dermal fibroblasts were cultured on the beads and were shown to attach and spread out in a morphology similar to that in a 2D culture plate. Cytodex 1 beads, a widely used commercial microcarrier, were used as control group for the beads. Measurements showed a higher attachment efficiency in the chitosan genipin microcarriers compared to Cytodex 1. Furthermore, upon the addition of trypsin, cells easily detached into a single cell suspension and did not require extended incubation periods or intense agitation as necessary for several commercial microcarriers. Proliferation assays were conducted on the beads for a period of two weeks, the fold growth of cells were counted and furthermore, cell numbers harvested after the culture period was counted. The cells harvested from microcarriers were reseeded onto a 2D culture plate to test their growth and proliferation ability. Mesenchymal Stem Cells were also cultured on the beads to evaluate their potential to be used for stem cell therapy.

P517 The use of pNIPAM-laponiteÒ hydrogel in 3D-cell culture of the intestine

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Human colonic adenocarcinoma cell lines: Caco-2 and HT29-MTX have been commonly used in *in vitro* culture systems as these cells have the ability to differentiate into enterocyte-like cells and mucus-secreting goblet cells respectively. This project aimed to develop a 3D cell culture model which mimics the natural epithelial layer of the native intestine, each cell line was cultured in three hydrogel systems, and cell morphology, survival and matrix production investigated over prolonged culture periods. Each cell line was investigated in suspension and layered cultures, using a novel pNIPAM-Laponite®, pNIPAM-DMAC Laponite® and alginate hydrogels. These were maintained under both static and dynamic culture conditions for up to 3 weeks. Cell viability was measured using Alamar Blue viability assay. Cell morphology was assessed following staining with: H&E and Alcian Blue-Periodic Acid Schiff (PAS). Subsequent scanning electron microscopy (SEM) assessment was performed to determine the morphology of cells grown in or on the pNIPAM-Laponite® hydrogel only. Immunohistochemical was used to investigate MUC2, MUC5AC and alkaline phosphatase production in cultured cells. Caco-2 and HT29-MTX cells were successfully cultured within and on the surface of each of the three hydrogels. However Caco-2 cells preferentially grew on the surface of the pNIPAM-Laponite hydrogels under dynamic conditions where they remained viable for the maximal culture period of 3 weeks. Under all other conditions cell viability was reduced. In contrast HT-29 MTX cells remained viable both suspended or in layered cultures. Interestingly, cells cultured on the surface of pNIPAM- Laponite® under dynamic culture formed villus-like structures and produced both acidic and neutral mucins. HT29-MTX cells were shown to expressed MUC-2 and MUC5AC, whilst there was no evidence of MUC2 and MUC5AC production in Caco-2 cells. SEM analysis of cells cultures showed the presence of cells within/on the surface of the pNIPAM-Laponite® hydrogel. The most effective system which supported both cell lines was culture on the surface of pNIPAM- Laponite® hydrogel under dynamic conditions. Future development of this model for co-culture could provide an effective 3D model of the intestine.

P518 Injectable Hydrogels of Cellulose Nanofibrils Crosslinked with Cell culture Medium for Tissue Engineering

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Polysaccharide biomaterials such as alginate, chitosan, hyaluronic acid and cellulose can form hydrogels similar to ECM due to their physicochemical structure. Typically, most polysaccharides require separate crosslinking steps to form a hydrogel network. On the other hand, cellulose nanofibres from plants or bacteria form colloidal dispersions in aqueous medium. For cell encapsulation and bioprinting, alginate has been extensively used due to its easy crosslinking by divalent cations, such as Ca^{2+}. Gelation of CNF aqueous dispersions by divalent or trivalent cations has also been reported in many studies. This study aimed to produce two CNF hydrogels with different surface chemistry and study their ability to form mechanically stable and injectable networks by ionic interactions with DMEM cell culture medium. The CNF hydrogels were produced using TEMPO mediated oxidation or carboxymethylation pretreatment before fibrillation. The interactions of fibroblasts with the hydrogels were then studied in terms of cell toxicity, attachment, proliferation, morphology and migration. Crosslinking by exposure to cell culture medium was found to allow gelation of the negatively charged CNF and improved their mechanical properties. Moreover, the physicochemical properties of carboxymethylated CNF hydrogel maintained cell proliferation and migration but adversely affected cell morphology and spreading. In contrast, TEMPO oxidized hydrogel was found to maintain cell spreading and characteristic morphology of fibroblasts. Taken all together, this study demonstrated that the TEMPO-oxidized CNF hydrogel, when crosslinked with DMEM, offer a promising hydrogel for tissue engineering applications.

P519 Tunable injectable pNIPAM-Laponite® based hydrogels for musculoskeletal regeneration

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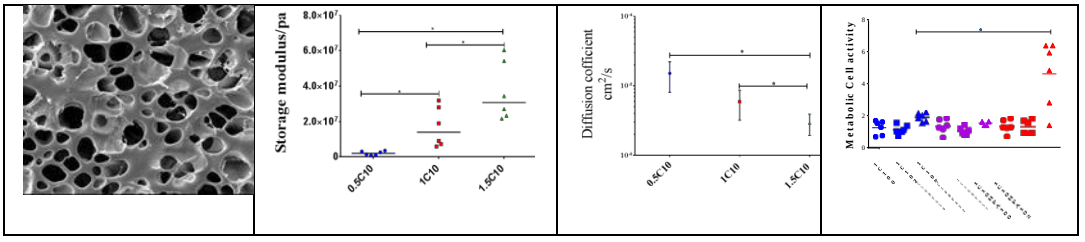
Aims: Damaged tissue has several options for treatment, including repair, replacement with a synthetic or natural substitute, or regeneration (Chirani, 2015). Injectable Hydrogels are attractive for use in biological applications such as tissue regeneration (Hunt *et al*, 2014). One possible application would be as a cell scaffold for bone regeneration; with the hydrogel providing an ideal environment for stem cells to regenerate bone tissue due to their high water content and similar microstructure. This study aims to investigate the effects of crosslink density and comonomer concentration, on the properties of injectable pNIPAM-Laponite® hydrogels, to determine their suitability to be used as a regenerative therapy.

Method: Hydrogel samples containing a range of comonomer (NIPAM, HPA) concentration and crosslink density (0.5-1.5%) were prepared via precipitation polymerisation; as described in previous work (Thorpe *et al*., 2016). The morphology and internal structure of the hydrogels were characterised via SEM (Nova Nano, FEI, Netherlands). Electron micrographs were collected from the samples and the pore sizes determined. The dehydration and rehydration rates of the hydrogels were determined using an FTIR (Nexus, Thermo Nicolet Corp., USA), coupled to a single reflection diamond ATR cell (Specac, UK). The mechanical properties of the hydrogel samples were determined using dynamic mechanical analysis (PerkinElmer DMA 8000) in compression mode at 25°C (± 2). Rat stem cells were embedded into different hydrogel compositions and cell viability assessed using Alamar Blue assay (Life Technologies, Paisley UK) in normal complete media following 0, 2 and 7 days incubation.

Results: Increasing the crosslink density of the hydrogels was shown to significantly decrease the pore sizes resulting in an increase in the stiffness and a decrease in the diffusion rate of water ingress. Incorporating HPA as a comonomer increased the measured pore size, significantly decreasing the stiffness but with only a small impact on the water diffusion rate. Increasing HPA concentration was also shown to reduce the rate of dehydration at 25°C. Cell culture results indicated there was no loss of viability in any of the hydrogel compositions tested. However, the total metabolic activity increased significantly in the composition with highest HPA content ($1C_{10}$ HPA10) after 7 days when compared to other hydrogel formulations.

Impact: The diffusion rate decreased as a result of a decrease in pore size and the greater number of clay platelets increasing the tortuosity of the diffusion path. The addition of HPA was shown to increase the mean pore size, decrease the stiffness and increase the water ingress rate slightly, but retard the dehydration rate due to the greater number of hydrophilic polymeric units able to bind with water. We speculate that metabolic cell activity is increased significantly at higher HPA concentration, due to increasing the pore size and diffusion rate of the water through the hydrogel. This study has demonstrated that the injectable hydrogel is tuneable to produce differential pore sizes, mechanical properties and cell behaviour and thus could be applicable to a number of diverse tissue engineering approaches.



P520 Self-setting injectable hyaluronic acid hydrogels for tissue engineering applications

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The field of injectable hydrogels is a thriving area of research for the development of breakthrough tissue engineering (TE) approaches. Owing to their hydrophilicity, biomimicry with native tissues and ease of modification, hydrogels are steadily gaining momentum for the restoration of damaged tissues. From a clinical perspective, injectable hydrogels are a strong added value, allowing the development of minimally invasive surgeries and reducing tissue disruption, pain and hospitalization stay. Among natural polymers used for TE applications, hyaluronic acid (HA) is increasingly gaining interest owing to its hydrophilicity, degradability and biological activity.

Using a chemistry previously developed within our laboratory, we report the design of a new self-setting injectable HA hydrogel, forming through the condensation of silanols at physiological pH. This hydrogel is mostly dedicated to bone tissue engineering where its intrinsic properties and degradability should provide a suitable and temporary environment for new bone in-growth. Upon injection, the scaffold sets in clinically relevant times without the need for toxic crosslinking agents. Interestingly, the hydrogel displays both physical and covalent interactions, making it ideal to fill bone defects and achieve intimate contact with surroundings tissues. Further mechanical characterization evidenced a highly elastic behaviour with breaking deformation higher than 100%, high fracture toughness and breaking stress. Its swelling ability (3000% in PBS) and degradation kinetics was found to be concentration-dependant. Permeability to oxygen and proteins diffusion was studied and evidenced as favourable for cell viability and tissue development. To provide a friendlier environment for osteoblastic cells adhesion, we then studied the addition of calcium phosphate granules or nanoparticles within the hydrogel. Results demonstrated a strong mechanical reinforcement, which is required for bone tissue engineering. In addition, cytocompatibility performed on osteoblastic cells demonstrated an excellent viability and good proliferative behaviour. Ongoing experiments will further study the differentiation of mesenchymal stem cells into the osteogenic lineage within the 3D scaffold. Later, *in vivo* studies will be conducted to study bone regeneration in rat cranial defects.

In parallel, using a “lego chemistry”, we are developing versatile extracellular matrices made from assemblies of silanized polymers. By varying the concentrations and polymers ratios, we are able to control their mechanical properties and degradation profiles. Preliminary results are highly encouraging and will be pushed further to develop new tissue engineeringapplications.

P521 Development of human liver extracellular matrix hydrogel for three dimensional cell culture and cell transplantation

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Background and Aims: Hepatocyte transplantation has been proposed as an alternative to orthotopic liver transplantation for treating end-stage liver diseases, however this method is limited due the poor cells engraftment into the liver. Furthermore, in vitro culture of hepatocytes is associated with de-differentiation and consequently decreased hepatocyte-specific function. Therefore, the development of human extracellular matrix (ECM) hydrogel, with retained ECM protein composition and 3D-architecture, could provide a physiological microenvironment able to promote in vitro survival and proliferation of hepatocytes.

Methods: Whole human livers unsuitable for transplantation were decellularised by retrograde perfusion, lyophilized and comminuted to a particulate form. Next, several protocols were employed to gel the ECM powder solution. Resultant ECM-hydrogels were characterized by Scanning Electron Microscope (SEM) and rheological measurement. Lastly, 0.5 million of human hepatoblastoma cell line (HepG2) were seeded on ECM hydrogels (n=10) and cells were cultured up to 10 days.

Results: The development of human liver ECM-hydrogel was achieved only by introducing our novel solubilisation and gelification steps. These steps were characterized by: i) solubilisation in protease free solution and ii) implementation of gelling polymers such as agar and agarose. Three-dimensional ultrastructure and porosity of ECM hydrogels have been confirmed by SEM. Rheological measurements have confirmed preservation of biomechanical properties after ECM gelification with stiffness values similar to human healthy liver. HepG2 cells seeded on ECM hydrogel were highly functional as confirmed by efficient and prolonged albumin secretion, while this was absent for the cells seeded on agarose hydrogels without ECM. Experiments are ongoing to compare HepG2 cell viability and protein/gene expression between ECM-hydrogels and 3D human liver scaffolds.

Conclusions: We introduced a novel protocol to develop human ECM-hydrogel starting from unsuitable organs for transplantation. Hydrogels are characterized by stiffness similar to that of normal human liver and they are able to provide an optimal 3D microenvironment for the growth of bio-engineered HepG2 cells which showed high metabolic functionality. Overall, these findings open new perspective for three-dimensional cell culture to be employed for in vitro disease modelling and in vivo cell transplantation.

P522 Self-assembled peptide gels for intervertebral disc tissue engineering

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Lower back pain represents significant cost to healthcare organisations and the economy. A number of factors are known to contrinbute to the problem, with intervertebral disc (IVD) degeneration being a major one. Tissue engineering is being considered as an alternative to current treatments which are not ideal and can address either the nucleus pulposus, the annulus fibrosus or both/whole disc. Hydrogels are being widely investigated by many researchers worldwide as they may offer a suitable microenvironment for the nucleus pulposus (NP) cells in particular, mimicking its natural gel-like structure. Self-assembling peptide hydrogels have been shown to be tunable in terms of various properties and have a nanofibrous architecture similar to that of many types of extracellular matrix and hence are being investigated for a variety of tissue engineering applications. This study investigates the potential of an FEFEFKFK peptide hydrogel for supporting NP cells and mesenchymal stem cell (MSC) responses. The gels were able to support the production of typical extracellular matrix components and their gene expression (as shown by fluorescence immunocytochemistry and qPCR), such as collagen II and aggrecan, by bovine NP cells, suggesting an appropriate microenvironment for this cell type. MSCs were also able to produce key extracellular matrix molecules, and the gels were able to support the gene expression of some novel NP markers, importantly without the addition of exogenous growth factors. This study therefore suggests these self-assembled peptide hydrogels provide a suitable 3D environment for NP cells and the differentiatipn of MSCs toward an NP-like phenotype, showing potential for IVD tissue engineering.

P524 Chitosan coated µicrocarriers fabricated via electrofluidodinamic atomization f or oral delivery applications

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One of main challenge in oral delivery currently consists in to the control of drugs or molecular release by the design of tailor made capsules able to deliver selectively therapeutic agents in the intestine, preserving the gastric stroke. For this purpose, drug-loaded carriers have to accurately designed to protect the active principle from the harsh environment of the stomach and, then, delivering it where the drug action or absorption is desired. Here, we have optimized a core-shell system – a core of cellulose acetate coated by a thin chitosan film - by a two step processbased on electrofluidodynamic technologies (EFDTs) and layer-by-layer (LbL) technique. EFDTs are emerging methodologies based on liquid atomization induced by electrical forces to obtain a fine suspension of particles from hundreds of micrometers down to nanometer size, for a wide variety of applications in biomedical and pharmaceutical field [1]. Due to their simple preparation process and cost-effectiveness, EFDTs may be integrated with layer by layer processes to better control the diffusion mechanisms through hydrophilic core matrix, thus providing a delayed release of drug in order to assure the right dose to the target.

In this work, morphological investigations by optical/electronic microscopy and selective staining tests allowed to evaluate main features of capsules (i.e., size, shape) and chitosan coating (i.e., thickness, homogeneity). These peculiar properties have been critically analysed to design chitosan coating with controlled dissolution to assure a delayed release just after the capsule transit in gastric compartment. Ketoprofen lysine salt kindly supplied by Dompè (Italy) was used to validate in vitro release in simulated gastric and Intestin fluids (SGF, SIF) and/or in the presence of physiological enzymes. All the results showed a promising behaviour of multi-layered capsules for oral delivery applications, showing a delay in the release time up to 3 hours – i.e., the drug should pass the in vivo gastric tract prior the complete dissolution of the external layer - followed by a sustained release of about 6 hours of the drug from the inner core ofcapsules.

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P525 Dense collagen/PLGA composite hydrogels as medicated wound dressings for the treatment of cutaneous chronic wounds: In vitro and in vivo evaluation

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Cutaneous chronic wounds are characterized by the absence of healing after six weeks. The classic treatment is the debridement of the wound bed followed by a compression method. When the treatment is not efficient enough, the application of wound dressings is required. To date, no dressings are appropriated to treat the different kinds and stages of wounds. Nowadays, research orientation is towards medicated wound dressings incorporating therapeutic molecules within biomaterials in order to favor skin repair or to prevent infection. Among drugs, spironolactone, an antagonist of mineralocorticoid receptors has been recently used to promote wound healing.

In this study, dense collagen/PLGA composite hydrogels have been developed to deliver spironolactone to the wound site in a sustained manner over a long period of time. To assess composite hydrogels as medicated wound dressing, hydrogel stability, drug loading and release kinetic of spironolactone have been analyzed.

Dense fibrillar collagen hydrogels concentrated at 40 mg/mL were incubated in a PLGA solution (7 KDa) containing spironolactone for 24 hours. Then the mixtures were rinsed in PBS to closely associate the hydrophobic polymer with the collagen network. This procedure permitted to obtain composites with high mechanical properties and an improved resistance against in vitro degradation by collagenase. The elastic modulus measured in composites was two times higher than that measured in pure collagen hydrogels. After freeze-drying and rehydration, the composite hydrogels swelled up to 10 times their dried weight and recovered their original shape. The ultrastructural analysis by transmission electronic microscopy revealed the presence of PLGA domains (around 500 nm in diameter) scattered within the network of collagen fibrils. Compared to pure collagen hydrogels, the drug loading in composites was 5 times higher and the drug release rate was quasi constant over the first two weeks. Unlike pure collagen hydrogels, no burst release was detected. The released spironolactone from composites retained its activity, thereby evidencing the absence of degradation during the composite synthesis. Cell viability, evaluated by Alamar Blue, showed the absence of cytotoxic effect of composites hydrogels on fibroblasts and keratinocytes. Subsequently, the effect of PLGA/collagen composite hydrogels was evaluated in vivo in a model of impaired wound healing in mouse. Applied on cutaneous wounds, spironolactone loaded composite hydrogels improved wound closure by 50% and permitted a complete re-epithelialization after 9 days.

Taken together, these results show that dense collagen/PLGA composite hydrogels are promising medicated wound dressing for the treatment of chronic wounds as they deliver constant doses of spirolactone and promote skin repair in vivo.

P526 Click-crosslinkable collagen hydrogels for skeletal muscle tissue engineering

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Despite the robust capability of skeletal muscles to regenerate, complete loss of muscle functionality can occur. A suitable biomaterial can be used as a template to guide skeletal muscle tissue repair. In this research, we assessed collagen hydrogels as the scaffold material to promote matrix formation to overcome this clinical problem.

The objectives were to develop a novel cross-linking strategy which required a smaller quantity of initiator than traditional photopolymerizable collagen hydrogels, with the aim to prevent toxicity towards the cells. This strategy was implemented to produce collagen hydrogels with varied mechanical and physical properties to quantify growth and maturation of C2C12 cells to assess the optimal mechanical and physical properties for muscle regeneration.

Collagen was thiolated by a substitution reaction using 2-iminothiolane hydrochloride (Traut's reagent) at pH 7.4 without the need of an additional base. A collagen hydrogel was produced by means of a thiol-ene photo-click reaction of the thiolated collagen and poly(ethylene glycol) norbornene (20,000 $g.mol^{-1}$). Click chemistry is a novel cross-linking strategy when applied to collagen with the added advantage of requiring a smaller amount of initiator compared to traditional photopolymerization.

Three of the novel gels (G' ~ 3 - 6.2 kPa) and a commercial gel (PhotoCol®) (the control) were used to compare the growth and maturation of C2C12 from myoblasts to myotubes. Cell adhesion, spreading, proliferation and viability were analysed. Myotube fusion index and immunohistochemistry at 3 and 7 days were performed to explore muscle tissue formation.

Collagen functionalization showed that 80% of the total lysine residues of the collagen molecule was thiolated with triple helical preservation of 90%. Gelation kinetics of the thiol-ene click reaction showed that no further elastic properties were produced after 5 minutes of low intensity light exposure. Varied amounts of the cross-linker produced gels with differing mechanical (G' ~ 3 - 6.2kPa) and physical properties (swelling ratio 1500 - 3000%). Good cell viability was observed at 1, 3 and 7 days and preferential muscle tissue formation and myotube alignment was observed on the novel gels compared to the control. The click-chemistry collagen hydrogels could hold great potential as new scaffold materials to promote matrix formation after loss of muscle functionality.

P527 Fabrication of injectable hydrogels by modifying carboxymethyl cellulose (CMC) for preventing undesired tissue adhesion

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The post-operative tissue adhesions cause pelvic pain, infertility, and intestinal obstruction. There are many types of anti-adhesion methods such as pharmaceutical adhesion prevention, adhesion prevention barrier. Hydrogel barriers for anti-adhesion have many advantages such as biodegradability, non-toxicity, non-preform, flexibility, high permeability of oxygen and nutrients. We developed the in situ injectable crosslinked-hydrogel fabricated by modified carboxymethyl cellulose (CMC) with tyramine and subsequent enzyme reaction using horseradish peroxidase and hydrogen peroxide (H_2O_2). The modified CMC with tyramine was confirmed by ATR-FTIR. The biodegradation behaviour was investigated by weight loss and scanning electron microscopy. In vivo animal test demonstrated dramatic decrease of post-operative tissue adhesion by applying in situ injectable hydrogel. The injectable hydrogel made of modified CMC has great potential for use as an anti-adhesion barrier.

P528 Modification of alginate to fabricate in situ injectable hydrogel for wound healing

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Alginate is one of the widely used natural materials because it has good solubility in water and biodegradable properties, as well as the excellent non-toxicity when injected into a human body. Alginate hydrogels can be easily formulated to carry drugs and/or cells by simple mixing followed by a chemical or physical curing process. Injectable hydrogels offer a wide range of attractive benefits, as an easy-to-use medical device for various applications, including drug delivery and tissue engineering. In this study, injectable alginate hydrogels were fabricated by modification of alginate with tyramine and subsequent enzymatic crosslinking reaction. Their gelation, mechanical and degradation properties were systemically investigated by varying the concentration of horseradish peroxidase and hydrogen peroxide. The modified alginate hydrogels were analyzed by ATR-FTIR, ^{1}H-NMR in order to confirm the introduction of tyramine. Rheological properties were also tested according to adjusting the amount of hydrogen peroxide, horseradish peroxidase and concentration of modified alginate solutions. The author investigated human adult fibroblasts behavior in Dulbeco's Modified Eagle's Medium with hydrogel and animal study in Sprague Dawley Rat for 14 days after implantation with modified hydrogel. The modified alginate hydrogels were successfully synthesized. The results for gelation time and mechanical strength revealed that both the rate and degree of cross-linking in alginate hydrogels were fairly well controlled by changing the hydrogen peroxide and horseradish peroxidase concentrations. In addition, the degradation rates were controllable by adjusting the degree of cross-linking. The injectable alginate hydrogels have sufficient value for biomedical applications.

P529 Construction of polyethylene glycol-based three-dimensional scaffold customized to the stimulation of human dermal fibroblasts proliferation

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In vivo, human skin is known to be a tissue organized systematically and three-dimensionally with dermal fibroblasts, melanocytes, and keratinocytes. Accordingly, three-dimensional (3D) culture of each cell constituting skin tissue has been requested strongly for constructing artificially *in-vivo*-like skin tissue and conducting precisely drug screening and toxicity test against skin in *in-vitro*. Unfortunately, to date, there are no 3D culture systems inducing effectively proliferation of each cell constituting skin. Therefore, as a first step toward constructing synthetic 3D microenvironments optimized to proliferate human dermal fibroblasts (HDFs) among a variety of skin cells, we tried to construct polyethylene glycol (PEG)-based 3D scaffold with mechanical strength stimulating proliferation of HDFs. The PEG-based 3D scaffold was formed by the conjugation of vinylsulfone-functionalized multi arm PEG (PEG-VS) and dicysteine-containing peptides with an intervening matix metalloproteinase (MMP)-specific cleavage site (herein referred to as crosslinker) through a Michael-type addition reaction, with the incorporation of monocysteine-containing adhesion peptides with RGDSP. Firstly, in order to determine MMP-specific cleavage site stimulating proliferation of HDFs in the hydrogels, the expression of *MMP1*, *MMP2*, *MMP3*, *MMP7*, and *MMP9* in HDFs was estimated using real-time PCR and significantly the highest expression was detected in *MMP1*, compared to the other MMP genes. Subsequently, the culture of HDFs for 14 days in 10% (w/v) 3-arm PEG-based hydrogels with crosslinkers containing MMP1- and multi MMP-specific cleavage site were conducted, respectively. As a result, the significant increase of cell proliferation was detected in HDFs cultured in 3-arm PEG- based hydrogels with crosslinker containing MMP1-specific cleavage site, compared to those with multi MMP-specific crosslinker. Moreover, in the culture of HDFs for 14 days in 10% (w/v) 3-, 4-, or 8-arm PEG-based hydrogels with crosslinker containing MMP1-specific cleavage site, mechanical strength derived from 3-arm PEG-based hydrogels showed significantly stronger stimulation of HDFs proliferation than those of 4- and 8-arm PEG-based hydrogels. Finally, HDFs were cultured for 14 days in 5, 7.5, 10 and 12.5% (w/v) 3-arm PEG-based hydrogels with crosslinker containing MMP1-specific cleavage site, respectively, and the proliferation of HDFs cultured in 7.5% (w/v) 3-arm PEG-based hydrogel was significantly increased, compared with the other PEG concentrations. From these results, we suggest that synthetic 3D scaffold derived from 7.5% (w/v) 3-arm PEG-based hydrogel with crosslinker containing MMP1-specific cleavage site can support effectively proliferation of HDFs and mechanical properties of 3D scaffold internal may be one of important factors in regulating physiology of HDFs.

Keywords: Three dimension, PEG-based hydrogel, Mechanics, Human dermal fibroblasts, Proliferation

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P530 Application of a modified pullulan hydrogel for injectable anti-adhesion agent

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Post-operative peritoneal adhesions are common and give serious complications for human. They can cause pelvic pain, infertility, and potentially lethal bowel obstruction. There are many requirements for polymeric material forms such as film, membrane and hydrogel type to be used as tissue adhesion barriers. They should include flexibility, non-tissue adhesiveness, biodegradability and non-toxicity in the body. Polysaccharides (pullulan) which are from various kinds of natural materials are useful for tissue engineering scaffolds due to their biocompatibility, biodegradability and non-toxicity. And hydrogel type biomaterials are able to provide the best possible conditions for cells. In this study, pullulan was modified with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to introduce carboxyl and phenyl groups as crosslinking sites. And modified pullulan hydrogel was fabricated using HRP (peroxidase from horseradish) for injectable anti-adhesion barrier. The modified pullulan hydrogel was characterized by several spectroscopic methods, in vitro and in vivo test. From in vivo animal test, it was observed that hydrogel were significantly effective in preventing post-operative adhesion and wound healing.

P531 Formation of capillary structures in in vitro trachea model

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Introduction: The replacement of pulmonary organs like trachea is a stiff challenge in clinic. The substitute must be functional, biocompatible, non-toxic, in best case from an autologous source as well as stable in shape and size. Current approaches show first achievements but still the treatment of pulmonary organ diseases is unsatisfactory. In this study, we present an *in vitro* organoid trachea model with a tri co-culture of endothelial cells, fibroblasts and respiratory epithelial cells in hydrogel blends made of collagen and agarose as well as fibrin hydrogels.

Material and Methods: Hydrogel blends were moulded with embedded endothelial cells from umbilical veins (HUVECs), nasal fibroblasts (HNFs) and respiratory cells from nasal conchae (HRECs). Scaffolds were based on fibrinogen on the one hand, polymerized by thrombin and calcium chloride addition to fibrin. Primary culture of HRECs was expanded within one week and seeded on hydrogels as primary culture to obtain best results. Pre-cultivation under submerged conditions was performed for 7d followed by air-liquid interface cultivation for additional 14 and 21d. Evaluation was performed by fluorescence –and two-photon laser scanning microscopy (TPLSM) of CD31, pan-cytokeratin, PAS and MUC5AC staining as well as electron microscopy visualizing cilia formation.

Results: Experiments revealed on the one hand capillary network formation in hydrogel gels. This was shown with three-dimensional stack images of the TPLSM and further quantification via Imaris. Additionally, the effect of HRECs on capillary network formation was investigated. Results showed stimulating effect on the capillary formation when seeding HRECs on the hydrogel surface. Cultivation period was set to 14 and 21d in air-liquid interface investigating whether cilia formation and differentiation occurs. 14d cultivated samples already showed differentiation of HRECs while 21d cultivation revealed ciliated epithelium on fibrinhydrogel.

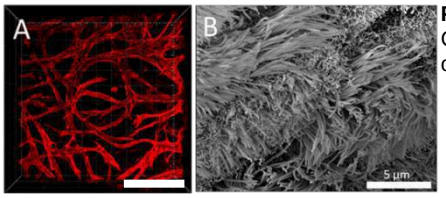

Figure 10 A: TPLSM image of capillary-like structures stained with CD31. Scale bar: 200 µm. **B:** scanning electron microscopy image of the respiratory epithelium. Scale bar: 5µm.

Discussion and Conclusion: This study showed successful combination of hydrogel based scaffolds supporting capillary network formation and ciliated epithelium. Based on these results further approaches in pulmonary tissue engineering can be achieved. The main focus can be put on the co-culture of more than two different cell types to achieve more functionality of tissue engineered constructs.

Acknowledgment:

We gratefully acknowledge the financial support of the Deutsche Forschungsgemeinschaft DFG (grant FI 975/23-1).

P532 Thermally triggered hydrogel delivers mesenchymal stem cells and induces differentiation towards NP phenotype and restores mechanical function following injection into bovine intervertebral disc

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AIMS: We have previously reported the development of a synthetic Laponite® crosslinked pNIPAM-co-DMAc (NPgel) hydrogel delivery system which has the potential to deliver mesenchymal stem cells (MSCs), via small bore needles (26G) which decrease the chance of inducing damage to the annulus fibrosus[1]. hMSCs incorporated into NPgel and cultured in 5% O_2, induces differentiation into nucleus pulposus (NP)-like cells without the use of additional growth factors[1]. However, clinical success of NPgel is dependent on the capacity to restore mechanical function to the IVD, ability to integrate with surrounding tissue to prevent extrusion and supporting viability and differentiation of incorporated MSCs into the correct cell phenotype following injection into the disc. Here, we investigated several IVD repair strategies following injection into bovine NP tissue explants, including: MSCs alone, acellular NPgel or hMSCs incorporated within NPgel. This study tested the hypothesis that the delivery of hMSCs within the NPgel would aid scaffold integration and promote differentiation of MSCs towards the correct NP cell phenotype within native NP tissue. In addition the ability of NPgel to restore mechanical function following injection into digested bovine IVDs was investigated. Together these data ascertain the capacity of this hydrogel to be used both as a cell delivery vehicle and provide mechanical support in treatment of IVD degeneration.

METHODS: Bovine NP tissue explants[2] were injected with media (control), hMSCs alone, acellular NPgel or hMSC incorporated within NPgel and maintained at 5% O_2 for up to 6wks. Injected MSCs were stained with CFSE stain for identification following injection. Cell viability was assessed by Caspase 3 immunohistochemistry (IHC). Histological assessment of the matrix was performed, alongside IHC for aggrecan, chondroitin sulphate and collagen type II to assess phenotypic characteristics of both native NP cells and injected MSCs. The hydration degree of NP tissue explants and mechanical characterisation, was performed following 6 weeks in culture. In addition NPgel was injected into collagenase digested bovine caudal discs and mechanical analysis performed to assess the injectability of the biomaterial and to ascertain the capacity of the NPgel to restore mechanical function of the degenerate IVD. All data (performed in at least triplicate) was found to be non-parametric and hence statistical comparisons were performed by Kruskal-Wallis with a pairwise comparisons (Conover-Inman) post hoc test performed ($p \leq 0.05$).

RESULTS: CFSE positive MSCs were identified in all NP tissue explants where MSCs had been injected either alone or incorporated within the NPgel, throughout the 6 week culture duration. Cell viability was maintained in all explants throughout the 6 week culture duration. Where hMSCs were delivered via NPgel, the hydrogel integrated with native NP tissue and cells were shown to be producing NP matrix components: aggrecan; collagen type II and chondroitin sulphate. No significant difference in the mechanical properties was observed between NPgel injected NP tissue and media injected controls following 6 weeks in culture. The hydration degree of NP tissue explants injected with acellular NPgel or hMSC incorporated within NP gel was significantly higher than NP tissue explants injected with media or MSCs alone following 6 weeks in culture. Finally hydrogels injected into collagenase digested bovine discs, demonstrated rapid solidification, filled micro and macro fissures, were maintained within the disc during loading and restored young's modulus back to levels of non-degenerate bovine IVDs.

IMPACT: Here, we have demonstrated that hMSC incorporated within NPgel and injected into NP explants, integrate with native NP tissue and promote differentiation towards the NP phenotype. Moreover we have demonstrated that the NPgel can be delivered by minimally invasive injection, maintained within the IVD during mechanical loading and restore the mechanical function of degenerate discs; thus could be used both as a cell delivery scaffold whilst providing mechanical support as a treatment strategy for IVD degeneration. The use of a combined cellular and mechanical repair approach, through the use of the NPgel developed here, is particularly promising since it is hypothesised that the mechanical support of the NPgel, to restore disc height, would provide immediate symptomatic pain relief, whilst the delivery of MSCs targets the underlying pathogenesis of IVD degeneration and would provide a long term, gradual regeneration of an extracellular matrix which biologically functions akin to native NP tissue as a treatment strategy for LBP.

ACKNOWLEDGEMENTS: Biomolecular Sciences Research Centre, Materials and Engineering Research Centre, Sheffield Hallam University and School of Engineering, Manchester Metropoilitan University for funding.

P533 Effective treatment of atopic dermatitis using PVA/Alginate hydrogel containing extract of houttuynia cordata

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that usually begins in infants or childhood, characterized by intractable pruritus, xeroderma, and relapsing eczematous skin lesions. For the treatment of AD, a variety of products are being developed such as creams, lotions, salves and sprays. However, the majority of the product is rapidly disappeared within 15 minutes owing to permeation and evaporation from the skin. In this study, we prepared Houttuynia cordata (HC; extract used as an anti-inflammatory agent for treating AD in traditional Korean herbal medicine)-loaded polyvinyl alcohol (PVA) hydrogel embedded in mildly cross-linked alginate (PVA/mcALG hydrogel). The hydrogel can provide a moisturizing effect when applied on the skin, and form a film after skin coating and thus is easily detachable. We evaluated the anti- AD effect of HC-loaded PVA/mcALG hydrogel for 4 weeks using a dinitrochlorobenzene (DNCB)- induced BALB/c mice model. The ear thickness and scratching behavior using hindlimb were investigated once a week. After 4 weeks of the treatment (on the final day of the experiment), level of immunoglobulin E (IgE) in serum was determined by ELISA. Also, skin and spleen samples from each mouse were collected and skin samples stained with hematoxylin and eosin (H&E) and toluidine blue. And then, epidermal thickness and the number of mast cells in the skin layer of mice were measured. The HC-loaded PVA/mcALG hydrogel treatment reduced ear thickness, scratching behavior, IgE levels, epidermal thickness, and the number of mast cells as well as spleen size. From the results, we could suggest that the HC-loaded PVA/mcALG hydrogel may be a promising effective therapeutic system for the treatment of AD.

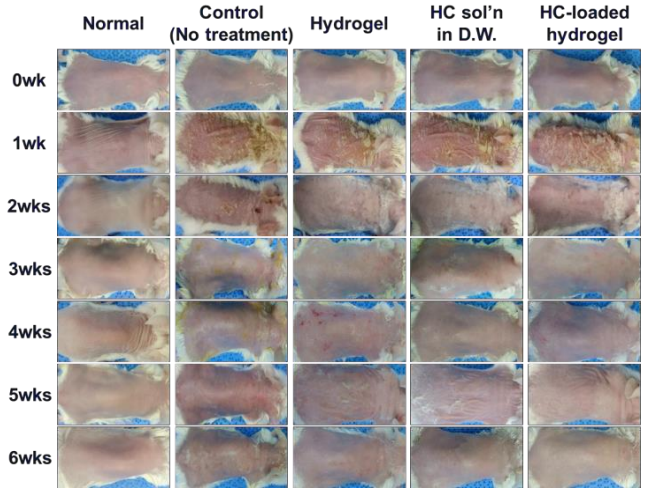

Figure 1. Photographs of preclinical features of the AD treatment.

P535 Collagen/poly(ethylene glycol) diacrylate cryogels prepared by UV irradiation: tunable macroporous structures for tissue engineering applications

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UV irradiation of acrylate-based precursors in water solutions is a fast and versatile method to obtain hydrogels with well-defined crosslink densities and mechanical properties, that show potential for cell encapsulation and tissue engineering. The formation of an interconnected, macroporous network within the hydrogel is fundamental to cell-material interactions and can be achieved by combining the UV crosslinking with several pore-forming strategies. In this work, macroporous poly(ethylene glycol) diacrylate (PEGDA)–based cryogels were synthesized by UV irradiation of frozen precursor solutions. PEGDA was chosen due to its peculiar non-fouling character, which allows the engineering of substrates with tunable bioactivity. In this regard, several cryogel formulations were prepared by adding different amounts of Type I collagen to the PEGDA solutions, with collagen providing extracellular matrix (ECM) mimicry. PEGDA solutions (700 Da, 10% w/v), containing VA-086 as a photoinitiator, were frozen under controlled freezing conditions (-20°C, with a freezing rate of -1°C/min) in a freeze-dryer (Virtis Advantage). After 1 hour at -20°C, the frozen samples were exposed to UV light (365 nm) for 3 minutes and finally swollen in distilled water for the removal of unreacted precursors. Cryogels containing collagen were synthesized as described above, by adding the appropriate amount of collagen (0.1 or 1% w/v) to the starting PEGDA solution. The gelation yield, the swelling ratio, the degradation rate and the mechanical properties of the cryogels were evaluated. Morphological analyses were also performed on both swollen and dry cryogels by means of fluorescence microscopy and/or micro-computed tomography, to analyse the effect of collagen on the cryogelation-induced porous structure. The experimental findings showed that the gelation yield was slightly increased for higher collagen concentrations, while the swelling ratio, the compressive elastic modulus of the cryogels and the weight loss after 28 days in PBS at 37°C seemed to be independent of the collagen amount. The morphological analysis demonstrated that a macroporous structure, with well-interconnected pores and robust pore walls, was formed for each cryogel formulation, and notably the pore size and pore size distribution were found to progressively decrease and get narrow as the collagen concentration was increased (Fig. 1). Preliminary cell seeding studies with 3T3 fibroblasts also showed the cytocompatibility of the developed cryogels, which thus hold promise for the development of 3D stiff microenvironments with tunable pore sizes, suitable for in vitro tissue engineering.

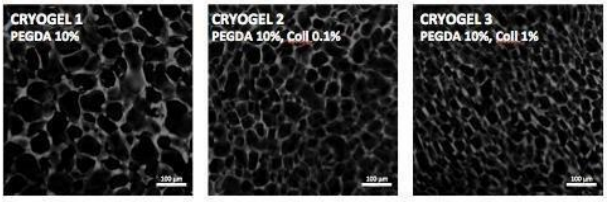

Fig.1. Effect of collagen concentration on the porous cryogel structure.

P537 Mechanical properties of hydrated free-standing films from modified hyaluronan measured by nanoindentation

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[1]*Contipro a.s., Dolni Dobrouc, Czech Republic,* [2]*Anton Paar TriTec SA, Peseux, Switzerland*

Introduction: Free-standing films of hydrophobized hyaluronic acid (HA) are developed as temporary barriers for prevention of tissue adhesion after surgery. Evaluation of mechanical properties of films in the wet state is important for assessing their *in vivo* behaviour. Tensile testing is problematic due to difficult mounting of the soft and thin film and considerable sample consumption. However, nanoindentation methods can measure elastic modulus down to a few kPa and require relatively small samples. Monitoring of the film properties during its *in vitro* degradation is also possible. Here we present results of nanoindentation testing of films from HA with varying degree of substitution by lauric or palmitic acids (acyl chain lengths C_{14} or C_{16}, respectively).

Methods: Clear films of HA derivatives about 20□m thick were prepared by solution casting. Their swelling ratios were evaluated after 24 h immersion in phosphate-buffered saline (PBS). Elastic modulus of film immersed in PBS was measured with Bioindenter (Anton Paar). The instrument was equipped with a 1 mm diameter ruby ball probe.

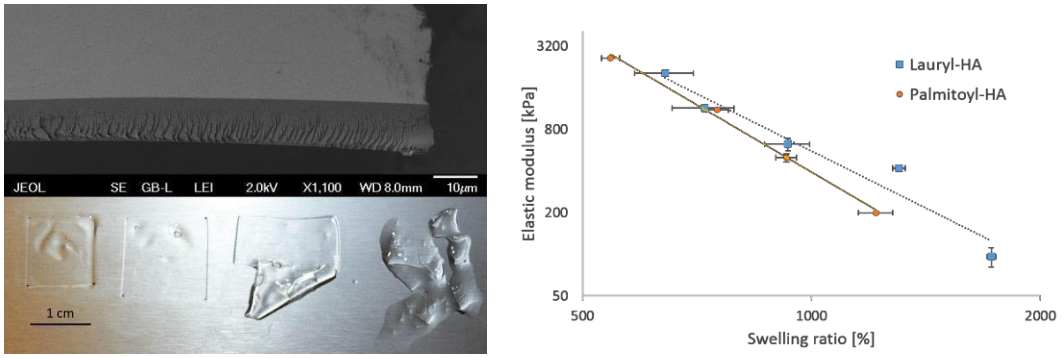

Fig.1: Cryo-SEM image of the palmitoyl-HA film and film samples swollen to different degree. The graph (both axes in log scale) shows a power dependence between swelling ratio and elastic modulus obtained by nanoindentation for lauroyl- and palmitoyl-derivatives of hyaluronic acid.

Results: Elastic moduli of films correlate with their swelling ratios while the effect of acyl chain length is only minor (Fig. 1). Nanoindentation testing provided reliable data with standard deviation of about 5 %.

Conclusions: Mechanical properties of free-standing biopolymer HA films can be reliably measured in the wet state by nanoindentation. As the film swelling ratios depend on the degree of HA substitution by acyl chains, the elastic modulus of the films can be fine-tuned for the desired application

P538 Fabrication of Poly(vinyl alcohol) hydrogel blended with red sea cucumber enhancing cell proliferation of neonatal normal dermal human fibroblast

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When skin wound occur, hydrogel patches have been generally used as wound dressing for wound healing. Poly (vinyl alcohol) (PVA) is one of the commonplace hydrogel patch's materials which have non-toxicity, non-carcinogenicity, biocompatibility and easy processing. Red sea cucumber (RSC) is widely found in Jeju Island in Korea and It has cell proliferation on neonatal normal human dermal fibroblast (NHDF). So, we designed to blend PVA hydrogel patches with RSC and fabricated without chemical crosslink by using freezing/thawing method to make more effective PVA hydrogel-patches on wound healing. Then, we evaluated swelling property in water and mechanical property to know hydrogel patch's mechanical characterization and intermolecular interactions of hydrogel affected by RSC were elucidated using FTIR and cell proliferation was measured by MTT assay. As a result, Hydrogel patches blended with RSC observed significant increase of cell growth without cytotoxicity. In addition, hydrogel indicated a high water absorption and mechanical properties are suitable for wound dressing. Although further studies, including in vivo and signalling pathway for wound healing, will be studied, the comprehensive results suggest that RSC hydrogel patches are promising candidates for wound dressing and tissue engineering applications.

P539 Introduction of strain-stiffening features into elastin-like recombinamers by polyisocyanate hybridization

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INTRODUCTION: Stress-stiffening is a feature displayed in cytoskeletal proteins as a mechanic response, which is rarely found in synthetic polymers and recombinant proteins. One way to overcome the absence of material characteristics is the formation of hybrid systems, e.g. natural hybrids (like wood, bone) are ubiquitous and synergize the beneficial properties of multiple materials into a superior matrix. In this work we covalently-cross-linked elastin-like recombinamers (ELRs), which have shown to form biocompatible and cell adhesive hydrogels [1], with polyisocyanopeptides (PICs), a synthetic polymer that stress-stiffens and is able to form thermoresponsive hydrogels at very low concentrations. [2]

METHODS: ELRs were derived by recombinant bioproduction and further modified with functional cyclooctyne or azide groups. [1] The PIC polymerization was carried out with Ni(ClO4)22H2O as catalyst in toluene at room temperature according to Kouwer et al.[2]. The products were routinely characterized with IR and circular dichroism spectroscopies, and AFM and NMR. Rheology samples were dissolved in (cold) demineralized water. Measurements were carried out in parallel plate geometry with heating/cooling rates of 2 ºC min. Nonlinear rheology in the gel phase was carried out using a pre-stress protocol [3]. For scanning electron microscopy swollen samples were frozen from 37ºC in liquid nitrogen and cut before, freezedrying.

RESULTS: The covalently cross-linked PIC-ELR hybrids are able to form hydrogels that are stable below their transition temperature, additionally the hybridization allows for lower ELR concentrations, without impairing the integrity of the hydrogel. Moreover, at low ELR concentrations a stress-stiffening of the hybrid gel was observed, with temperature and concentration dependent hysteresis. On the other hand, high concentrations of ELRs (>40 mg/mL) led to pure ELR behaviour.

DISCUSSION & CONCLUSIONS: The introduction of PIC and the simultaneous reduction of the ELR concentration, enabled ELR hybrids that show a strain-stiffening. In this hybrid system the properties of the low concentrated material are superimposed by the higher concentrated one, and the ratio at which both materials' characteristics are present is very narrow.

In conclusion, we were able to create a hybrid system with extraordinary mechanical properties, with a high potential for tissue engineering applications, caused by the biocompatibility of the ELRs.

P540 Strategies to improve the mechanical properties of fibrin scaffolds for tissue engineering of skin

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Tissue-engineered skin has been widely used in clinical for the last 30 years; however, many of those advances are inaccessible for low-income patients. To reduce costs, we aimed for developing a scaffold using fibrin, a raw material both cheap and readily available obtained from human plasma. It has been widely mentioned that fibrin can be used as scaffold for tissue engineering, yet there are few reports who employ directly human plasma to create scaffolds; one of the most important limitations of using human plasma to make fibrin gels is the low mechanical stiffness obtained, limiting its use in surgical applications. In this work, three strategies to improve the mechanical properties of fibrin gels from human plasma (HP) were evaluated: the first one involved increasing fibrinogen concentration by using human plasma cryoconcentrated (HPCC); the second one was mix together HP with alginate, a biomaterial widely used in tissue engineering with better elastic behavior; the third was to combine the previous two strategies.

The rheological behavior of gels formed by using the three alternatives was evaluated. As expected, the gel obtained by using only HP is weak and difficult to handle; however, when fibrinogen concentration is increased 10 folds, the elastic behavior improves considerably (elastic modulus ~ 10 kPa) and it is possible to manipulate the gels with surgical instruments without breaking them. Similarly, when HP is mixed with sodium alginate, the elastic modulus increases up to values very close to HPCC gels. Finally, when HPCC and sodium alginate were mixed together, the gel´s mechanical characteristics were slightly reduced compared to those of HPCC gels.

None of the three gel types was cytotoxic for a murine fibroblastic cell line (L929) as evaluated by using MTT per ISO-10993-5. Results also confirmed cell adhesion of the same cell line in all three cases. Current efforts are focused on evaluating gel stability on physiologic conditions.

P545 3D culture of adult stem cells in heparin-HA hydrogel

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A hydrogel system composed of heparin and hyaluronic acid (Hep-HA) was prepared by photopolymerization of thiolated heparin and methacrylated hyaluronic acid. Human adipose derived mesenchymal stem cells (hADSCs) were encapsulated and cultured using this hydrogel. hADSCs well were spread, proliferated, migrated well inside the hydrogel, whereas cells did not show similar cellular activities when control hydrogels composed of heparin and PEG or PEG and hyaluronic acid were used. Similarly, the expression of hyaluronidases and adipogenic differentiation of cells in an induction media were achieved efficiently only in Hep-HA hydrogel, compared to the control hydrogels. Thus, both heparin and hyaluronic acid moieties were necessary for 3D culture of hADSCs without additional modification of hydrogel.

P548 Hydrazone cross-lined injectable gelatin-hyaluronic acid hydrogels for regenerative medicine

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Introduction: Hyaluronic acid (HA) based hydrazone crosslinking injectable hydrogels have been used as artificial extracellular matrix (ECM) for bone regeneration owing to its simplicity and versatility [1]. However, HA is a nonadhesive polymer, thus HA hydrogels are usually combined with cell adhesive polymers, such as gelatin to improve cell adhesion capacity [2]. A new carbonyldihydrazide (CDH) modified gelatin was developed for the preparation of ECM mimic HA–gelatin hydrogels for regenerative medicine applications.

METHODS: Aldehyde modified hyaluronic acid was prepared following published protocol [3]. CDH modified gelatin was prepared following standard EDC coupling in presence of N - hydroxybenzotriazole (HOBt). 10 fold excess of dihydrazides (with respect to the carbodiimide) was used to promote monosubstitution. Degree of hydrazide modification was determined using trinitrobenzenesulphonic acid assay. Hydrogel was developed by mixing HA aldehyde (6-8 % modification) and gelatin hydrazide solutions with solid content of 16 mg/ml with a volume ratio of 1:1.

RESULTS & DISCUSSION: Carboxyl group of gelatin was partially modified with carbonyldihydrazide and degree of substitution can be easily tuned by alternating amount of carbodiimide. When using 0.5 mmol EDC per gram of gelatin, concentration of substituted hydrazide was 0.15 mmol/g. Hydrazide concentration increased to 0.23 mmol/g when EDC amount was 1.5 mmol/g.

Gelation starts within 1 minute after mixing and finished after 24 hours at room temperature. Frequency sweep was performed with a frequency between 0.1-10 Hz. The storage moduli(G') were determined to be 470 Pa, and reduced to 340 Pa after 24 hours swelling in 10 mM PBS (pH7.4). The gel is stable at physiological condition. After 6 months incubation at physiological conditions the hydrogel maintained shape.

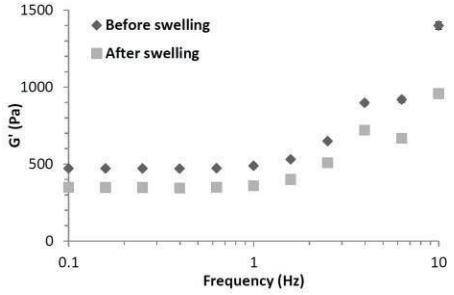

Figure 1. Frequency sweep of HA-gelatin hydrogel before and after 24 hours swelling.

P549 Discover bio-mimetic hydrogels for stem cell culture

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Various types of cells, especially stem cells, respond to an array of signals from their environment. Current *in vitro* cell culture methods recapitulate selected parameters; however, the complex interplay of matrix, soluble factors and cell-cell interactions is lacking. As a result, stem cell behaviour is altered through the process of cell culture.

In order to achieve higher biological relevance and therefore stem cell behaviour that is closer to the *in vivo* milieu, the *in vitro* microenvironment should recapitulate key parameters. By nature, these parameters vary between different stem cells, and include adhesion molecules, soluble effectors as well as interaction with other cells. The challenge is to identify the optimal combination of these parameters, in order to create biomaterials for individual celltypes.

We have developed a non-covalent modular hydrogel for creating biomimetic matrices that can incorporate a large variety of key parameters for stem cell culture. We have developed an array of biomimetic hydrogel matrices consisting of glycosaminoglycans and different concentrations of the well-known adhesion peptide sequence RGDSP. We demonstrated the versatility of our biomimetic hydrogel by culturing human umbilical vein endothelial cells (HUVEC), mesenchymal stromal cells (MSC) and neural precursor cells (NPC). The array of biomimetic hydrogels promoted varying levels of proliferation and differentiation behaviour, indicating the necessity of tailored microenvironments for individual cell types. These results show that key parameters of stem cell microenvironments can be modulated by our biomimetic hydrogel arrays.

In contrast to many commercially available hydrogels, our biomatrices are modular, biologically relevant, and chemically defined. Moreover, they are easy-to-implement in various forms from surface coatings to cell-laden materials, due to their non-covalent nature. In conclusion, we provide a hydrogel platform technology, which enables reliable environmental conditions for the culture of primary stem cells.

P550 A synthetic HA-Fibrin hybrid proteoglycan for joint preservation and repair

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Procore Bio Med, Nes Ziona, Israel

We designed a novel synthetic (hybrid) proteoglycan mimic, combining viscous, high molecular weight HA with the regenerative, multifunctional fibrinogen to create a defined fibrinogen-HA co-polymer. Upon activation by thrombin and calcium, this conjugate further polymerizes to form a fibrin-HA based, highly stable, viscoelastic hydrogel named RegenoGel™. RegenoGel™, is a superior highly stable viscosupplement gel for treating OA with expected joint preservation and regenerative potential unclaimed by other currently available products. RegenoGel™ is based on ProCore's proprietary HAProLink™ technology, joining a high molecular weight HA with a regenerative Fibrinogen matrix. HAProLink™ is based on a proprietary Hyaluronic Acid (HA) chemistry that enables targeted, non-destructive conjugation to proteins of choice. This technology allows better control of the final product compared to standard HA-protein conjugation methods, eliminating the formation of undesired side products. RegenoGel™ was formed to provide an optimal 3D environment for cartilage surface regeneration.

To date, following marketing approval by the Israeli Ministry of Health, RegenoGel™ was administered to more than 50 patients suffering from mild to severe osteoarthritis. Patients treated with Regenogel report on almost immediate pain relief that is sustained over time in most cases for at least six months after a single injection.

With the aim of improving the mechanical properties of the hydrogel, a library of conjugates prepared using different formulations and compositions is currently being analyzed. Finding a correlation between the Fibrinogen:HA ratios in the conjugate and their mechanical properties is essential for the optimization and long term formation of the product.

P551 The role of Indian hedgehog and parathyroid hormone related protein in the intervertebral disc

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Low back pain has been related to intervertebral disc (IVD) degeneration. During early IVD degeneration, notochordal cells (NCs) are replaced by chondrocyte-like cells (CLCs) in the centre of the IVD, the nucleus pulposus (NP). Since dogs experience back pain and IVD degeneration with similar characteristics as humans, they are considered a suitable animal model for human IVD degeneration. Current treatments for human and canine IVD disease do not lead to repair, so there is need for treatments resulting into functional IVD restoration. To develop such agents, further knowledge of the pathogenesis of IVD degeneration and the pathways involved is required. Numerous pathways could play a role in IVD degeneration, e.g. Wnt/β-catenin and BMP signalling. However, some interesting pathways have not been studied yet, for instance the Parathyroid hormone-related protein (PTHrP) and Indian hedgehog (Ihh) pathway. Ihh and PTHrP form a growth-restraining feedback loop in endochondral ossification, in which PTHrP inhibits Ihh and Ihh stimulates PTHrP production. Previous work indicated a positive correlation betweenIhh expression and osteoarthritis, a process resembling IVD degeneration. Additionally, Ihh promoted chondrocyte hypertrophy and mineralization, while PTHrP suppressed these processes which also occur during IVD degeneration. For these reasons, this study aimed to determine the largely unexplored role of PTHrP and Ihh in the IVD.

PTHrP, PTHR1 (PTHrP receptor), Ihh, Ptc (Patched; Ihh receptor), and Smo (Smoothened; transmembrane protein that activates downstream hedgehog signalling) gene and protein expression was determined in different phases of IVD degeneration: in NC to CLC transition and in early-severely degenerated IVDs. For this purpose, NPs from early-severely degenerated canine and human IVDs were analysed.

Ihh, Ptch, Smo, PTHrP, and PTHR1 expression was detected in all IVD end plates. Ihh, Ptc, Smo, and PTHR1 expression decreased from healthy (Thompson grade I) IVDs towards early degenerated (Thompson grade II-III) IVDs. Additionally, the expression of Ihh, Ptc and PTHrP increased from early towards severely degenerated (Thompson grade IV-V) IVDs. PTHrP expression decreased with ageing in healthy (Thompson score I) canine, but not in healthy human IVDs. No clear spatial distribution expression pattern within the NP was detected for any of the studied proteins; expression was scattered throughout the NP in NC and CLC single cells and clusters (if present).

The results of the current study imply that Ihh and PTHrP signalling is active in the healthy and degenerated IVD. However, it remains to be determined what their role is in maintenance of the NC phenotype and degenerative/reparative processes within the ageing IVD, e.g. by studying Ihh/PTHrP signalling in calcified IVDs and by performing functional *in vitro* studies (ongoing work). Since Ihh and PTHrP signalling appear to play a role during IVD health and disease, targeting these signalling pathways may be a therapeutic approach to prevent or retard IVD degeneration in the future. However, the exact role of Ihh-PTHrP signalling in the IVD needs to be substantiated first.

P552 Biological responses of dental pulp stem cells in biomimetic chitosan-gelatin scaffolds in vitro and in vivo

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Introduction: Biodegradable scaffolds from naturally occurring chitosan-gelatin (Ch-Gel) blends have attracted great interest in tissue engineering of various tissues, such as bone, cartilage and peripheral nerves. The aim of this study was to evaluate the biological responses of dental pulp stem cells (DPSCs) seeded in Ch-Gel scaffolds in vitro and in vivo for targeted mineralized tissue (dentin/bone) regeneration.

Materials and Methods: Scaffolds were prepared at a composition of 40%-60% Ch-Gel with 0.1 wt glutaraldehyde as crosslinker. DPSC cultures were established from healthy donors and characterized for stem cell markers with flow cytometry. Scaffolds were seeded with DPSCs in Complete Culture Medium (=CCM: a-MEM with 15% FBS, 100 mM L-ascorbic and antibiotics/antimycotics) with or without previous single exposure to rhBMP-2 (100 ng/mL for 24 h) as additional morphogenetic trigger. Live/dead staining visualized by confocal microscopy was used for evaluation of cell viability; real time PCR for expression of osteo/odontogenic genes; scanning electron microscopy (SEM) and X-ray powder diffraction (XRD) for structural/chemical characterization of the regenerated tissues; finally, subcutaneous implantation of DPSC-seeded scaffolds on the back (dorsum) of immunocompromised mice (NOD/SCID) for assessment of ectopic mineralized tissue formation.

Results: Scaffolds with interconnective pores of average size 100 μm were prepared and supported cell adhesion, spreading and viability over time. Upregulation in expression of osteo/odontogenic genes, including alkaline phosphatase (ALP: 3.5-fold), bone morphogenetic protein 2 (BMP-2: 5.2-fold), dentin sialophosphoprotein (DSPP: 25.2-fold), bone sialoprotein (BSP: 36.1-fold), Osterix (36.4-fold) and bone gamma-carboxyglutamate protein (BGLAP: 5.2-fold) was observed. SEM images showed newly-formed tissue consisting of calcium and phosphorous generated inside the scaffolds, while XRD indicated a partial transformation of the amorphous calcium-rich phase to biological apatite. Analysis of in vivo specimens retrieved after 6 weeks from immunocompromised mice showed abundant, densely nucleated extracellular matrix covering the entire structures, with Alizarin-Red-positive mineralized areas that were more prominent in rhBMP-2 pre-treated structures.

Conclusions: Ch-Gel hybrid scaffolds supported attachment, viability, osteo/odontogenic differentiation and effective biomineralization by DPSCs in vitro and in vivo. These results provide a promising strategy for their application in targeted mineralized tissue (dentin/bone) regeneration.

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P553 Impact of gap-junctional intercellular communication on adipogenic differentiation of adipose-derived stem cells

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Introduction: The knowledge of tissue-inherent processes such as cell-cell interaction is of particular importance in tissue engineering in order to understand and drive tissue development. Direct intercellular communication between cells via Connexin 43 (Cx43)-generated gap junctions enables the functional coupling of cells and the exchange of ions, secondary messengers and metabolites, which has been shown to modulate differentiation processes, determination of stem cell fate and tissue homeostasis [1,2]. Adipose-derived stromal cells (ASC) play a pivotal role in adipose tissue engineering approaches for reconstructive surgery, however, little is known about the impact of gap junction-mediated communication in these cells. Thus, in this study the expression of gap junctions and the significance of gap junction-mediated cell-cell contacts in the adipogenic differentiation of ASC were investigated.

Methods: ASC were seeded with different densities (5.000, 25.000 and 100.000 cells/cm^2) and cultured in growth medium or adipogenic medium. Expression of Cx43 was characterized by qRT-PCR analysis, Western blot and immunohistochemical staining at different time points. Presence of functional gap junctions was evaluated by flow cytometry using a fluorescent dye-transfer assay. Inhibition of gap junctions was performed by addition of 18α-glycyrrhetinic acid (AGA). Adipogenesis was assessed by histological staining, analysis of cellular triglyceride content and gene expression analysis of adipogenic marker genes.

Results: Cx43 expression in ASC was demonstrated on mRNA and protein level and was shown to be greatly influenced by initial cell seeding density. Functionality of gap junctions was proven by successful dye transfer between cells as elucidated by FACS analysis. Adipogenic differentiation of ASC was shown to be dependent on cell-cell contacts. Differentiation rate was distinctly higher at higher seeding densities and inhibition of gap-junctional functionality by 18α-glycyrrhetinic acid (AGA) markedly compromised adipogenesis resulting in decreased lipid accumulation and reduced expression of adipogenic marker genes.

Conclusion: Altogether the results of this work demonstrate the relevance of direct cell-cell communication via gap junctions in the adipogenic differentiation of ASC and furthers our understanding of cellular cross-talk and interaction. This may aid in the more rational design of tissue engineering approaches in the future.

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P554 Enhanced delivery of magnetic nanoparticles by GET

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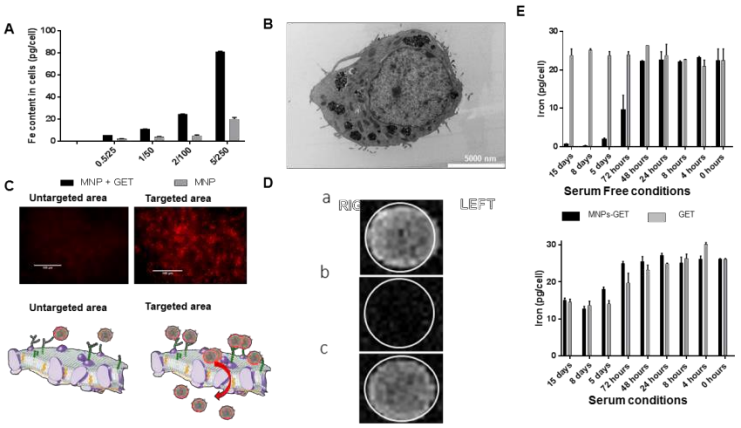
Magnetic nanoparticles (MNPs) are able to interact with a wide range of biological entities on a cellular and molecular level made possible by sub-cellular dimensions and tailorable properties. They are biocompatible with paramagnetic properties allowing for manipulation through a magnetic field opening a whole range of biomedical applications, including, non-contact manipulation, MRI tracking and remote cell activation. MNPs are a very promising technology for translation of cell based therapies. However to achieve their full potential, MNPs must be functionalized to ensure efficient and consistent uptake of the particles by the cells or relevant tissue.



GET (GAG-binding enhanced transduction) system is based on a novel fusion protein that couples a membrane docking peptide to heparan sulfate glycosaminoglycans (GAGs) with a Protein Transduction Domain (PTD) and has been reported to improve delivery of different cargoes into cells.

In this study we explore the use of GET to deliver MNPs. We have characterized and quantified delivery of GET-MNPs to cells *in vitro*. We have demonstrated targeted delivery potential. Finally, magnetically labelled MSCs using GET show significant hypointensity implying they GET-MNP enables efficient delivery of MNP to facilitate MRI imaging.

Figure 1. A) Iron uptake in NIH3t3s cells at different GET/MNPs concentration ratios (uM GET/ug MNPs). B) TEM imaging of MNPs in NIH 3t3s. C) Targeted delivery of a red fluorescent version of GET with MNPs under a static magnetic field. D) *In vitro MRI of cell seeded collagen gels. 10^5 hMSCs were labelled with 25ug MNPs using a) no transfection agent b) P21 and c) protamine sulphate and MR imaged using 2.3T animal scanner. Areas of hypointense (black regions) are indicative of MNP labelled cells..* E) Iron uptake in NIH3t3s after long term pre conjugation of GET-MNPs in the absence (left) and presence (right) of serum.

The combination of MNPs and GET system (GET-MNP) will provide a multifunctional system capable of: a) efficiently delivering therapeutics to cells b) providing good contrast for imaging purposes in vivo c) targeting the therapeutic/cells to a specific area of the body by applying a magnetic field.

P555 Influence of fibre diameter on the tissue reaction of wound dressings - preliminary results of a histological animal study

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Introduction

Natural and synthetic biomaterials are widely used in the treatment of wounds. They also play an increasing role in regenerative medicine for the repair of skin. However the interaction between the chosen biomaterials and the tissue at the implantation site is not fully understood. In particular, the effect of different fibre diameters of the used biomaterials on the induction of fibrosis and the formation of the foreign body reaction are not completely known.

In the present study we analysed the interaction between wound dressings with different fibre diameters in an animal model with respect to fibrosis and foreign body reaction.

Materials and Methods

For our experiments we used 16 explanted and formalin-fixed skin regions of 4 pigs in an animal study. The explantation occurred on day 25 after subcutaneous implantation of two types of semicrystalline polymers (LGTC 7030 & LG 9010) with different fibre diameters (LGTC 7030: 26µm, 68µm, 105µm, 176µm & LG 9010: 22µm, 23µm). From each region hematoxylin & eosin- as well as Goldner staining were performed from both the centre and the periphery of the implantation site. To analyse fibrosis, we made random 10 images of the region of interest of each slide at a hundredfold magnification. The fibrotic area of each image was measured with ImageJ. The foreign body reaction was evaluated in the HE-stained slides by counting multi-nucleated foreign body giant cells in the region of interest of 30 fields of view (400x magnification) under the microscope.

Results

No correlation could be detected between the fibre diameter within the different materials and the extent of tissue fibrosis. LG 9010 showed in the central region a tendency towards less fibrosis for the 23µm fibre diameter compared to the 22µm fibre diameter. Moreover, also in the evaluation of the foreign body reaction no statistically significant correlation could be found for the different fibre diameters. However, tendencies for a correlation between the fibre diameter and the tissue foreign body reaction were seen in the central region of LGTC 7030. Thus, there are less foreign body cells in specimens with thicker fibre diameters (105µm and 176µm) compared to the thinner fibres (68µm).

Discussion

Our preliminary results revealed no correlation between the extent of fibrosis or foreign body reaction and the fibre diameters of the different biomaterials. Future studies should address the issue, which further parameters have a significant influence on the reactions. The results regarding the described trends open interesting perspectives for our understanding of the fibrous and foreign body reaction. In ongoing studies the impact of the distribution of cells and fibres within the three-dimensional context is being analysed.

P556 A pulmonary heart valve engineered from natural biomaterials

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For paediatric patients, a tissue engineered heart valve (HV) offers the potential of a prosthesis that can grow and remodel with the patient throughout their life; however many tissue engineering approaches have failed due to cell-mediated contraction, which prevents valve leaflets maintaining a tight seal over time. Having previously developed a dimensionally stable natural biomaterial composite of fibrin, collagen and glycosaminoglycan (FCG) [1] and upscaled this FCG scaffold into a HV shape, the aim of this study was to examine the effect of dynamic conditioning on tissue engineering of a functional pulmonary HV.

To fabricate the HV, smooth muscle/fibroblast cells extracted from the vein of human umbilical cords were encapsulated within a HV shaped FCG construct. The constructs were statically cultured for 7 days, followed by 14 days of dynamic conditioning in a custom-made perfusion bioreactor which mimicked the ventricular pumping action of the heart [2]. Valve functionality was evaluated using high speed video and ultrasound; histological staining was used to assess extra cellular matrix deposition by cells. Valve performance was measured in a custom built flow loop according to ISO 5840 (Cardiovascular implants- cardiac valve prostheses) and burst strength of the construct was recorded using custom built equipment.

The HV demonstrated excellent coaptation of valve leaflets following dynamic conditioning. Alignment of α-smooth muscle actin and deposited collagen III confirmed appropriate remodelling of the construct, with staining of fibronectin showing remodelling in process throughout the construct. This valve passed the acceptance criteria for regurgitation fraction, mean pressure difference and effective orifice area for pulmonary valves according to ISO 5840. Analysis of burst strength showed a significant two-fold increase in both HV leaflet and HV wall tissue.

This study is the first demonstration that a functional pulmonary HV that achieves ISO requirements can be engineered through dynamic conditioning of a natural biomaterial scaffold of fibrin, collagen and glycosaminoglycans. As well as therapeutic applications in HV repair, this approach can also be used as an *in vitro* platform for drug screening, device testing or analysis of vascular cell interactions and disease states.

P557 Expansion at "physiologic" hypoxia attenuates replicative senescence of adipose tissue-derived stromal cells

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Multipotent mesenchymal stem/stromal cells (MSCs) of adults are considered as a perspective tool in cell therapy and regenerative medicine. Unfortunately, autologous cell therapy does not always provide positive outcomes in elder donors, perhaps as a result of the alterations of stem cell compartments. Ex vivo expansion is an essential step of MSC proceeding tor cell therapy. Long-term cultivation leads to MSC replicative senscence and lost of quality. The mechanisms of stem and progenitor cell senescence and the factors engaged are investigated intensively. Modification of cell culture conditions is a promising approach to improve the quality of expanded cells. According to the current data, reduction in O_2 to the tissue-related level in cellular microenvironment ("physiologic" hypoxia) can affect the features of MSCs. Recently we have demonstrated an increase in the proliferative activity and CFU-F number of adipose tissue- derived stromal cells (ASCs), while, on the contrary, the adipogenic and osteogenic differentiation decelerated. Global microarray analysis of ASCs expanded at 5% O_2 revealed the differential gene expression of 558 genes in comparison with ASCs at 20% O_2. Wherein, the expression of genes involved in proliferation, cell metabolism and signaling was upregulated, while that of the ones responsible for the connective tissue matrix and intracellular cytoskeleton components decreased.

Here we elucidated the effects of tissue-related O_2 on ASCs functions in replicative senescence *in vitro* model. ASCs at ambient (20%) O_2 (12-21 passages) demonstrated an increased average cell size, granularity, reactive oxygen species level, including anion superoxide, lysosomal compartment activity, and IL-6 production compared to 5% O_2. Long-term expansion at both O_2 levels did not lead to immortalization that was evidenced by the low level of *hTERT* transcription and encoded enzyme activity. Decreased ASC viability and proliferation, as well as the change of more than ten senescence-associated gene expression were detected. Among them the upregulation of several genes was noted: *CCND1 SERPINE1, PAI1. CD44.* Downregulation of *CDKN1C, NOX4*, and *CREG1, ID1,* was determined. In long-term ASCs the expression of only 11 genes changed with 5% O_2, compared to 16 ones at 20% O_2. *CCND1, CD44, SERPINE1, NOX4, ID1*, and *CDKN1C* were upregulated as well as under 20% O_2, but lessintensively.

Expansion of ASCs at 5% O_2 resulted in an attenuation of the most of detected senescence-associated changes, which may be considered as *in vitro* cellular senescence deceleration. Thus, the O_2 level of in vitro milieu affects the cellular senescence events via the fed-back loop of mitochondria-ROS-mitochondria-lysosomes and can decelerate it. The data on the effect of oxygen level on cellular replicative senescence are definitely of interest to modern gerontology. At the same time, standard cell culture condition under atmospheric O_2 (20%) may be associated with an increase in intracellular disorders and are less favorable compared to the "physiologic "hypoxia (5% O_2).

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We concluded that the in vitro O_2 level affects the cellular senescence via the fed-back loop of mitochondria-ROS-mitochondria-lysosomes and can decelerate it .These data would be of considerable interest for researchers and clinicians working in the modern gerontology and regenerative medicine

P558 Understanding matrisomal cues to recreate a pro-regenerative intervertebral disc microenvironment

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Intervertebral disc (IVD) degeneration, which occurs with age, usually originates low back pain. Degeneration is accompanied by extracellular matrix (ECM) depletion causing nucleus pulpous (NP) extrusion and IVD destruction. Current therapeutic options are inadequate. Novel clinical solutions such as the decellularization of young healthy IVDs have been under investigation. However, a thorough study of ECM composition depending on age and developmental stage is needed to design and select the ideal scaffolds for IVD regeneration.

As so, iTRAQ LC-MS/MS profiling was performed on healthy bovine NP natural scaffolds from foetus, young and old animals to define the NP matrisome. The enrichment of Collagen XII and XIV in foetal NP natural scaffolds, Fibronectin and Prolargin in elder tissues and Collagen XI in young ones was independently validated.

We report the first matrisome database of NP natural scaffolds with varying age and developmental stage, which is key to unveil the molecular cues that should be integrated or avoided when developing the next-generation biomaterials. Either alone or in combination with cell-based therapies currently under study, these materials should aim to recover tissue homeostasis by recapitulating early developmental stage microenvironments (Figure 1).

Figure 1 – Ideal microenvironment for IVD regeneration

P559 Proof of concept study on preventing post-operative abdominal adhesion

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***Introduction**:* Post-operative abdominal adhesion (PAA) is a major complication leading to medical and economical problems. The concept of reperitonealization (adhesion-free healing of deperitonealized surfaces) has been attracting tremendous attention to prevent adhesions. However, the drawbacks associated with autologous graft limit its clinical application, especially for prophylactic use. Thus, regeneration of the serosal surface by providing a source of mesothelial progenitor cells via cell-therapy or tissue engineering is considered feasible to prevent PAA. The present study aims to assess the effect of above different strategies on PAA prevention by: an autologous peritoneal graft and a bi-layer cell sheet composed of fibroblasts (FB) and mesothelial cells (MC), in a pre-established rat model with standardized peritoneal lesions and typical morphometry of the adhesion.

***Materiel and Method**:* The standardized peritoneal lesion on the lateral abdominal wall was induced in adult male rat in an area of 0.5 x 1.0 cm by electrocoagulation and four interrupted sutures. Twenty rats with these lesions were randomized into one of three treated groups or non-treated group (control). Different treatments received by rats were as following: (1) autologous peritoneal graft with the side of mesothelial cells exposed to the abdominal cavity (Group MC); (2) autologous peritoneal graft with the side of subserosa containing fibroblasts exposed to the abdominal cavity (Group FB); (3) cell sheet consisting of autologous mesothelial cells and fibroblasts (Group CS). Fourteen days after the operations, abdominal adhesions of all rats were evaluated by macroscopic observation and histological assessment.

Results: Macroscopic observation revealed that in Group MC, there was no adhesion on the surface of autologous peritoneal graft covering the area of the lesion. In the other three groups, to the contrary, every rat obviously showed abdominal adhesion existed on the induced lesion. Histological assessment revealed that mesothelial cells were still present on the grafted lesion region of the rats in Group MC, but no mesothelial cell was observed in the samples from the rats in other groups.

***Conclusion**:* This study clearly demonstrated the proof of concept that mesothelial lining is paramount to PAA prevention. Providing exogenous MC source via cell sheet engineering could be a promising therapy, but still limited by the size or severity of damage area. The lack of supporting connective tissue matrix may explain such limit. Tissue engineering, via incorporating mesothelial cells in the biodegradable scaffolds, might provide a powerful prophylactic material for postoperative adhesion prevention.

P560 Dealing with healing after incisional bladder wounding

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Aim:

The process of wound healing is not as well characterized in inner organs, such as the urinary bladder, as in the skin**.** We hypothesize that increased knowledge in bladder wound healing is important in regenerative medicine and for an increased understanding of the healing process after surgical repair. We aimed at describing histological changes and timing of different events in normal bladder healing. To do this we performed standardized incisional wounds and surgical closure in a small animal model.

Methods:

A total of 88 Sprague-Dawley rats were used in the study. Half of the rats were wounded in the urinary bladder and half were sham operated with abdominal incision only. In the bladder-operated animals, a 1cm longitudinal midline incision was made in the dome of the bladder and closed again with running 7-0 non-absorbable mono-filament sutures. The animals were sacrificed at 10 different time points post operation and bladders were collected and fixed in 4% formalin. Bladder tissue histology was examined with H&E and immunoassays to quantify inflammatory cells, growth factors, blood vessels and cell proliferation. Collagen I and III content was examined with picrosirius and fast-green staining.

Results:

We observed all classical phases of the wound healing process; Inflammation day 0 to 5, characterized by CD68 (blood monocytes and tissue macrophages), followed by proliferation (ki67) day 2 to 8 and a decrease of inflammatory cells. At day 2, a higher proliferation index was found in the basal layer of the urothelium compared to controls. Two weeks post wounding we identified the maturation phase by the observation of a reduced amount of both inflammatory and proliferating cells and signals of collagen deposition. Four weeks after wounding both inflammation markers and proliferation rate of different cells in the tissue was similar to control samples.

Conclusions:

Healing of the incisional wound in a rodent urinary bladder includes all the classical phases of the wound healing process: inflammatory, proliferative and maturation. Differences included time and length of each phase when compared to the skin wound healing process in rats and humans. This knowledge could be essential for optimizing healing conditions in urogenital reconstructive surgery in healthy and diseased bladders. Further work will explore whether our findings can be extrapolated to humans.

P561 Low level light therapy stimulates wound healing in a diabetic mouse model in a wavelength dependent manner

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Low level light therapy (LLLT) has been shown to positively modulate wound healing processes. So far, most studies are being performed with lasers in the red to near-infrared spectra. Recently, we showed that also shorter wavelengths can significantly influence biological systems such as nitric oxide (NO) metabolism. Therefore, the aim of this study was to evaluate and compare the therapeutic effects of pulsed REPULS-LED light at different wavelengths on wound healing in a diabetic mouse wound healing model.

A dorsal excision wound was created at the back of diabetic (db/db) mice and was monitored over a period of 28 days. Every second day the wound was subjected to LED illumination (REPULS) with a wavelength of either 470 nm (blue), 540 nm (green) or 629 nm (red), each at 50mW/cm2 and compared to a non-treated control group. Wound size and wound perfusion was assessed and correlated to wound temperature and light absorption of different wavelengths in the tissue.

Red and green light therapy positively stimulated wound healing. Red light led to a significant higher reduction in wound size at day 12 post surgery while green light showed a strong trend. Accordingly, the wound healing rate up to day 12 was 60% higher in both the red and the green light treated group. Blue light was ineffective in this setting. Light absorption was wavelength-dependent and was associated with significantly increased wound perfusion as measured by laser Doppler imaging in the red light treated group. Shorter wavelengths ranging from green to blue significantly increased wound surface temperature, while red light, which penetrates deeper into tissue, led to a significant increase body core temperature.

In summary, wound treatment with pulsed red or green light resulted in improved wound healing in diabetic mice. Since impeded wound healing in diabetic patients poses a severe, ever-increasing socio-economic problem, LED therapy may be a cost-effective and easily applied supportive treatment for diabetic wound therapy.

P562 Cooperative regulation of hyaluronic acid hydrogel and hypoxia in disc regeneration

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Introduction: Hydrogel injection has been proposed as a promising therapy for disc degenerative diseases. With good biocompatibility and parallel biomechanical characteristics, hyaluronic acid (HA) has been widely researched as disc regenerative hydrogel. However, the bio-mechanisms of HA regenerating degenerative disc are not fully uncovered. Our group synthesized the HA injectable hydrogel biomaterial to treat disc degenerative diseases. In this study, we further explore the bio-mechanisms of HA promoting the differentiation of MSCs into nucleus pulposus cells.

Methods: The ASC52telo hTERT immortalized adipose derived mesenchymal stem cells (ASC52telo cells) and human bone marrow mesenchymal stem cells (hBMSCs) were utilized in this study. Cells were seeded in HA hydrogel or in dish and put in normoxia (21% O2) or hypoxia (2% O2) environment. RT-qPCR technique was used to analyse the expression of CD44 and HIF1a. ASCtelo cells were incubated in 6mg/mL cobalt chloride medium and detected the expression of CD44 by RT-qPCR and Western-blot technique. Luciferase report assay was used to explore whether HIF1a transcriptionally activates CD44.

Results: CD44 and HIF1a were both increased in HA hydrogel in hypoxia environment ($P<0.05$). CD44 was also increased in cobalt chloride culture medium ($P<0.05$). Luciferase report assay indicated that HIF1a can directly transcriptionally activate CD44 expression.

Discussion: Nucleus pulposus cells live in hypoxia environment. Biomaterials should support MSCs to adapt the hypoxic condition and also to promote differentiation into nucleus pulposus cells. CD44 is a main ligand of hyaluronic acid. High expression of CD44 contributes to differentiation of MSCs into nucleus pulposus cells. The mechanism that CD44 is transcriptionally induced by HIF1a further explains the roles of HA in regeneration of disc degenerative diseases.

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P563 Temporal intracellular characterisation of stem cells with external stimulation

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The control of stem cell fate via external stimulation is a vital contribution to the advancement of tissue engineering for regenerative medicine, and there are many external factors at play when cells interact with biomaterials[1]. Highly sensitive temporal characterisation using Atomic Force Microscopy (AFM) and micro-Raman spectroscopy (RMS) of living human mesenchymal stem cells (hMSC) elucidates the cellular response and mechanisms during applied external electrical stimulation, with dynamic time points unachievable through conventional methods. AFM directly measures single cell elasticity and morphology changes due to mechanics such as reorganisation of the cytoskeleton, and RMS of living cells can determine the progression changes in biomolecular composition. The external stimulation is provided by a biocompatible conductive polymer platform, capable of electrical and mechanical stimulation.

The hMSC are able to successfully adhere and spread on the conductive polymer platform, with no adverse effects, over long time periods (14 days) [2,3]. A biphasic pulse stimulation is applied to the conductive polymer electrodes with the cultured cells in a live cell arrangement in order to deliver the external stimulation to the cells, whilst also simultaneously performing AFM and Raman measurements. Initial experiments demonstrate changes in the elasticity of hMSCs was observed during and post-stimulation.

Using conductive polymer actuating microchips [4] we are also able to deliver both electrical and mechanical stimulation to individual hMSC. Direct electrical or mechanical stimulation combined with cell modulus measurements and cell biochemistry spectra is a novel type of measurement in understanding the immediate response of living cells to external stimulation, and how this response may be appropriated to control stem cell fate. Once these response mechanics are better understood, the process can be improved and finely-tuned, creating a more efficient approach to tissue engineering via ‘smart’ biomaterials.

P564 Designing therapeutic delivery scaffolds to recapitulate children's bone regenerative potential in adults

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INTRODUCTION:

In situ bone tissue engineering strategies rely on the regenerative potential of the patient stem cells. However, there is a decline in the capacity of mesenchymal stromal cells (MSCs) to form bone with age. Within the native cellular microenvironment, MSCs are presented with a myriad of cues that are responsible for directing their behaviour. Particularly, biophysical cues have been demonstrated to have a profound effect on directing the behaviour of these cells. We hypothesise that age-dependent changes in MSC behaviour involve mechanosensitive signalling pathways, which reduce the ability of adult cells to respond to biophysical cues, in comparison to cells from children that are still undergoing bone development. The current work aims to identify a therapeutic target that when used in combination with a delivery scaffold, will recapitulate the enhanced bone regenerative capabilities of children, in adults.

METHODS:

Firstly, we evaluated the effect of age on cell mechanosensitivity by comparing the response of 11-12 year olds children-MSCs (C-MSCs) and 20-30 year olds adults-MSCs (A-MSCs) when seeded on 10 (soft) and 300 (stiff) kPa type I collagen-coated polyacrylamide (PAA) gels. As indicator of mechanosensitivity, nuclear location of Yes-Associated Protein 1 (YAP) was measured. A customised PCR array containing 96 genes for osteogenesis, angiogenesis and mechanotransduction pathways was performed. Functional osteogenic differentiation was measured by an alkaline phosphatase (ALP) activity and calcium deposition assays. To assess angiogenesis, conditioned media from C-MSCs and A-MSCs were collected at day 3 and used for tube-like formation assay, as well as for placental growth factor (PGF) and vascular endothelial growth factor (VEGF) ELISA assays. Once the age-altered mechanosensitive target - c-Jun N-terminal kinase 3 (JNK3) - was identified the role of the T1A, a JNK3 activator, was tested in 2D as an enhancer of the osteogenic potential of A- MSCs.

RESULTS AND DISCUSSION:

Results demonstrated the increased mechanosensitivity of C-MSCs compared to A-MSCs, as evidenced by a greater increase in YAP cytoplasmic-to-nuclear translocation from soft to stiff substrates which correlates with the stiffness-dependent increases in ALP activity and mineralisation in C-MSCs. Moreover, a clear age-dependent activation of proangiogenic signalling in C-MSCs was observed with increased release of placental growth factor (PGF) and vascular endothelial factor (VEGF) which in turn induced the formation of longer tubules by endothelial cells. In addition, a stiffness-induced up-regulation of NADPH oxidase 1 (NOX1), vascular endothelial growth factor receptor 1 and 2 (VEGFR1 and VEGFR2) and WNT inhibitory factor 1 (WIF1) was observed by means of a customised PCR array. Notably, the stiffness dependent up-regulation of the mechanosensitive gene c-Jun N-terminal kinase 3 (JNK3) in C-MSCs was revealed which led us to investigate the role of JNK3 in osteogenesis. We inhibited its activity by using a JNK3 specific inhibitor (SR- 3576) in C-MSCs cultured on stiff gels and observed a significant decrease in the ALP activity of C-MSCs while no statistical effect was seen in A-MSCs. In order to enhance the osteogenic response of A-MSCs by activation of the JNK3 with T1A, A-MSCs were treated with T1A and the ALP activity was measured. T1A-treated A-MSCs cultured on stiff substrates had higher ALP activity than the ones on soft substrates and similar to levels seen in C-MSCs. T1A crossed the cell membrane of A-MSCs successfully, which was tracked by its fluorescent label and no effects on cell viability or proliferation were observed. Thus, we demonstrated that JNK3 is a mechanosensitive gene capable of modulating osteogenesis and its therapeutic potential was corroborated by measuring the ALP activity of A-MSCs cultured on stiff gels when treated with the JNK3 activator. This work presents new insights in age-altered mechanosensitivity of MSCs, which opens new avenues to develop new therapeutic target-specific scaffolds for recapitulating the enhanced bone regenerative potential of children in adults.

ACKNOWLEDGMENTS:

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P565 A continuous pore size gradient PLLA scaffold for osteochondral regeneration

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Introduction: Osteochondral (OC) scaffold-based regenerative approaches in the joint are challenging since the scaffold must provide mechanical strength while also mimicking the local cartilage and bone microenvironments. Thermally Induced Phase Separation (TIPS) (1) can produce scaffolds with a wide range of pore size morphologies/distributions (2). Here, we produced by TIPS a poly-L-lactide (PLLA) scaffold with a continuous pore size gradient along the sample thickness, from ~70µm diameter on one side to ~200µm diameter on the opposite surface. A novel, patent pending microphysiological tissue system (MPS) bioreactor (3) that allows separate control of the chondral and osseous environment while permitting communication across the OC junction (4), similar to in vivo conditions, is used to validate the TIPS-generated pore-gradient PLLA scaffold.

Materials and methods: Asymmetric thermal histories were imposed on a PLLA foam (4% wt PLLA in 87:13 wt/wt dioxane/water), cooling each sample surface at different rates (25°C/min on the fast cooling side and 1°C/min on the slow cooling side) to create a monotonously variable pore size along sample thickness. After rinsing in distilled water, cylindrical scaffolds were punched out and uniformly seeded with 800,000 bone marrow hMSCs (IRB: University of Washington), then placed in the MPS with the small pore side in the upper chamber (UC) and the large pore size in the lower chamber (LC). After 7 days of growth medium perfusion at 2 ml/day, chondrogenic medium (CM) and osteogenic medium (OM) were supplied to the UC and LC, respectively, at a flow rate of 2 µl/s. After 4 weeks, engineered OC tissues were collected and analyzed by RT-PCR and histology.

Results and Discussion: At 4 weeks, the constructs exhibited strong alcian blue staining on the UC side and alizarin red staining in the LC side, better than control construct fabricated in photocrosslinkable methacrylated gelatin. Constructs cultured only in CM or OM exhibited stronger alcian blue staining on the small pore side, or stronger alizarin red staining on the large pore side. RT-PCR confirmed upregulation of chondral genes (COL2, ACAN, SOX9) in the upper part of the construct (small pores) and of osseous genes (RUNX2, BSPII, OPN) in the lower part (large pores), compared to day 0. A spatially defined, biphasic differentiation of hMSCs within the engineered PLLA OC constructs is observed, with small pores favoring chondrogenic differentiation, likely because of enhanced cell-cell contact, while larger pore size favored osteogenic differentiation, possibly due to easier nutrient movement. PLLA constructs performed better than methacrylated gelatin OC controls for histology and RT-PCR.

Conclusion: Our monolithic, biodegradable scaffold with gradient pore structure is a promising scaffold for OC repair, providing mechanical stability and tailored, local cues for chondro- and osteoinduction.

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P566 Quality and quantity control cell culture with microgravity for to enhance vasculogenesis of peripheral blood endothelial progenitor cells

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Introduction: We recently developed a serum-free, ex vivo cell expansion system called Mononuclear Cell Quality and Quantity Control Culture System (MNC-QQc), using peripheral blood mononuclear cells MNCs which increase the number and the vasculogenic property of endothelial progenitor cells (EPCs) for enhanced vasculogenesis and tissue regeneration from a small amount of peripheral blood. Recently, the effect of microgravity (MG) was reported to enhance the potential of various stem cells. Therefore, we investigated whether combination of MG will further enhance the function of MNC-QQc system to increase the number and function of peripheral blood EPCs.

Methods: MG culture condition was established using disposable cell container (DCC) on 3D-clinostat (Mitsubishi Heavy Industries, Ltd., Tokyo, Japan). MNCs were isolated from peripheral blood in healthy volunteers (n = 8). Cells were cultured in MNC-QQc under four different conditions: (1) normal MNC-QQc (Normal Control; NC), (2) earth gravity during 7 days in DCC (Earth Gravity; EG), (3) MG during 7 days in DCC (MG), and (4) MG for 3 days followed by EG for 4 days in DCC (Microgravity and Earth Gravity; ME). After 7 days of MNC-QQc, total cell number and percentage of CD34-positive cells were measured by FACS analysis, as an indicator of EPCs. The vascular regeneration ability of MNC-QQc cells was evaluated by identifying definitive EPC colony-forming units (dEPC-CFU) and primitive EPC CFU (pEPC-CFU) in colony forming assays (EPC-CFA).

Results: While none of the culture conditions changed the total number of cells,

the MG and ME groups showed significantly higher number of CD34

-positive cells than the NC group [MG vs NC (4.37 ± 2.65 vs 1.32

± 0.31, $p < 0.05$) and ME vs NC (4.74 ± 2.96 vs 1.32 ± 0.31, $p < 0.05$)]; there was no significant difference between the EG and NC groups (3.76 ± 2.78 vs 1.32 ± 0.31). The total EPC-CFU number did not differ among the four groups (NC: 944.4 ± 646.9, EG: 1043.0 ± 710.2, MG: 1084.7 ± 645.6, ME:1487.4 ± 528.3). However, the dEPC-CFU number, which demonstrates the differentiation potential of EPCs, was significantly increased in ME compared to that in NC (1329.1 ± 573.4 vs 688.3 ± 513.0, $p < 0.05$) and other groups.

Conclusion: The results indicated that MNCs with QQ culture under MG increases the CD34-positive fraction with higher vasculogenic potential compared to those in EG culture. MNC-QQc in combination with MG-EG conditions may be a more effective EPC expansion culture method and is a potentially valuable tool for therapeutic vasculogenesis and tissue regeneration

P567 Implementation and verification of an efficient, precise, reproducible and accurate automatic quantification method for measuring mesenchymal stromal cell morphological parameters

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Introduction: The use of human mesenchymal stromal cells (MSCs) plays a significant role in many areas of regenerative medicine. Biophysical methods and especially the control of geometric MSC morphology are particularly promising for controlling MSC differentiation. Thus, measuring cell shape becomes increasingly important. A few quantitative methods for measuring cell morphology are available, but key questions such as the image thresholding-dependent reproducibility and resolution-dependent accuracy of cell morphology descriptors is not available. **Aim:** The aim of this study was to 1) quantitatively measure a range of cell morphology parameters that allowed evaluation of a large number of cells within a short time and 2) compare the efficiency, precision, reproducibility and accuracy of manual vs. binary thresholding and image resolution-dependency. **Method:** Calcein-stained human bone marrow-derived MSCs on compressed collagen sheets were recorded by fluorescence microscopy as a mosaic image from 10 × 10 individual images with 10-x magnification. Using ImageJ manual vs. binary thresholding with and without contrast enhancement was compared by statistically comparing the results for the 5 cell shape descriptors Major Axis, Circularity, Aspect Ratio, Roundness and Solidity, and the total number of cells/normalized area. **Results:** Binary thresholding with contrast enhancement had no advantages over binary thresholding without contrast. However, binary thresholding was significantly faster (6.3 times), more precise when comparing different users, and more reproducible than manual thresholding ($p < 0.05$). Manual thresholding was only reproducible for the number of cells. For all shape descriptors measured, significant differences were found between the binary vs. manual method ($p < 0.001$). The Major Axis showed the highest differences between the two methods (21.6%). The differences for the remaining shape descriptors ranged from 3.4% to 11.8% between the two methods. To examine whether manual vs. binary thresholding showed resolution-dependent differences, captured MSC images were halved in their resolution to obtain a series of MSC images with decreasing resolution. For all images and all shape descriptors, the two methods showed resolution-dependent significant differences in Circularity ($p > 0.070$), a descriptor that can be used to quantify MSC filopodia. Comparing the calculated shape descriptors for each resolution to the highest resolution image, significant deviations to the highest resolution occurred in binary thresholding but at equal or lower resolution than in manual thresholding. Resolution-related accuracy was therefore more favourable with binary thresholding. **Conclusion:** The measurements and comparisons performed for this work show that binary thresholding is significantly more efficient, more precise and more reproducible than manual thresholding. Moreover, when assessing MSC Circularity binary thresholding has a better resolution-dependent accuracy, whereas other shape descriptors at different resolutions were accurately quantified. As ImageJ is often incorporated into available approaches for measuring cell morphology, the here reported data will help many scientists to choose thresholding and image resolution, and to judge accuracy of individual shape descriptors.

binary thresholding can be used to analyze more images and thus more cells, providing a time saving and non-biased technique for quantifying cellular morphologies.

P568 Mimicking the early regenerative response of perivascular stem cells *in vitro*

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In vivo mesenchymal stem cells (MSCs) reside in the perivascular niche and are referred to as perivascular stem cells or pericytes. Isolation of CD45-CD34-CD146+ cells from different tissues has been shown to yield a highly proliferative and multipotent cell population. While these cells are well characterized *in vitro*, little is known about the regenerative potential of perivascular stem cells *in vivo*. Here, we aimed to simulate the microenvironment in the early stage of bone healing *in vitro* in order to study the regenerative response of perivascular stem cells.

Perivascular stem cells (CD34-CD146+) were enriched from human bone marrow mononuclear cells by MACS®. Different physiological and pathological microenvironments were simulated by using conditioned medium (CM) from cultures of primary human endothelial cells and osteoblasts (healthy bone), femoral head derived bone fragments (injured bone), and activated platelets (platelet-rich plasma (PRP), injury). Cells were incubated in the different media for 24h and analysed with respect to proliferation, gene expression, migration and osteogenic differentiation.

Perivascular stem cells showed a specific response to different microenvironments. Cell proliferation, measured by DNA quantification, was observed in all media with highest values (2-fold increase) in PRP-CM and bone fragment-CM. All media induced chemo-kinetic activity of cells, while chemotactic attraction of cells was only observed for PRP-CM. A strong upregulation of Cox2 gene expression, a key component in immunomodulatory signalling was detected for PRP-CM and bone fragment-CM. Similarly, these microenvironments induced an upregulation of VEGF gene expression. The osteogenic differentiation potential of perivascular stem cells was retained for all media.

Our results indicate that a microenvironment simulating bone injury elicits strong immunomodulatory and pro-angiogenic activity of perivascular stem cells. This suggests that in the early stage of bone healing the prime function of MSCs is in regulating the immune response and inducing neovascularization. Future studies will investigate the key components in CM driving this function, which might be potential targets to therapeutically stimulate the regenerative potential of perivascular stem cells.

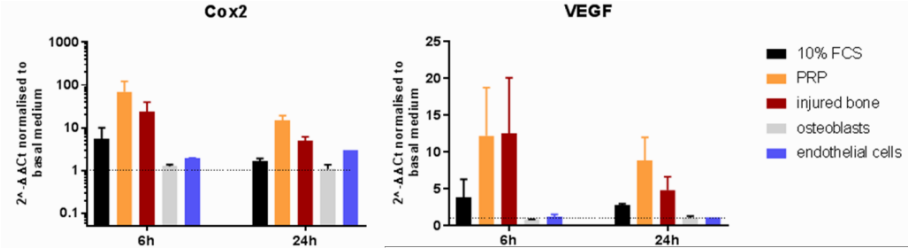

Figure 1. Gene expression of perivascular stem cells incubated in different microenvironments. Shown is the fold change of Cox2 and VEGF expression relative to cells incubated in serum free basal medium.

P569 Using bioprinting to investigate the mechanism of axon pathfinding and regeneration.

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Neuronal networks are critical for many body processes, such as brain function, movement and sensing. These neural networks are established during development and depend on precise axon guidance mediated by extrinsic molecular cues and extracellular matrix (ECM) proteins. Following spinal cord (SCI) or peripheral nerve injury (PNI), axon regrowth is impaired, leaving individuals with compromised movement or, in case of SCI, paralyzed. Our key objective is to elucidate how different ECM proteins and chemical cues determine axon outgrowth and pathfinding, thereby permitting the development of novel biochemical micropatterned materials for enhanced nerve regeneration.

To examine neuronal behavior under various conditions, inkjet printing, light induced photopatterning and microstamping techniques were compared to create an *in vitro* neuronal guidance assay. Light induced photopatterning resulted as a precise flexible tool to create patterns.

We made Fibronectin gradients and line patterns using light induced photopatterning. We used rat dorsal root ganglion (DRG) as a model system for peripheral nervous system (PNS) regeneration, and primary cortical neurons and cortical cell line from mouse as a model of the central nervous system (CNS).

Our results indicate that defined tracks of Fibronectin are able to guide axons directionality. Furthermore, Fibronectin gradients are able to support axon outgrowth. By performing live cell imaging technique, we will furthermore access the growth dynamics and speed of growth on these defined patterns.

We showed a technique to create defined protein patterns by using light induced photo-patterning as a tool for axonal pathfinding assays. Future work includes testing different ECM proteins and guidance cues for their guidance capabilities on neurons from the PNS and CNS. Knowledge about guidance abilities of defined biochemical cues will allow the design of micropatterned surgical repair methods for *in vivo* testing.

P570 Exogenously-delivered NO Stimulates Elastogenesis by Adult Human Aneurysmal Smooth Muscle Cells

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Introduction: Vascular diseases such as aortic aneurysms are typically characterized by endothelial dysfunction, variation in nitric oxide (NO) release, over-proliferation of aortic smooth muscle cells (SMCs), upregulation of matrix degrading enzymes, degradation of elastin matrix fibers, and compromise in SMC-elastin signaling homeostasis. Here, we investigate the benefits of exogenously delivering NO cues to regulate cell proliferation, matrix synthesis and deposition (total protein, glycosaminoglycans, elastin, desmosine), lysyl oxidase enzyme, and iNOS/ cytokines/ chemokines release by human aortic aneurysmal SMCs (Hu-AA-SMCs) within 2D or 3D biomimetic cultures.

Materials & Methods: Hu-AA-SMCs were isolated from abdominal aortic aneurysm tissue segment of a 54 year old male and cultured for 21 days, either on 2D collagen-1 coated substrates or within 3D collagen hydrogels, either with or without 100 nM S-Nitrosoglutathione (GSNO). GSNO dose was optimized based on our previous study. Phenotypic and genotypic changes and protein release and deposition (biochemical assays, western blot analysis, RT-PCR, cytokine/chemokine assays) were quantified using our published protocols and compared under these conditions.

Results & Discussion: AA-SMC proliferation is lower in 3D collagen gels compared to their 2D counterparts, and adding GSNO marginally suppressed SMC proliferation in both 2D and 3D. Multi-fold increases in total protein deposited in cell matrix in both 2D and 3D cultures were noted with adding GSNO, compared to controls. Total protein deposited in cell matrix within 3D cultures was significantly higher than that in 2D counterparts, in the presence or absence of GSNO. While matrix elastin deposition appeared suppressed in 2D cultures, significantly higher matrix elastin was deposited within 3D cultures, compared to GSNO-free controls. GSNO stimulated release of more tropoelastin within 3D cultures, and the tropoelastin amounts were two orders of magnitude higher than matrix elastin. Similar results were noted in sGAGs release in pooled media and matrix layers. GSNO induced ~2.5-fold increase in LOX activity within 3D cultures, not seen in 2D cultures. LOX activity within pooled media from 3D cultures was multifold higher than that in 2D cultures, even in controls. Such increase in LOX protein functional activity is highly encouraging from a tissue engineering and in situ elastin regeneration standpoint within aneurysms. Desmosine crosslinking density was lower within cultures receiving GSNO. iNOS and TIMP-1 amounts were enhanced within 3D cultures receiving GSNO, and comparable to that noted in healthy SMC counterparts. Elastin gene expression in AA-SMCs, both in 2D and 3D cultures, in the presence or absence of NO, was significantly lower compared to healthy cells. Elastin gene expression was lower in 2D compared to that in 3D, which was reflected in their protein expression levels as well. Adding GSNO had no effect on iNOS gene within AA-SMCs, compared to controls. LOX gene expression was multifold higher within AA-SMC cultures compared to healthy SMCs, although adding GSNO suppressed such elevated levels. Base-level expression (i.e., 0 nM GSNO) of fibrillin-1 gene in AA-SMCs was modestly higher compared to their healthy counterparts, although such expression levels were suppressed within 2D cultures with the addition of 100 nM GSNO.

Conclusions: Results attest to the benefits of delivering 100 nM NO cues to suppress AA-SMC proliferation and promote robust ECM synthesis and deposition by adult human AA-SMCs, with significant applications in tissue engineering, biomaterial scaffold development, and drug delivery.

P571 Using tissue engineered scaffolds to understand the cellular aspect of bladder regeneration

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In the management of congenital and acquired bladder pathologies augmentation of the bladder is commonly performed using vascularized digestive tract patches. This can be linked to severe complications, such as metabolic disturbances and even cancer. Therefore tissue engineered grafts are a promising alternative. However, recent cellular based tissue engineered grafts tested in clinical trials showed less success than hoped. Therefore, new designs of tissue engineered grafts need to be explored in relevant bladder animal models. The pig is a good model to study bladder augmentation in terms of surgical methodology and functionality of clinical relevant graft sizes. The rat is an excellent model to understand the cell biology governing adequate bladder regeneration. The objective of this study was to better understand the cellular aspect of bladder regeneration process of collagen-based grafts without a synthetic material component.

A previously developed rat cystectomy model was used to implant engineered grafts. Cellular, acellular and growth factor (IGF-1) loaded grafts were implanted in respective groups (N=4 rats). 4 weeks after grafting, rats were sacrifice and the bladders were harvested. The cellular regeneration of grafted rat bladders was analysed by histology.

Natural derived collagen grafts without a synthetic material component showed a good bladder regeneration capacity. No severe inflammatory state was observed in any of the tested animals of the respective groups. Urothelium was covering the grafted luminal areas and muscle was infiltrating the grafts from the anastomosis sides. Cellular grafts did not show significant acceleration of cellular regeneration. However, slow released IGF-1 showed a dose depended and significant acceleration of cellular regeneration. The lesson learned from this rat bladder regeneration study is that natural derived material without a synthetic component showed a good regeneration potential in rat bladders, even without ex vivo cultured cells.

P574 Hyperactive mechanism of cardiac fibroblasts against doxorubicin-induced cardiotoxicity

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Introduction: In heart disease, the number of resident cardiac fibroblasts is increased by proliferation. However, it is not fully understood that the mechanism of these hyperactivity of existing cardiac fibroblasts. In this study, we evaluated the hyperactivity of cardiac fibroblasts by assessing cytotoxic responses to doxorubicin (DOX) which is an effective chemotherapeutic agent used to treat malignancies but it causes cardiac fibrosis and cardiomyopathy.

Materials and Methods: Mouse Cardiac fibroblasts (CFs) and mouse dermal fibroblasts (DFs) were treated with 10 μM Dox for 1, 4, 8 h. Cytotoxic responses to Dox in each culture conditions were evaluated by cell viability and intracellular calcium concentration.

Results: When cytotoxic response to Dox of each type of fibroblasts were evaluated using confocal microscopies, the cell viability of CFs was significantly higher than that of DFs. To evaluate protective role of CFs againstDox, it was evaluated that cytotoxic responses to calcium modulation. Interestingly, intracellular calcium concentration of CFs was fewer than that of DFs (Fig.1-2.). Calcium is a major signal transducers that response to multiple cellular processes, calcium expression levels need to be controlled. To elucidate the molecular mechanisms of calcium-doxorubicin interaction in CFs, the microarray analysis was performed and we identified candidate factors that were significantly upregulated in CFs compared with DFs.

Discussion and Conclusions: These findings indicate that hyperactivity of cardiac fibroblasts in heart disease might have relations to calcium-Dox interaction. The effects of these factors in CFs remains to be determined.

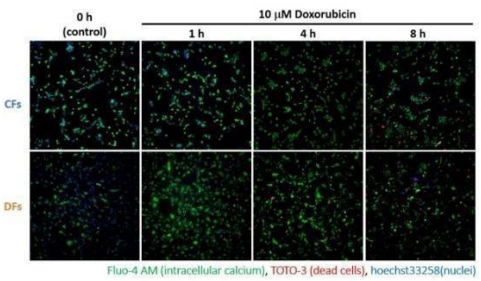

Fig.1. Immocytochemistry in each cell culture dish using a confocal microscopy. (x200 magnification).

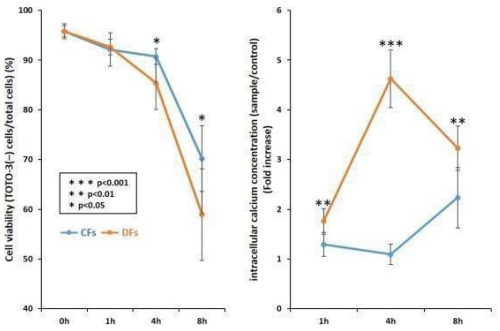

Fig.2. Line graph represents cell viability and intracellular calcium ion in each cell culture condition. (N = 3)

P576 Biofunctionalization of chemically-cured urethane pre-polymer coatings for next generation pro-healing cardiovascular implants

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Introduction: Current cardiovascular devices are associated with impaired healing of the diseased endothelial tissue, contributing to thrombosis and neointimal hyperplasia. Hence, new biomaterial platforms with in situ endothelialisation capability are highly desirable for development of 'off-the-shelf' pro-healing cardiovascular devices. Such capability can be introduced via surface biofunctionalization of bioactive molecules such as antibodies or functional peptides to augment homing of endothelial progenitor cells in peripheral blood. In this work, we present a novel chemical modification approach of urethane-based pre-polymers to generate composites with tuneable chemistry capable of immobilising pro-healing bioactive molecules to influence cell fate.

Methods: Isocyanate-terminated polycarbonate urethane pre-polymer (15wt% in tetrahydrofuran) was spin-coated onto circular 316L stainless steel coupons, followed by autocatalytic reaction with 25mM-500mM ethylenediamine (EDA) or propargylamine (PPA) to generate surface amine ($–NH_2$) and alkyne groups respectively. Mouse anti-CD34 antibodies were oxidised using 10mM sodium periodate and conjugated to the $–NH_2$ surfaces. Thiolated REDV peptides were reacted to the alkyne-terminated surfaces via thiol-yne click chemistry catalysed with 1.5mol% Irgacure 2959 and uv-irradiation.

Results: Chemical modification of pre-polymers with EDA yielded hydrophilic surfaces with reactive $–NH_2$ groups, and a treatment-concentration dependent formation of micro-ridges and nanofeatures. Human umbilical vein endothelial cells (HUVEC) seeded over a 7-day period on these coatings revealed an enhancement in adhesion and proliferation compared to untreated controls. Immobilisation of anti-CD34 antibodies onto the EDA-coatings were confirmed and optimised via direct enzyme-linked immunosorbent assay (ELISA). The anti-CD34 antibody-immobilised samples supported HUVEC growth, revealed higher CD34 antigen capturing capability as well as reduced platelet adhesion count compared to clean EDA-coatings. Early result with peripheral blood mononuclear cells on these surfaces showed typical cell morphology compared to standard fibronectin coated dish controls, suggesting its possibility to support CD34+ EPC homing. On the other hand, PPA treated surfaces yielded hydrophobic surfaces with terminal alkyne groups, as well as formation of nanofeatures. HUVEC did not favourably attach and grow on these surfaces. Immobilisation of functional REDV peptide was shown to moderately improve HUVEC adhesion and growth.

Conclusion: We have developed novel urethane-based coatings with tuneable chemistry capable of influencing cell response, and demonstrated immobilisation of antibody and functional peptide and their biological effect using distinct platforms. We envisage such platforms can be useful for controlled immobilisation of single or multiple pro-healing bioactive molecules to support the optimisation and investigation of the realm of in situ endothelialization in cardiovascular implants.

P577 Overcoming the defect: in vivo bioreactor as solution for generating new resource of autologous bone graft

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Critical size bone defect is still being one of the major problems in orthopaedic trauma. A bone defect has been defined as critical when the segment defect is at least 1.5-2.5 times the diaphyseal diameter of the bone. A defect this large will not undergo any process of healing when not treated. Treatment of bone defect possess an enormous challenge.

According to the diamond concept of bone healing, there are four main components needed to improve healing, namely osteogenic, osteoconductive, osteoinductive, and mechanical. The use of iliac crest bone autograft has been introduced for treating critical bone defect for a long time. However, the application is hampered by limited source.

Tissue engineering is currently exploring alternatives for generating new bone tissue. The base triad for bone tissue engineering is manipulation of osteogenic cells, growth factors and bioscaffolds. The first two in vivo bioreactor studies were successfully induced new bone formation on periosteum or vascular pedicle. Engineering bioreactor within human body, growing bone materials within periosteum is one of the new approaches. The environment of “periosteal pocket” is able to fulfill the role as bioreactor and scaffold necessary for bone growth. Constant stream of pluripotent stem cells and biomolecular signals that is necessary for bone formation are some advantages of this in vivo bioreactor.

An animal trial by Stevens et al. studied the possibility of generating mature autologous compact lamellar bone suitable for transplantation by injecting alginate gel to a periosteum pocket of tibia. After 8 weeks of observation, histopathological examination revealed a formation of mature osseous tissue which is similar to compact bone. Other study implanted beta tricalcium phosphate scaffold in a periosteal flap, and found that it was able to grow a well-vascularized and chondrocytes-rich lamella bones. Other than periosteum, some different body site has been studied to provide suitable environment for new bone formation, such as subcutaneous pouch, muscular pouch, abdominal cavity, omentum, axial vascular bundle, and arteriovenous loop.

Some issues still need to be covered on this new trending discovery. The amount of new bone that is able to be produced and the time required for growth need to be addressed in future research. Whether the amount of produced bone allograft can be predicted or even programmed is becoming the new question. These issues perhaps hold a new direction for bone tissue engineering as well as the future of regenerative medicine.

P578 Which endogenous regenerative pathways are activated in human adipose tissue following an injury?

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OBJECTIVE: We investigated the *in vitro* response of human subcutaneous adipose tissue (AT) to injury-associated stimuli in order to shed light on endogenous regenerative pathways activated following an injury. We first examined proliferative and differentiation capabilities of adipose-derived stromal cells (ASCs) isolated and cultured in human serum (HS) and treated with human platelet lysate (PL) and we investigated the PL effects on ASC inflammatory response in both physiological and inflammatory microenvironment. We then focused our attention on the PL-induced proliferative response of *in toto* AT in either normal or inflammatory condition in order to reduce the gap between *in vitro* results and *in vivo* events associated with the AT regeneration.

MATERIALS AND METHODS: ASCs were obtained by seeding into culture the stromal vascular fraction derived from human lipoaspirate while *in toto* AT was obtained by mincing subcutaneous fat derived from abdominoplasty waste. Both ASCs and AT were cultured in αMEM supplemented with 10% HS. For different treatments, PL and/or IL-1 were added to complete culture medium at a final concentration of 5% and 100U/mL, respectively. In particular, HS and PL were obtained from a pool of at least 10 healthy blood donors. HS was produced eliminating coagulation factors from fresh plasma by cryoprecipitation and by calcium chloride addition and clot removal. PL was produced starting from buffy coat samples obtained from whole blood. After several centrifugation steps, platelets were washed and re-suspended in phosphate-buffered saline at a concentration of 10x106 platelets/µL and the suspension was subjected to 3 consecutive freeze/thaw cycles followed by a high-speed centrifugation. The collected supernatant was used as PL.

RESULTS: ASCs cultured in HS showed a higher proliferation rate than the control ones expanded in FBS and a further increase in the cell growth was observed when PL was added to the culture. We confirmed the higher proliferation of ASCs cultured in HS and HS+PL by a clonogenic assay. After that, we investigated the proliferative pathways activated by PL and we found a strong but transient activation of Akt and ERK1/2 pathways starting from 30 minutes of PL treatment. We also observed that ASCs cultured in HS differentiated into more committed adipose cells without the need of any induction, when cell density increased. However, this committed population maintained the ability to give rise properly to osteoblastic and chondrocytic lineages, under suitable induction cues. In presence of IL-1, PL stimulated a very strong inflammatory response in sub-confluent ASCs, via pro-inflammatory IL-6 and -8 secretion, COX- 2 induction and PGE2 production. In particular, we found IL-6 and -8 production being transient. When *in toto* AT was cultured *ex vivo*, we observed a strong cell proliferation induced by PL in the AT outer layer and a PL-induced release of proliferating cells in both normal and inflammatory conditions.

CONCLUSION: Our data suggest that AT progenitor cells could be activated in response to an injury. In particular, PL recruits quiescent cells into the cell cycle and HS promotes their proliferation and differentiation in order to restore the tissue integrity. Moreover, AT takes part to create a transient inflammatory milieu at the wound site, as a response to PL.

P579 Multifactorial approaches for tenogenic phenotype maintenance and function

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Cell-based therapies require removal of cells from their optimal *in vivo* tissue context and propagation *in vitro* to attain suitable number. However, bereft of their optimal tissue niche, cells lose their phenotype and with it their function and therapeutic potential. Biophysical signals, such as surface topography and substrate stiffness, and biochemical signals, such as collagen I, have been shown to maintain permanently differentiated cell phenotype and to precisely regulate stem cell lineage commitment. Herein, we developed and characterised substrates of variable rigidity and constant nanotopographical features to offer control over cellular functions during *ex vivo* expansion. We show that human adult dermal fibroblasts, tenocytes, and mesenchymal stem cells attach, align, elongate and deposit aligned extracellular matrix on the grooved PDMS substrate surface of all 3 stiffnesses (Fig.1). Preliminary *in vitro* data indicate that surface topography and substrate stiffness play crucial role in maintaining cell phenotype and the prevention of phenotypic drift *in vitro*.

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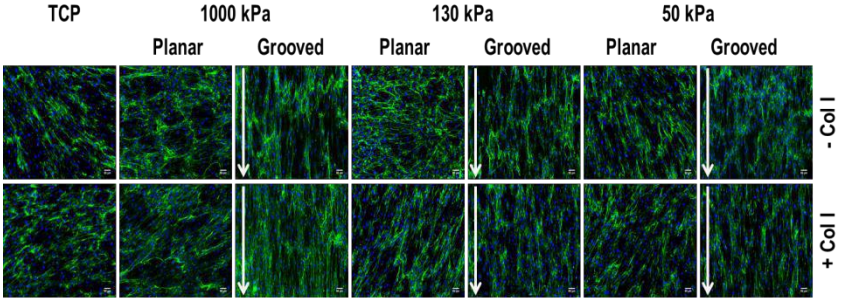

Fig. 1: Human tenocytes align and deposit aligned matrix (fibronectin) on grooved PDMS substrates of varying stiffness up to 14 days in culture.

P580 Hyaluronan-based core-shell nanofibres for wound healing and drug delivery

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Introduction: Over last years, novel and nanomaterials-based wound dressings and drug delivery systems have been studied extensively for their unique properties. Nanofibres-based wound dressings possess exceptional properties including high specific surface with small pore size enabling drug delivery and release *in situ* and keeping the wound site moisturized and opened to an air exchange at the same time. The wound site may be also stimulated by the matrix degradation products promoting the cell motility and accelerating the wound closure. In the research presented, we developed a novel hyaluronan-based inorganic-organic core-shell nanofibrous matrix and tested its potential as a novel wound dressing. Hyaluronic acid shell was tested as a wound healing promoter based on its ability to stimulate RHAMM receptors resulting in increased cell proliferation and protein synthesis[1].

Methods: Nanofibres were produces by needle-less electrospinning. Spinning solution for the silica core was produced via modified sol-gel method in which tetraethylorthosilicate (TEOS) was used as a precursor. Shell covering the silica core was based on high molecular weight hyaluronic acid (HA). The formed nanofibres were characterized in terms of morphology, chemical properties, wettability, degradation kinetics and biocompatibility. The effects on fibroblasts (3T3) and keratinocytes (HaCaT) proliferation and wound closure *in vitro* were also studied in comparison to bare silica nanofibres.

Results: The obtained SiO_2-HA core-shell (CS) nanofibres were smooth in morphology, with diameter ranging from 150 nm to 450 nm in the mean. Formation of the HA-based shell representing a minor component of the nanofibre mass was confirmed to have a crucial effect on degradation kinetics over the first 96 hours by keeping the fibrous morphology of the system and slowing down the core degradation and drug release. In CS nanofibres the degradation reached less than 40% of the bare silica nanofibres after 48 hours. Biocompatibility evaluation proved a good cytocompatibility and increased proliferation for both tested cell lines. The scratch-wound healing assay performed *in vitro* on HaCaT cells verified the HA-shell based CS nanofibres have a positive impact on wound healing and possess ability to hasten wound closure.

Discussion and Conclusions: Novel inorganic-organic core-shell nanofibres were confirmed a novel biodegradable and biocompatible material able to maintain nanoporous structure over its degradation. Its ability to support wound healing and accelerate the wound closure highlights its potential to perform as a matrix for sustained drug delivery in wounds and wounds and ulcers treatment.

Acknowledgements: The research reported in this paper was supported in part by the Project of Ministry of Education, Youth and Sports in the framework of the targeted support of the National Programme for Sustainability I (CZ.1.05/2.1.00/01.0005) and the SGS project 2017 (21066/115) at the Faculty of Mechatronics at the TUL.

P581 Pattern induced osteodifferentiation of MSCs: an approach to mimic bone hierarchical structure

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Considering the complex hierarchical structure of bone, biomimicking the micro/nano level features is an integral part of scaffold fabrication for successful bone regeneration. Hence, we aim to study the effect of physical cues on cell alignment, proliferation and differentiation. Micropatterns (20 µm) were made using photolithography on electropun fibers of SU8 photoresist. *In-vitro* Cell proliferation assays (Alamar blue and Picogreen), protein estimation (BCA assay), osteodifferentiation (ALP staining), live dead assay (FDA/PI) and cell alignment study (Imaje J analysis) was done. Our findings show a similarity with the helicoidal plywood model of the bone. This resembles the *in-vivo* anisotropic multi-lamellar bone tissue architecture. Also, the patterned surface induced osteodifferentiation of MSCs even in absence of osteoinduction medium and this shows the effect of geometric cues on cellular functions. This can serve as a model system to study the effect of topographical cues not only for bone but other tissues as well, owing to the accuracy and ease of the fabrication technique used.

P582 Circulating microRNA analysis upon bone injury

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Bone injury triggers both local and systemic responses. Immune cell populations and levels of signalling mediators, such as cytokines, are altered at systemic level[1]. MicroRNAs (miRNAs) are small RNA molecules that regulate different pathways and condition cell phenotype, by targeting messenger RNAs (mRNAs). miRNAs circulate in plasma, within protein complexes or inside cell-secreted extracellular vesicles (EV), including exosomes. Thus, they might regulate cell function in locations distant from their site of production and release. In the current work, we aimed to characterize the profile of circulating miRNAs upon bone injury, and to investigate the most affected signalling pathways.

A critical size bone femoral defect was performed in Wistar rats and response analysed at 3 (3d) and 14 (14d) days post-injury (PI). Non-operated (NO) animals were used as control. Blood was collected and centrifuged for plasma collection. EV circulating in plasma were isolated by differential (ultra)centrifugation. Plasma miRNAs were profiled by qRT-PCR array. The most differently expressed miRNAs were analysed by Ingenuity Pathway Analysis (IPA) software to determine the signalling pathways they regulate. Presence of candidate miRNAs in plasma EV was tested by qRT-PCR, upon proteinase K and RNAse A digestion.

Overall, we observed a timely controlled miRNA expression pattern. Comparing 3d PI to NO and 14d PI animals, injury led to a down-regulation of circulating miRNAs in total plasma. Several members of the let-7 miRNA family, classically associated with cell proliferation inhibition, were significantly up-regulated at 14d PI, when compared with 3d PI. In agreement, bioinformatics analysis of miRNA expression profile showed an inhibition of Ago2 miRNA processing pathway at 3d PI comparing with NO animals, which was then activated at day 14, when compared with 3d PI animals. In addition, the IPA analysis indicates a correlation between the miRNA expression profile and an up-regulation of cell proliferation pathways at 3d PI, which were then down-regulated at 14d PI, returning to basal levels. At day 14d PI, pathways related with stem cell differentiation were activated, when compared with day 3 PI. In particular, Gene Ontology analysis was most significantly associated with “Cell cycle” (3d PI versus NO), and with “Cellular development” at 14d PI versus 3d PI, which includes cell differentiation processes. Importantly, let-7c was detected inside plasma EV.

In conclusion, our results demonstrate a systemic and timely controlled miRNA expression profile after bone injury, likely related to the regulation of the response to injury. More importantly, the presence of let-7c inside EV supports the future clinical application of EV as delivery carriers of importantly regulated miRNAs for improved bone injury regulation.

Acknowledgements: Work supported by AO Foundation-Switzerland (S-15-83S); FEDER funds through the COMPETE 2020 - POCI, Portugal 2020, and Portuguese funds through FCT (POCI-01-0145-FEDER-007274, SFRH/BD/85968/2012, SFRH/BD/112832/2015 and SFRH/BPD/91011/2012). GAC is The Alan M. Gewirtz Leukemia & Lymphoma Society Scholar, and supported by NIH/NCI grants 1UH2TR00943-01.

P583 HIF1A overexpression using protein transduction domain induces angiogenesis in Huvec

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Hypoxia inducible factor-1 alpha (HIF1A) is an important transcription factor for angiogenesis. And protein transduction domain (PTD) has been used to transfer genes in recent studies. But PTD is not used to induce the expression of HIF1A. This study is aimed to use novel PTD (Hph- 1-GAL4; ARVRRRGPRR) to overexpress HIF1A gene and identify the effect of angiogenesis in vitro and in vivo.

HIF1A was transfected using Hph-1-GAL4 in HUVEC. And the expression of HIF1A and HIF1A target genes were analyzed by quantitative real-time PCR (qPCR) 2 and 4 days later respectively. In vitro tube formation assay was identified using Diff-Quik staining. HIF1A and Hph-1-GAL4 were injected subcutaneously into the ventral area of each 5-week-old mouse. All plugs were retrieved after 1 week and the relative expression of HIF1A and HIF1A target genes were evaluated by qPCR. And each matrigel plug was evaluated by hemoglobin assay and HE staining.

HIF1A and HIF1A target gene expressions were significantly higher in HIF1A transfected HUVEC than control HUVEC in vitro. And total tube length was higher in HIF1A transfected HUVEC than control. In vivo matrigel plug assay, the amount of hemoglobin per weight of matrigel was significantly higher in HIF1A treatment group than PBS treatment group. Boold vessels were identified in HIF1A treatment group through HE staining. And HIF1A, VEGF, and CD31 expressions were significantly higher in HIF1A treatment group than PBS treatment group.

In conclusion, it might be helpful to transfer the gene and regenerate tissues effectively when using Hph-1-G4D to overexpress HIF1A.

P584 Biofunctionalisation of electrospun constructs with fibrillin-1 for anterior cruciate ligament repair and regeneration

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Anterior cruciate ligament (ACL) graft ruptures can occur due to the modular mismatch of the two structures at the integration site, as well the slow rate of bone healing[1]. Electrospun fibrous constructs are able to emulate the native extracellular matrix (ECM) architecture at the nano-level, whilst providing adequate mechanical properties[2]. To improve cell response and to mimic ACL ECM at the enthesis site, the effects of biofunctionalising electrospun constructs[3] with fibrillin were explored.

Medical grade poly-e-caprolactone PCL (15% w/v) was electrospun into aligned fibrous constructs and sterilised. Constructs were plasma treated (air) and re-sterilised before being fibrillin coated. Canine anterior cruciate ligamentocytes (cACL) were seeded onto constructs and investigated for adhesion, morphology, metabolic activity and proliferation for up to 14 days. ECM protein production was evaluated using immunocytochemistry. Two-way ANOVA with Tukey HSD post-hoc was used to identify significances.

Scanning electron microscopy (SEM) visualised aligned electrospun constructs with an average fibre diameter of 3.42µm (±1.11µm). The Live/dead assay revealed the attachment, native cACL morphology and 90% viability of cACLs on all constructs: PCL only, PCL+Plasma (P+P), PCL coated with fibrillin (P+F) and PCL+Plasma+Fibrillin (P+P+F) and controls (Tissue culture plastic/TCP, TCP+Fibrillin/TCP+F) at all time points. A significant increase in metabolic activity was observed on P+P+F compared to PCL, TCP and TCP+F at all time points ($p<0.05$). Immunocytochemistry revealed accelerated and increased production of fibrillin, fibronectin and collagen 1A1 on P+P+F and P+P constructs compared to PCL only and P+F constructs at day 7 and 14 (statistically insignificant). New ECM deposition also appeared to follow the direction of the fibrous constructs as early as day 7, particularly on P+P and P+P+F constructs.

Biofunctionalised PCL constructs with plasma and fibrillin have demonstrated their ability to support and improve biocompatibility with cACLs as well as increase new ECM production. Topographical cues increased ECM remodeling in the direction of the fibres. This could result in the removal of clinical side effects associated with ACL replacements and improve long-term health benefits for ACL rupture patients.

We acknowledge University of Liverpool for their collaboration, with funding by the UK Regenerative Medicine Platform Hub, EPSRC, Coordenadoria de Aperfeicoamento de Pessoal de Nivel Superior (CAPES) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq.

[1]Ho B *et al.* (2016) *J Pediatr Orthop* *https://www.ncbi.nlm.nih.gov/pubmed/27379789* (online). [2]Pauly HM *et al.* (2016) *J Mech Behav Biomed Mater* **61**:258-270. [3]Mizutani N *et al.* (2014) *J Artif Organs,* **17**(1):50-59.

P585 Activin A and alkaline phosphatase affect hair inductive potency of human DP cells

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Recent studies have shown that the hair-inducing capacity (trichogenicity) of dermal papilla (DP) cells is restored when 3D culture rather than 2D culture is employed. However, little is known about why there is a restoration in trichogenicity when dissociated DP cells are prompted to form spherical structures. With the hope of identifying trichogenic genes of human DP cells, we used proteome profiler array as well as Affymetrix gene array. We found that a number of genes and secretory proteins including activin A and alkaline phosphatase (ALP) were upregulated in the DP spheres compared with 2D-cultured DP cells. We then explored the role of activin A and ALP in trichogenicity of human DP spheres by adopting small interfering RNA (siRNA)-mediated gene knockdown approach and in vivo hair reconstitution assay. We observed that human DP spheres with knock-down of those genes are severely impaired in hair follicle induction when combined with mouse epidermal cells. Our data strongly suggest that activin A and ALP affect hair inductive potency of human DP cells.

P586 Non-contact wettability assessment system for cultivated cells

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We have developed a method for assessing surface wettability without any direct contact. At the first step in this method, an air-jet is given from a nozzle to a target surface covered with a liquid. The air-jet can remove the liquid covering the target surface around a part just under the nozzle. This removal size of liquid is an index of surface wettability, because low wettability surfaces repel the liquid. This method enables us to assess the wettability of target surface under a liquid such as a plate-cultured cellular tissues (N. Tanaka et al., Biomaterials, 34, 9082-9088, 2013). When we apply this method to engineered tissues, both particle contamination and cell damage must be suppressed. To remove particles from the air-jet, we employed a particle filter, which was often used for semiconductor industry, into the compressed air line in the developed system (Fig. 1A). Furthermore, human manual operation, which might be the main source of particles, was reduced by introducing both image processing and motorized alignment device. Using a particle counter, the air-jet from the system was confirmed to include no particles, although great number of particles was detected in the original compressed air (Fig. 1B). To evaluate the tissue damage by the air-jet application, we determined the pressure level of air-jet application at which the cells detached. Confluent mouse skeletal myoblasts C2C12 never detached from the culture dish by the air-jet application within a pressure of 100 kPa. Finally, we assessed the wettability of cultivated C2C12 tissue surface. The squeezed diameter of culture medium covering on C2C12 cells was automatically determined within 4% variation (Fig. 1C). Through this study, we confirmed the system was contamination-free and compatible with cell culture. This method would be useful for assessing surface wettability on cultivated cells.

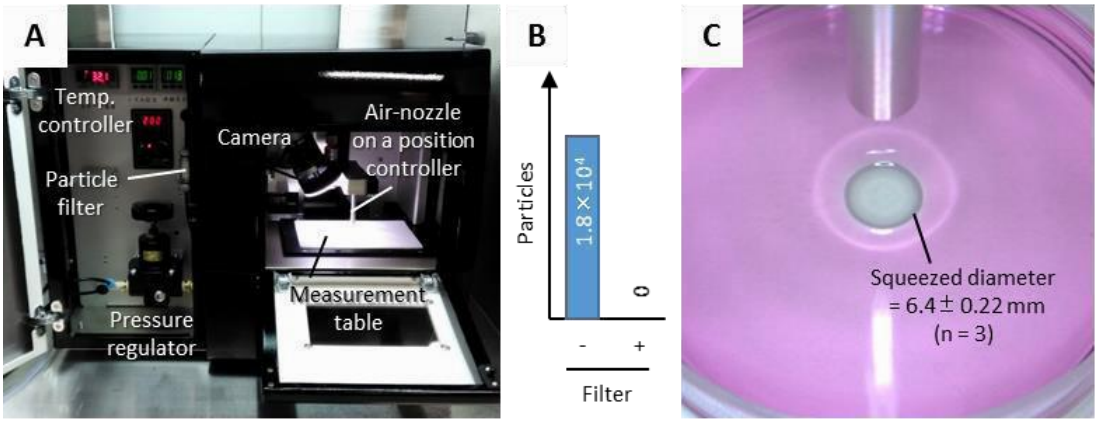

Fig. 1 Non-contact wettability assessment system for cultivated cells. (A) System overview. (B) Particle counting results. (C) Liquid squeezing by air-jet and squeezed diameter determination.

P587 Covalently coated cell stretching devices for osteogenic differentiation of human adipose stem cells

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Cells can sense and adapt to the prevailing mechanical environment *in vivo*. By mimicking such stimulus *in vitro*, the behaviour and differentiation of stem cells can be guided and modelled. Furthermore, new and more effective differentiation methods are needed for tissue engineering applications to make the process faster, more cost-efficient and to avoid the use of expensive and contradictory growth factors.

In the current study, we aimed to investigate the effect of equiaxial stretching on the attachment and osteogenic differentiation of human adipose stem cells (hASCs) using a polydimethylsiloxane (PDMS) based cell stretching device (Figure 1). As a highly hydrophobic material, pristine PDMS does not support cellular attachment, and physisorbed protein coating does not withstand dynamic loading. Therefore, we developed and characterized a durable covalent coating method for PDMS which supports the attachment and viability of hASCs during mechanical stimulation.

The hASCs were cultured under static and dynamic (cyclic equiaxial strain of 2 to 5 %) conditions on covalently coated PDMS substrate up to 10 days. Based on DNA amount and ALP activity analyses, our results indicated that stretching delayed proliferation and promoted osteogenic differentiation of hASCs. Immunocytochemical vinculin detection and actin staining with phalloidin revealed that stretching also reduced the size of the cells and intensified focal adhesions and actin cytoskeleton.

The developed stretching system can be utilized in the future for studying the effect of different stretching parameters on stem cell behaviour. Furthermore, our results suggest that equiaxial stretching could be used as an additional osteogenic differentiation method for hASCs in bone tissue engineering applications in the future.

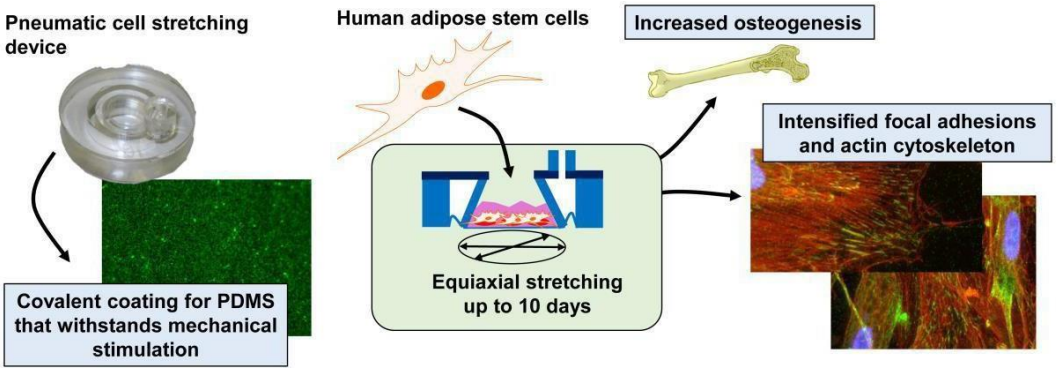

Figure 1. Graphical abstract.

P588 The mechanical stimuli can modulate intracellular calcium oscillation: suggesting pathological model or relevance without chemical cues

Yan Ru Wu[1], Ji Won Shin[1], Yun Gyeong Kang[1], Su Hyang Kim[1], Mi Jin Kim[1], Eun Jin Lee[1], Ji Eun Kim[1], Jung-woog Shin[1,2]

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Introduction: Intracellular calcium ([Ca^{++}]i) oscillation is one of the key parameters in evaluating cellular functions. The ([Ca^{++}]i) oscillation and its modulation has been widely investigated especially in blood vessel endothelial cells (ECs). However, in modulating its dynamic responses only chemical stimuli have been utilized so far. This study adopted hydrostatic pressure to modulate its dynamic responses as ECs are continuously exposed to blood pressure.

Methods: Human umbilical vein endothelial cells (HUVECs) were placed under different pressure conditions: 100mmHg (normal blood pressure), and 200mmHg (hypertension). Fluo-4AM calcium indicator was used for real time recording of [Ca^{++}]i oscillation on a confocal microscope. After 10 minutes baseline recording, the pressure was engaged for 0, 1, 5, 10, 15 minutes. The responses were also recorded for 10 minutes after releasing pressure. All the data were analysed to calculate number of peaks, frequencies, magnitudes by Matlab (R2015a, Mathworks, Inc.).

Results: The results showed [Ca^{++}]i oscillation is sensitive to the magnitude of hydrostatic pressure. The responses after pressure was released were interesting and showed the potential of modulating [Ca^{++}]i oscillation by mechanical stimuli only. When normal pressure was engaged the following responses tended to recover to normal oscillation. However, higher pressure, mimicking hypertension, was engaged the following patterns did not tend to return to normal oscillation. This trend was more observable when the duration of higher pressure engagement got longer.

Discussion: This study showed that mechanical stimuli can modulate [Ca^{++}]i oscillation without any chemical cues. This study and suggested simple method can be extended to investigate various studies in relation to [Ca^{++}]i oscillation. Especially this study is believed to contribute in making [Ca^{++}]i related pathological model oscillation.

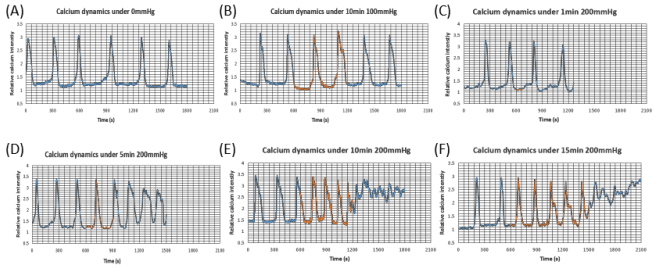

Figure 1. Representative images of calcium oscillation under different pressure: (A) P=0 mmHg.,(B) 100mmHg for 10min. (C) 200mmHg for 1min. (D) 200mmHg for 5min. (E) 200mmHg for 10min. (F) 200mmHg for 15min. Note the red line corresponds to being pressurized.

Acknowledgement: This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea(NRF-2014H1C1A1073148) and by the NRF Grant (NRF-2015M3A9B6073642).

P589 In vitro differentiation of human mesenchymal stem cells into esophageal epithelial cells: a preliminary study for esophageal tissue engineering

Yan Ru Wu[1], Ji Won Shin[1], Yun Gyeong Kang[1], Su Hyang Kim[1], Mi Jin Kim[1], Eun Jin Lee[1], Ji Eun Kim[1], Jung-woog Shin[1,2]

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Introduction: Mesenchymal stem cells (MSCs) have been given particular attention and been used widely due to their multipotency and low immunogenicity. However, the differentiation of MSCs into esophageal epithelial cells has been rarely reported. Moreover, protocol for this has not been established, here we aimed to induce MSCs differentiation into esophageal epithelial cells in vitro.

Methods: Human MSCs (hMSCs, Lonza) were stimulated with different dose of all-trans retinoic acid (ATRA, Sigma) to promote epithelial differentiation. The growth pattern and viability of cells were evaluated by MTT assay and Live/Dead staining, respectively. The expression of epithelial-specific markers (CK8, CK18, P63, and CK4) was assessed by real-time polymerase chain reaction (RT-PCR).

Results: We observed a slower growth rate and good viability when hMSCs were stimulated by chemical agents in all groups. Slower proliferation was found in relatively lower concentration(1uM, 5uM) than in higher concentration(10uM). Increased epithelial-specific markers expression was observed in all ATRA groups by 2 to even 5 times than control, lower concentration showed higher expression than higher concentration group. In all group observable shape changes were not found.

Discussion: The present results suggest that hMSCs can be differentiated into esophageal epithelial progenitor cells under proper chemical stimulation within 2 weeks. Also, the lower concentration less than 5uM was found to provide better condition for differentiation. Further studies for longer cultivation is recommended, especially with proper scaffolds and introduction of mechanical cues to provide biomimetic environments.

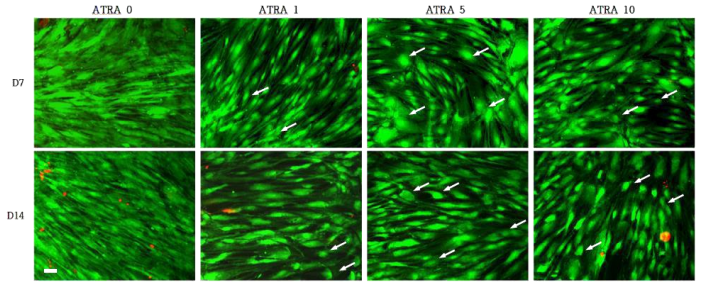

Fig. 1: Cell morphology of hMSCs stimulated by different concentration of ATRA. We can observe that some cells (white arrow labeled) showed squamous morphology, however most cells kept long and thin.

Acknowledgement: This research was supported by a grant of the Korea Health Technology R&D Project through the KHIDI (HI16C0362, the Ministry of Health & Welfare, ROK) and by the NRF Grant (NRF-2014K2A2A7066637).

P590 Multilayered composite scaffolds induce osteogenesis and chondrogenesis in vitro: possible osteochondral potential

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Healing of osteochondral defects is highly challenging. Whereas bone tissue demonstrates a high regenerative potential, cartilage shows poor self-healing capacity. Scaffold-based cell applications show an attractive opportunity in treatment. Yet, it remains challenging to combine the regeneration of both tissues simultaneously on one scaffold. Chitosan and collagen-based scaffolds have shown good induction of MSC chondrogenesis. Calcium-phosphate addition may improve osteogenesis. In this study, we developed a multilayer scaffold for osteochondral regeneration. Our hypothesis is that such layered scaffold could direct the differentiation of stem cells into osteo- or chondrogenesis by mimicking the biomechanical and structural properties of both tissues as well as the interface. A three-layered gradient biomimetic scaffold was developed using a chitosan-collagen composite by freeze-drying. The scaffold was characterized using FTIR, DRX, SEM, and µCT. Calcium phosphate was added to the upper layer (Fig1A). The lower (cartilage) part is composed of chitosan-collagen only with gradual decrease of chitosan content. For cellular assessment, the scaffolds were cut into 3mm cylinders and seeded with human adipose-derived mesenchymal stem cells (hAMSCs) at different densities up to $1x10^6$ cells per scaffold. The scaffolds showed good biocompatibility in vitro as indicated by LDH and live-dead staining after 3, 7 and 14 days. In addition, good cell attachment and proliferation (PicoGreen assay) was observed. Cell seeding density of $1x10^5$ was selected as optimal to support a continuous cell proliferation up to 14 days. This seeding density also supported a homogenous cell distribution through the whole scaffold (Fig1B-D). Alizarin red staining, RunX2, ALP, Coll I and Osteocalcin expression showed that osteogenesis occurred mostly in the upper part of the scaffold (rich in calcium phosphate). Meanwhile chondrogenesis occurred in the lower part of the scaffold as indicated by Safranin O staining, Sox-9, Coll III, aggrecan and versican expression (chitosan-collagen part).

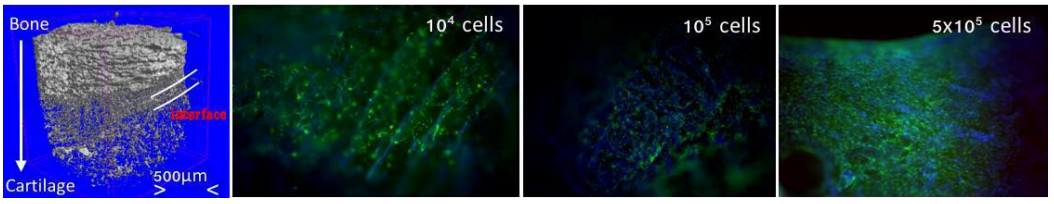

Fig 1. A) µCT scan of the three-layered scaffold. B-D) Live-dead staining of hAMSCs seeded on the scaffold after 14 days of culture.

Our study shows that each layer of the multilayered scaffold presented a specific potential for induction of osteo- or chondrogenesis. Clear tissue-specific matrix deposition and mineralization were detected when scaffolds were cultured for long observation times. Overall, our results indicated the potential of multilayer biomimetic scaffolds for osteochondral regeneration. Moreover, this type of scaffold may represent a valid alternative for the regeneration of the interface between different tissues in TE field.

P591 Human bone marrow stem cells direct seeding in three-dimensional printing scaffolds for bone regeneration in a critical-sized calvarial defect model

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Background: There are many options for critical sized bone defect, including autologous bone graft, allograft, xenograft, artificial materials. However, it has several limitations. There have been many reports that human bone marrow stem cells (hBMSCs) have the potential to undergo osteogenic, chondrogenic differentiation. The use of hBMSCs together with a 3D printed scaffold is an attractive option that may facilitate new bone formation.

Purpose: The purpose of this study is to investigate the osteogenic capacity of undifferentiated hBMSCs, seeded into three-dimensional (3D) printed polycaprolactone - tricalcium phosphate (PCL-TCP) scaffolds of mice.

Materials and Methods: The hBMSCs were isolated from human iliac bone. Nude mice (n = 32) were included in this study. A 5mm sized circular calvarial defects were created in the parietal bone of mice. There were three groups of nude mice in this study: group A, no treatment as a blank control; group B, scaffold alone; group C, scaffold with hBMSCs. Mice were euthanized at 2, 4 and 8 weeks after the procedure. Micro-computed tomographic (Micro-CT) scanning, Immunohistochemistry stain, and Quantitative Real-Time PCR (qRT-PCR) were performed at 2, 4, 8 weeks postoperatively.

Results: Micro-CT images revealed significant differences between PCL-TCP scaffolds with hBMSCs(group C) and the other two groups, as well as between group B and group A. Defect sites implanted with PCL-TCP scaffolds with hBMSCs showed higher staining intensity and had the highest osteogenic-related gene expressions. These results showed significantly improved new bone formation and osseointergration in PCL-TCP scaffold with hBMSCs engrafted calvarial defects, while the other two groups showed little healing.

Conclusion: The hBMSCs enhance the new bone formation without the need for pre-differentiation, and PCL-TCP scaffolds confirmed its suitability for bone tissue engineering. This study supports that hBMSCs direct seeding in scaffolds could be an effective treatment for human skeletal defects.

P592 Bi-layered polymer-reinforced bioprinted composites for osteochondral defect regeneration

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We have recently demonstrated that bioprinted alginate-polycaprolactone (PCL) composite constructs containing bone marrow MSCs could undergo endochondral ossification *in vivo*, supporting the development of a functional bone organ [1]. The objective of this study was to extend this strategy to include an overlaying layer of stable articular cartilage through the additional deposition of a co-culture of fat pad derived stem cells and chondrocytes. We then assessed the capacity of this bioprinted construct to induce regeneration of a critically sized osteochondral defect in a caprine model.

Constructs evaluated following 5 weeks *in vitro* culture demonstrated that the cells within the constructs were still viable (Fig. 1 b,c) and had produced a cartilaginous-like tissue, staining positively for chondral markers. The constructs were then implanted into the femoral condyle (Fig. 1 d,e,f) and repair tissue was observed to form by 6 months macroscopically (Fig. 1 g, h, j). MicroCT analysis revealed that there were non-mineralised regions within the defect, typically at the base of the repair tissue, which corresponded with the presence of residual material from the implant. Histological analysis of the repair tissue demonstrated that robust hyaline-like cartilage could be achieved within the chondral region of the repair tissue, demonstrating the potential of this bioprinted cell-based approach.

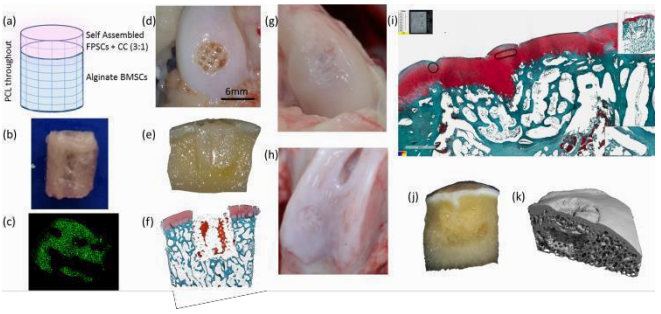

Figure 1. (a) Schematic and (b) macroscopic image of the 2 layers of the bioprinted construct. (c) A confocal microscopy image demonstrated the viability of the self-assembled cells in the top layer of the construct immediately prior to implantation. (d, e, f) Images of the bioprinted construct within the osteochondral defect site at day 0. (g, h, j) Macroscopic appearance of the repair tissue following 6 months. (i) Histological evaluation demonstrated the hyaline-like cartilage layer that formed within the defect site by 6 months (k), while microCT analysis demonstrated the distribution of mineralised tissue matrix within the bone region of the osteochondral defect.

This study provides insight into the regenerative capacity of this bioprinted, cell-based approach, and points to the necessity of ensuring construct degradation occurs over an appropriate time scale. Residual polymeric material was detected in a number of the regenerating defects and appeared to hinder complete remodelling of the bone region. Future work aims to address this issue by enhancing the degradation rate of the bioprinted polymeric component, while maintaining the chondroinductive function of the implant.

[1] Daly *(et al.)*, Adv Healthc Mater, 5(18):2353-2362, 2016.

P593 Additive manufacturing of 3D nanocomposite magnetic scaffolds

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Over the past years, great attention has been devoted to the development of polymer-based scaffolds for the repair/regeneration of damaged tissues. The idea to export the basic principles of magnetism to tissue engineering has opened a fascinating research. In the design of 3D nanocomposite magnetic scaffolds, the main goal is to obtain structures able to attract bioaggregates or cells stimulating tissue regeneration, by means of magnetic force gradients. 3D well-organized cylindrical poly(ε-caprolactone) (PCL) and PCL/magnetic nanoparticles (MNPs) nanocomposite scaffolds were manufactured through 3D fiber deposition technique by alternatively plotting fibers along selected lay-down patterns. 3D mathematically defined porous polyethylene glycol diacrylate (PEGDA)/MNPs scaffolds with a diamond architecture were prepared through stereolithography technique. Furthermore, hybrid coaxial and bilayer magnetic scaffolds were produced by combining such techniques. The performances of the designed scaffolds were assessed by means of experimental/theoretical investigations. In particular, Micro-computed tomography (Micro-CT) and scanning electron microscopy (SEM) were employed to assess morphological features. Mechanical analyses were performed at different scale levels through nanoindentation, tensile and compression tests. In order to study the effect of a static and a time-dependent magnetic effect on cell adhesion/spreading, human mesenchymal stem cells (hMSCs) were statically seeded. Micro-CT and SEM analyses showed that 3D fiber deposition and stereolithography are able to manufacture morphologically-controlled scaffolds. Results from compression and tensile tests highlighted that beyond a specific limit of MNP amount, by further increasing the nanoparticle concentration, the mechanical features of the nanocomposite scaffolds decrease. In order to locally map mechanical properties, nanoindentation tests were performed showing interesting results in terms of hardness and reduced modulus. Magnetic analysis revealed a superparamagnetic behavior of these materials. Results obtained from the biological analyses showed that prolonged exposure time to the static or dynamic magnetic field seems to negatively affect cell viability. Confocal analyses highlighted an increase in adhered number and a more evident spreading of hMSCs. On the other hand, the Alamar Blue assay and ALP/DNA measurements provided a quantitative evaluation of cell viability/proliferation and differentiation, respectively.

P594 Design of additive manufactured structures with functional gradients for interface tissue engineering

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Interfacial tissue engineering (ITE) purpose is tissue repair/regeneration by mimicking continuous biological and chemico-physical gradients. A 3D scaffold with a biomolecule gradient could be considered as a valuable candidate to reproduce some of the important tissue properties. The main goal of this research was to optimize a two-step functionalization procedure in which a 3D well organized poly(ε-caprolactone) (PCL) scaffold was aminolysed with a continuous gradient of amino-groups (NH_2) and successively, a collagen gradient was achieved through carbodiimide reaction. 3D scaffolds were produced by means of an additive manufacturing technique. As it is widely recognized, PCL has been employed for tissue engineering but it lacks of biological motifs for cell recognition. The aminolysis represents a simple way to covalently graft NH_2 groups, free to interact with other biomolecules. To this aim, PCL surfaces were modified by dipping the structures in a 1,6-hexanediamine/isopropanol solution. NH_2 density gradient was obtained along the length of the structure by controlling the time and the surface exposure to the reactive solution. Collagen type I was covalently immobilized using a carbodiimide reaction. Ninhydrin and hydroxyproline assays, contact angle measurements, confocal laser scanning microscopy (CLSM), Fourier transform infrared spectroscopy (FTIR), collagen staining and biological analyses were performed to characterize the surfaces and gradients.

The NH_2 concentration spans from $5*10^{-7}$ mol/cm^2 (15 min processing) to $15*10^{-7}$ mol/cm^2 (45 min processing). The PCL-NH_2 structures were treated with Rhodamine B Isothiocyanate to visualize the gradient by using CLSM. Results showed an 8-fold increase of fluorescence intensity from the neat PCL to the region aminolysed for 45 min. Results from hydroxyproline assay revealed that the immobilized collagen amount increases with the NH_2 concentration. Furthermore, the collagen presence improves the hydrophilicity of the materials. Microscopy imaging highlighted the collagen distribution whilst FTIR confirmed its grafting. Biological analyses showed that MG63 are better adhered and spread on collagen-based materials.

In conclusion, a method to obtain a collagen gradient on 3D morphologically-controlled PCL scaffolds was successfully proposed. Future trends will be devoted to the development of 3D additive manufactured scaffolds characterized by both chemical and morphological gradients.

P595 Protein surface modifications for cell membrane attachment in tissue engineering and regenerative medicine

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Tissue engineering encompasses the utilization of biochemical and physical factors to create structures that mimic native tissues serving to improve or replace damaged cells, tissues and organs. Hypoxia is a continuous problem in the development of large 3D osteocyte and chondrocyte tissues *ex vivo* for autologous transplantation. This is due to the inherently poor diffusion co-efficient of oxygen in water in conjunction with an inability to successfully mimic *in vivo* vascularization limiting the size of viable constructs and subsequent integration with host tissues. To address this problem we have developed surfactant conjugated haem-protein nanoconstructs capable of binding to the surface of human mesenchymal stem cells (hMSCs). These nanoconstructs provide an additional reservoir of oxygen and thereby limit the onset of central zone necrosis typically observed in large scale tissue engineering. Chemical modification (cationization) of the protein (myoglobin) surface has been shown to increase oxygen affinity meaning oxygen is only released in response to the onset hypoxia with no large scale changes in secondary structure observed. Subsequent conjugation with an amphiphilic surfactant enables priming of the hMSC surface in 2D cultures without impacting differentiation or inciting significant cytotoxicity with membrane localization persisting over several days. Primed hMSCs are able to undergo 3D tissue engineering using standard fibronectin-coated PGA scaffolds towards osteocyte and chondrocyte lineages with a reduction in the extent of central zone necrosis yielding constructs of a significantly larger size and quality. Future functionalization with other relevant proteins such as growth factors and enzymes in addition to the priming of differing cell types or alternatively scaffolds may enable improvements in directing differentiation and the development of extra cellular matrices to generate constructs more akin to native tissues.

P596 Two-cell bioprinting for bone tissue engineering application

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3D printing technology is becoming widely prevalent because of its achievable control on a range of biomaterials as well as cells[1]. Co-culture of mesenchymal stem cells (MSCs) and endothelial cells (ECs) has shown higher osteogenic differentiation potential compared to MSC monocultures[2]. Combining these cells with three-dimensional (3D) biomaterial scaffolds provides a promising strategy for engineering bone tissues. However, the cells are organized as needed in tissues by chance rather than by engineering. Three-dimensional (3D) printing can be used to position these two types of cells with biomaterials to produce a 3D structure for use as scaffolds[3]. The aim of this study is to achieve enhanced osteogenesis on a 3D printed alginate based hydrogel scaffold using MSCs and ECs, which would be bio-printed directly. MSCs and ECs were obtained from umbilical cord by enzymatic digestion. Bioink was prepared by mixing cells with hydrogel. Alginate based scaffold was printed using a 3D-bioprinter (Biobot, USA). The 3D printed scaffold was checked for cell viability using FDA/PI staining. Cell proliferation was assessed by Alamar Blue assay. The osteogenic differentiation potential and DNA quantification studies are also evaluated. Our preliminary results show good cell viability on 3D printed alginate based scaffolds (Figure 1). Both the cells survived for few weeks and could be positioned as per the requirement. We anticipate that the findings of this study will show the application of 3D bioprinting technology in the field of bone tissue engineering, by achieving enhanced osteogenesis using two cell types, on our 3D printed alginate based scaffold.

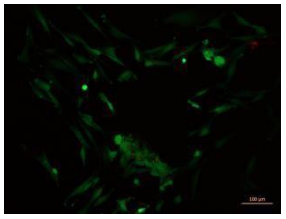

Figure 1: FDA-PI staining of 3D printed scaffold showing viable cells

P598 Steps forward to bioprinting of vascularized bone tissue equivalents

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Bone tissue is one of the most frequently transplanted tissues. Since procedures like the transplantation of autologous bone bear risks, though, regenerative medicine and tissue engineering reach to face those problems by engineering bone substitutes by using suitable materials and living cells. A crucial factor is the vascularization of the constructed tissue to ensure supply of the included cells with nutrients and oxygen. For the fabrication of such vascularized bone tissue equivalents, evolving manufacturing techniques like bioprinting can be used to construct geometrically defined three-dimensional structures.

We developed bioinks on basis of methacrylated gelatin that can - by further addition of hydroxyapatite - either support the osteogenic differentiation of stem cells and formation of a bone matrix, or the formation of vascular structures by endothelial cells. The inks and resulting hydrogels, whose material properties like swellability, viscosity and elasticity can be adjusted to the requirements, were characterized. Additionally, the bioactivity of the hydrogels regarding the support of vessel-formation (Fig. 1A) and osteoconductivity (Fig. 1B) was verified. The bioinks were then used to build up various geometries (Fig. 1C) via a microextrusion-based printing system, which were afterwards cultured for up to four weeks under suitable co-culture conditions. Evaluation of the hydrogels by mechanical analysis and staining of specific proteins and structures like collagen, proteoglycans, PECAM-1 and markers of basal lamina showed formation of a bone matrix, as well as the extensive generation of capillary-like hollow structures. Those processes were significantly increased in structures where bone and endothelial cells were co- cultured, in comparison to monocultured cells. Additionally, we could show the successful perfusion culture of printed constructs made of multiple bioinks and cell types, allowing the build- up of structures with higher dimensions (Fig. 1D).

In conclusion, we were able to develop bioinks and a printing process which allow the successful build-up of bone tissue equivalents containing capillary-like structures in relevant sizes and geometries.

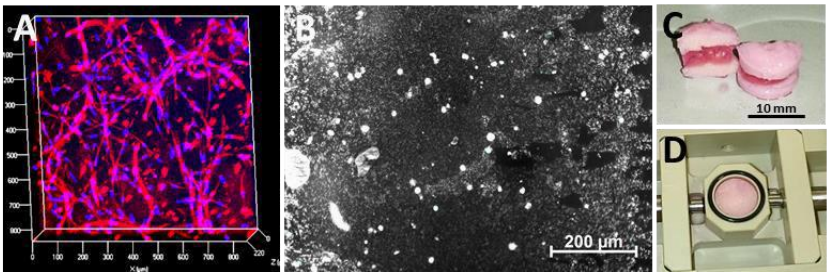

Figure 1: (A) Formation of extensive vascular networks in the developed hydrogel after 7 days culture (red: PECAM-1; blue: DNA). (B) Collagen-staining (white) in hydroxyapatite-modified hydrogel after 28 days of culture. (C) Bioprinted cell-laden construct made of two different hydrogels. (D) Perfusion-culture of a printed hydrogel construct.

P599 In vivo analysis of the 3D-scaffold biodegradation according to the level of autofluorescence

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One of the most important criteria in bone graft engineering is the scaffold biodegradation rate. There have been many studies in which the biodegradation of scaffolds has been measured in vitro. But there is only a small number of studies offering noninvasive methods of controlling the rate of biodegradation in vivo. The fluorescence imaging of implanted scaffold could be a convenient method to access the scaffold biodegradation in vivo.

During the two-photon polymerization of scaffolds (2PP-scaffolds) the fluorescent nontoxic fragments are included into the resulting scaffold structure. The fluorescence analysis of the 2PP-scaffolds' biodegradation was performed using the ImageJ 1.43u software (NIH, USA). In the fluorescence images the area of each defect with the implanted scaffold was identified, and the 'Integrated density' parameter was calculated. This parameter can be used as a criterion for evaluation of the scaffold biodegradation according to the autofluorescence level. The area of the scaffold and the proportion of the new bone were measured with the 'Area' parameter.

After the implantation into cranium of mice, the 2PP-scaffolds represented high autofluorescent structures with clear borders. After 5 weeks, the autofluorescence level decreased by nearly a half of its initial value. The area of the implanted 2PP-scaffolds was also decreased to 75.3%. By 10 weeks, the area and the level of the autofluorescence signal from the 2PP-scaffold had decreased by three-times compared with the week 5. Thus, such strategy of scaffold biodegradation assessment according to the level of autofluorescence can provides an effective and long-lasting method of assessing the rate of the scaffold biodegradation and the native tissue formation in vivo.

P601 On demand fabrication of bone grafts by direct ink writing: from tomography to patient specific implants

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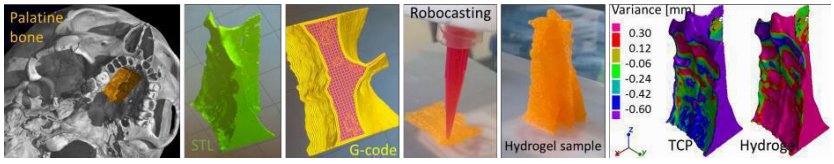
Introduction: Bone defects are traditionally treated with generic shape implants and several times the adaptation of the bone defect to the implant is required. A real progress in bone reconstruction would come with the custom manufacturing of bone implants with adjusted structure that provides both mechanical reliability and optimal bone regeneration. The aim of this work is to show that current progress in 3D-imaging and robocasting allow the fabrication of patient specific bone implants.

Materials and methods: Tomographic images (voxel size of 15 μm) of archaeological bones were used as model of real clinical images. The palatine bone was virtually dissected from the skull and converted into stereo lithography (STL) file using VG Studio software. The threshold value in the histogram was carefully selected to produce a virtual surface free of errors. Afterwards, using a G-code file generator (Slic3r), the printing settings were stablished, *i.e.* fill pattern (rectilinear) and fill density (50 %). The G-code was loaded in the robocasting device (*Pastecaster*) and the palatine bone was printed using either beta tricalcium phosphate (TCP) paste (10 mm/s at 20 °C) or alginate-gelatine hydrogel (5 mm/s at 28 °C), using a 250 μm nozzle. While TCP was sintered during 30 min at 1100 °C, hydrogel was crosslinked with $CaCl_2$ solution followed by carbodiimide hydrochloride (EDC). Printed bones were analysed by microtomography (GE phoenix v|tome|x) and accuracy of the reconstructions was determined against the original STL file.

Results and discussion: Figure shows the progress of the fabrication from the skull to the geometric comparison. Dimensional variance with respect the original file is shown in colour code: green indicates good fitting, red and blue, positive and negative deviations.



From left: tomographic image of skull, STL file of palatine bone, preview of fill pattern, robocasting, hydrogel sample and dimensional analysis for TCP and hydrogelsamples.

Additive manufacturing will bring benefit to patients when incorporated into the clinical practice, its combination with clinical tomography or magnetic resonance imaging will allow the clinicians to design, select from a variety of materials and fabricate by themselves on demand grafts. For example, bone grafts with accurate dimensions were fabricated either with a bioactive ceramic or a hydrogel. Consolidation of the grafts might be also possible in the health care centre, using standard dental ceramic sintering furnace or biofriendly crosslinking agents. In case of ceramics, sintering slightly reduces the size of the printed graft, in contrast, hydrogel swelling produce the opposite effect. Although that moving from concept to clinical use requires training, translational research and proper regulatory frame, the current state of the technology makes this change possible. Furthermore, additive manufacturing equipment is currently becoming more affordable.

P602 Development of flexible hybrid films for tissue engineering

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Introduction Polymer-ceramic hybrid biomaterials were developed by a mechanism of self-crosslinking of elastic polymer and rigid ceramic particles for their applications in tissue engineering materials. Diverse tissues and organs require biocompatible biomaterials with different mechanical properties and flexibility when they applied in tissue engineering. As examples, while bone tissue engineering requires biomaterials with high mechanical properties, cartilage tissue engineering used polymeric biomaterials with medium mechanical and flexible properties. The biomaterials were expected to show elasticity and strength depending on their roles as a biomaterial and applications in engineering of diverse tissues.

Experiments Glycosaminoglycan solution was mixed with ceramic solution at defined ratios and concentrations. While the hybrid samples in films and hydrogel were chemically analysed by FTIR, UV-Vis and NMR, they were also biologically evaluated by cell responses such as cell adhesion and proliferation well as cell toxicity by using bone cells. Releases of diverse bioactive molecules such as bovine serum albumin, tetracycline and DMOG were tested by loading them in the biomaterials in medium. The samples showed excellent mechanical and chemical properties as observed. Their biological properties were also very good as observed by CCK-8, cell live and dead assay. The loaded bioactive molecules were released over time. The fabricated samples showed excellent properties in terms of biological activity and mechanical properties for tissue engineering.

P603 Adhesive, remineralising and antibacterial composites for vertebral fracture repair

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Introduction: Vertebral compression fractures are a common complication of osteoporosis and cancer metastases and across Europe, osteoporotic vertebral fracture prevalence is 12% of the population. Vertebroplasty is the recommended treatment and involves injection of PMMA cement into the vertebra under fluoroscopic control. However there are a number of limitations to this approach including difficult to control mixing / setting, high heat and shrinkage during polymerisation, limited bonding to bone, low material strength, high modulus, and infection risk. Recently, light cured dental composites with added apatite bonding monomers, monocalcium phosphate monohydrate (MCPM) for collagen remineralisation and antimicrobial polylysine (PLS) release have been developed. The aim of this study was to produce an equivalent dual paste chemical curing composite with setting and mechanical properties suitable for vertebroplasty.

Methods: Experimental composite formulations consisted of dimethacrylate monomers with chemical activated initiator / activator. The powder phase contained glass fillers, calcium phosphates and PLS. Monomer conversion and calculated polymerization shrinkage were assessed using FTIR-ATR (n=3). Biaxial flexural strength and modulus of elasticity after 24hr immersion in water were also obtained (n=8).

Results: Addition of glass fillers of appropriate size (7, 0.7µm) ensured that the composites have good handling properties and could be rapidly mixed from a double-barrelled syringe using a fine automatic mixing tip. The setting kinetics of the composites can be finely controlled to give a suitable inhibition period (60 secs- 5 mins) to provide time for injection. This is followed by rapid set and high monomer conversion (70-80 %) to minimise leakage and cytotoxicity problems respectively. Shrinkage during polymerisation never exceeds 5% and heat generated reaches a maximum 0.1 KJ/cc both of which are lower than for PMMA cements. The set materials display moderate strength of approximately 90MPa to ensure fracture stabilisation and at the same time relatively low modulus (~2GPa). High strength helps aid stabilisation whilst lower modulus reduces cement stiffness and adjacent bone fracture due to stress shielding.

Conclusion: Experimental bone composites exhibited higher monomer conversion and strength with lower modulus. These may help reduce the risk of toxic monomer release and adjacent vertebral fracture. These composites are therefore a promising material for vertebroplasy. Further studies will aim to demonstrate biocompatibility and anti-bacterial properties.

P604 Evaluation of injectable gellan-gum hydrogel for articular tissue cartilage regeneration

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INTRODUCTION: Gellan-gum is a water-soluble anionic polysaccharide produced by bacterium Sphingomonas elodea(formerly Pseudomonas elodea), and widely used in tissue engineering owing to its a biocompatibility, acid resistance, enzymatic resistance, biodegradability. But it is boiled at least 90℃for perfectly dissolution. In this study Gellan-gum soulution at 1, 2, 3wt% were used. Gellan-gum is highly sensitive to temperature and rapidly gelation at RT. we maintain temperature 45℃ before cell seed. Injectable Gellan-gum had possibility of applications for future usage in cartilage tissue engineering.

METHODS: Injectable Gellan-gum scaffolds at 1, 2, 3wt% were used. All samples characteristics were studied by FTIR, compressive strength, porosity and SEM. Rabbits chondrocyte cultured in scaffold was evaluated by Confocal Confocal Laser Microscopy (CLM), MTT assay, RT-PCR, SEM.

RESULTS: The gellan-gum is stored in a syringe to maintain the gelling of gllan gum constantly we observed. no significant difference in cell differentiation for 1,2,3% Gellan-gum. 2% gellan gum can be used as injectable because the rate of gelation at room temperature is slow

1 wt% 2 wt% 3 wt%

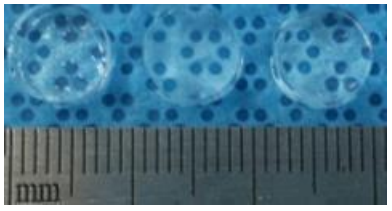

Fig. 1: Gross image of 1, 2, 3 wt% Gellan-gum hydrogel.

DISCUSSION & CONCLUSIONS: We found that Gellan-gum hydrogel of 2wt% solution can be used injectable when Gellan-gum maintain constant temperature.

ACKNOWLEDGEMENTS: This research was supported by Bio-industry Technology Development Program(KMFAFF, 112007-05-4-SB010), Technology Development Program(814005-03-2-HD020)

P605 Surface influence of bioactive calcium phosphate- or calcium carbonate-containing silica/collagen xerogels on osteoblasts, osteoclasts and macrophages

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Aim. The implantation of biomaterials into bone is a complex process regarding immune reaction as well as its influence on osteoblasts and osteoclasts. The study aims to smart materials which are able to downregulate the release of pro-inflammatory cytokines by macrophages. Moreover these smart materials should act on osteoblasts and osteoclasts in terms of bone formation and resorption that can be affected by the material surface and/or the ion concentrations of their environment.

Methods. A homogenous suspension of fibrillar bovine collagen was mixed with tetra-ethoxysilane, which was hydrolysed to orthosilicic acid, (B30) and a calcium phosphate phase (B30H20) or a calcium carbonate phase (B30CK20). A xerogel resulted after atmospheric drying of the intermediate hydrogel. Studies concerning calcium release, bioactivity and degradation of xerogels were performed. Human mesenchymal stem cells were cultivated and differentiated on xerogel samples of different composition to investigate osteogenic differentiation. Monocytes, isolated from human buffy coats, were used to perform osteoclastogenesis or were matured and polarized into active macrophages. Biochemical methods were applied for quantitative analysis of cell behaviour and laser scanning microscopy as well as scanning electron microscopy for qualitative analysis. *In vivo* studies in rats were performed using metaphysial defect model.

Results. The xerogels B30, B30H20 and B30CK20 showed bioactive behaviour with regard to consuming calcium and phosphate ions from their environment, overlaid by a calcium release for B30H20 and B30CK20. Osteogenic differentiation was influenced by calcium concentration mediated by xerogels. Despite of highest calcium concentration for B30, proliferation and osteogenic differentiation was enhanced for B30CK20 compared to that of B30 and B30H20. The formation of multinucleated cells was observed on all xerogels. After 21 days, B30 and B30CK20 were partly fragmented in presence of osteoclast-like cells. Osteoclast-specific marker TRAP 5b was affected by xerogel surface.

Xerogels B30 and B30H20 showed smaller surface pattern, B30CK20 showed larger pattern. Moreover xerogel granules with particle sizes of 425-710 µm and <125 µm, respectively, were embedded in a collagen matrix to adjust larger and smaller pattern, respectively. Pattern of different scale were investigated with regard to macrophage polarization. During maturation of monocytes to macrophages pro- (M1 phenotype) and anti-inflammatory (M2 phenotype) cytokines were released. After 7 days of culture, pro-inflammatory cytokines interleukin (IL)-1β and IL-6 were downregulated for samples with larger pattern compared to smaller ones.

Impact of the investigation. The study is a step further in creating smart materials which influence osteoblasts, osteoclasts and macrophages by direct cell-material-interaction. Ion release from xerogel, its composition and morphology play a key role in affecting reaction of cells cultured on the material surface. Moreover we could show for the first time that macrophage polarization works on organic/inorganic composites with irregularly shaped pattern.

P606 A novel micro-computed tomography procedure for the quality control of personalized 3D-printed implants: an illustration using stereolithography-printed scaffolds

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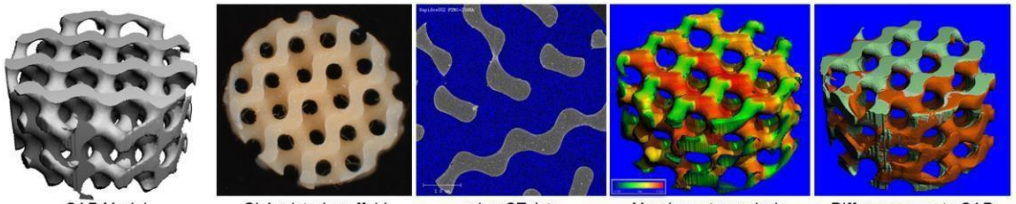
Additive manufacturing has shown great potential in the field of biomedical science. This technology offers a relatively simple means to manufacture objects with complex structural features at high spatial resolution. It is therefore ideally suited for manufacturing patient specific implants. Successful clinical reports of patient specific implants manufactured using 3D printing foster great promises, particularly in the field of craniomaxillofacial reconstruction. However, along with the ability to produce highly defined prostheses, the community still needs tools to ensure the quality of the final products.

Here, we propose the use of high resolution micro-computed tomography (microCT) as a quality control (QC) tool for personalized 3D-printed implants. MicroCT has become a relatively mainstream technology, with specifications covering most implant dimensions and capable of resolving the smallest 3D-printed structures.

As a proof of concept, we analysed fifteen 3D macroporous composite scaffolds (Ø 6 x 3.5 mm) were fabricated using a stereolithography (SLA) system (Envisiontec Perfactory[3]® SXGA[+]) with photo-crosslinkable resins based on poly(trimethylene carbonate) and various mineral (hydroxyapatite, HA) content. After production, the SLA-fabricated scaffolds were microCT scanned at 3 µm resolution (uCT100 SCANCO); their shape and morphometry were characterized from the image data. Then, SLA's degree of precision was quantified by registering microCT scans to the computer model and mapping the differences.



We found that with 20% HA, most morphometric parameters where within acceptable limits. The scaffolds' mineral content was found to have a statistically significant impact on morphometry, shape and homogeneity, but it did not have an influence on correlation factors between scaffolds and computer model.

In conclusion, additive manufacturing technologies hold great promises in the field of tissue engineering, and particularly in patient-specific implants. However, before large scale clinical studies can be foreseen, thorough quality processes need to be implemented. As perspective, the development of micro-computed tomography QC protocols would help ensuring the quality and precision of personalized implants preoperatively in a sterile and non-destructive way, and as such, has the potential to improve surgical outcomes.

P607 Titanium reinforced PLGA / collagen multi-layered osteochondral scaffold for repair of large osteochondral defects

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Osteochondral defects (OCDs), typically generated by repetitive trauma within the joint may alter the architecture or composition of the bone[1]. For large defects, there is currently no available effective treatment apart from joint replacement. We have developed a multi-layered osteochondral (OC) scaffold based on Titanium matrix reinforced PLGA/collagen system for large OCDs, and evaluated its mechanical stability and *in vitro* performance.

The Ti framework was fabricated by a 3D rapid prototyping technique, and the porous framework was filled with a collagen solution, reinforced with PLGA. The mechanical property of the resultant scaffold was tested, and its *in vitro* performance was evaluated in terms of cell attachment, morphology, viability and proliferation using sheep bone marrow mesenchymal stem cells. The *in vivo* performance was assessed in a clinical dog shoulder model.

It was observed that the scaffold has a gradient structure with respect to the mechanical properties, composition and porosity. The Ti matrix has a pitch size of 0.75mm with a porosity of 72%. It exhibits a compressive strength of 35 MPa and a Young's modulus of 0.55GPa which is within the range of trabecular bone. The *in vitro* cell assays performed on collagen/PLGA, PLA and Ti scaffolds confirmed their biocompatibility. *In vitro* evaluations revealed that the cells attach to the scaffold and colonize it uniformly as confirmed by staining of actin cytoskeleton and SEM examinations. The Live/Dead examination showed the viability of cells throughout 28 days of culture. It was observed that the scaffold supports cell proliferation shown by the continuous increase in alamarBlue activity over 28 days. In vivo evaluation in a clinical dog shoulder model has demonstrated that a stable fixation of the scaffold and new bone ingrowth into bone section of Ti matrix after 12 weeks post operation. The arthroscopic examination revealed cartilage regeneration and integration with the surrounding tissue, matching the curvature of the shoulder joint. We believe that the developed OC scaffold has the strength needed to bear the physical loads of the joints and the structure encourages consistent cartilage fill and a smooth cartilage surface.

In conclusion, we developed a multi-layered OC scaffold based on Ti/PLA matrix reinforced with PLGA/collagen system with suitable mechanical and biological properties. The *in vivo* evaluations demonstrated that the scaffold forms a strong mechanical fixation to the surrounding tissue and provides an appropriate physical environment to support the overlying cartilage heal.

Acknowledgement: This work was financially supported by The Arthritis Research UK (grant no: 21160) and Rosetrees Trust (project no: A1184).

P608 The influence of mechanical properties of 3D bioprinted hMSCs-laden alginate composite scaffolds on cell viability and morphology

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Accumulating evidence has shown that extracellular matrix (ECM) mechanical properties can modulate stem cell adhesion, migration, proliferation, differentiation, and signalling. However, most studies have focused on classical two-dimensional (2D) or quasi-three-dimensional environments, which cannot represent the real situation *in vivo*. Most of the current methods used to fabricate different mechanical properties invariably change the fundamental structural properties of the scaffolds, such as pore size and porosity. In our study, by using 3D bioprinting, we have developed novel 3D hMSCs-laden alginate (alg) composite scaffolds with different degrees of compressive modulus but with the same 3D microstructure to investigate the effect of scaffold mechanical properties on cell function. Firstly, a printing solution was produced by dissolving alg (0.8%, 1.3%, 1.8% and 2.3% (w/v)) in gelatine solution (4.09%(w/v)). Scaffolds were printed to generate cubic structures with dimensions of 10mm×10mm×2.5mm. The compressive moduli of the scaffolds were then calculated between 5% and 15% strain and resulted to 1.49±0.48, 5.67±0.13, 9.58±0.48 and 14.25±0.55 kPa, respectively, decreasing with culture time (figure 1A). One day after printing, all scaffolds showed similar microstructure and uniform interconnected macrospores of 500-600µm in diameter. To evaluate cell viability, a bioink was produced by mixing human mesenchymal stem cells (hMSCs) at a density of 5Mio/ml with each of the four printing solutions. Results showed that the cells survived the bioprinting process with high cell viability up to 14 days, and compressive modulus significantly influenced cell viability (figure 1B). More importantly, we found different cell morphologies for different alg concentrations. hMSCs spread inside the 0.8% alg scaffolds (figure 1C), grew along the pores and connected to each other on the surface in 1.3% alg group (figure 1D) or grew across pores in the 1.8% alg group (figure 1E). In contrast hMSCs were rounded and had very short processes in the 2.3% alg group (figure 1F). These findings demonstrate the importance of the 3D mechanical scaffold properties for cell viability and morphology. Based on these finding, further studies will focus on the influence of mechanical properties on osteogenic cell differentiation.

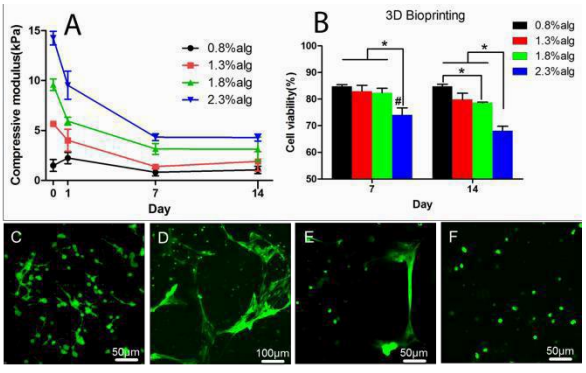

Figure 1 A) Compressive modulus changes in the different alginate composite scaffolds over a 14-day culture period. B) hMSCs viability in printed scaffolds at 7 and 14 days, *P<0.05, # P<0.05 the same group at different time points. C) Z projection of confocal GFP images showing hMSCs morphology within a 0.8%alg scaffold at 14 days. D, E, F) Confocal GFP images showing hMSCs morphology within 1.3%,1.8% and 2.3%alg scaffolds at 14 days.

P609 Evaluation of human hyaline and elastic chondrocyte differentiation in nanostructured fibrin-agarose hydrogels

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Introduction: Cartilage is a specialized connective tissue which provides structural support to several organs and joints. There are three types of human cartilage –hyaline, elastic and fibrous- with specific tissue distribution and different biomechanical, chemical and functional properties. The structure and function of cartilage can be affected by several pathologies, including trauma, congenital defects and neoplasm. Unfortunately, cartilage is characterized by a limited regeneration capability and the efficacy of the current surgical treatment is not optimal, and may be not always available. The aim of this study is to encapsulate human hyaline and elastic chondrocytes into natural nanostructured fibrin-agarose hydrogels (NFAH) and to evaluate the behaviour of the cells included in the NFAH by using histochemical and immunohistochemical techniques.

Methods: Human hyaline and elastic chondrocytes were isolated from small biopsies obtained from healthy donors. Cells were expanded and then encapsulated in fibrin-agarose hydrogels. Once encapsulated, constructs were nanostructured and kept in culture. After 1, 3 and 5 weeks, three constructs of each type were harvested, fixed in 10% neutral buffered formalin, included in paraffin and cut in 5 µm sections. Haematoxylin-eosin was used for histological evaluation. S- 100, cortactin and PCNA were evaluated by immunohistochemistry. The extracellular matrix (ECM) was characterized with alcian blue staining and orcein histochemistry (in the case of elastic chondrocytes), whereas collagen II, aggrecan, biglycan and versican were identified by immunohistochemistry.

Results: Histology revealed that encapsulation of both cell types in NFAH was successful. PCNA immunohistochemistry confirmed that hyaline and elastic chondrocytes were able to proliferate and increased their number over the time. Cells expressed S-100 and cortactin. Alcian blue staining was weak in constructs containing both cell types at all time periods, with orcein being positive in the case of the elastic cartilage. Collagen II and aggrecan expression were more abundant in constructs made with elastic chondrocytes than in tissues containing hyaline cartilage cells, but the rest of proteoglycans analysed here showed similar results.

Discussion and conclusions: This study demonstrated that NFAH may be successfully used for the encapsulation of human hyaline and elastic chondrocytes, and these cartilage constructs showed promising results. The immunohistochemical analyses of the constructs ECM demonstrated that elastic chondrocytes were able to produce more essential molecules than hyaline cartilage with time, suggesting that elastic cartilage could be reproduced in the laboratory with higher efficiency than other cartilage types. Future cell differentiation and molecular studies are in need to generate more efficient hyaline and elastic cartilage substitutes.

Acknowledgements: This study was supported by Fundación Progreso y Salud, Consejería de Salud, Junta de Andalucía, Spain, Grants PI-0653-2013 and PI-0401-2016.

P610 Effects of FGF18 on OA human articular chondrocytes embedded in a novel FBG:HA hydrogel

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Introduction. Current treatments for cartilage lesions such as microfracture, osteochondral graft and autologous cell implantation are often associated with fibrocartilage formation, donor site morbidity and further OA progression.[1] Hence, there is an interest in the development of new approaches for cartilage repair, such as the use of hydrogels as cell carrier platforms and the controlled delivery of bioactives that promote matrix production and tissue healing.[1] Herein, we describe the use of novel Fibrin:Hyaluronic Acid (FBG:HA) chemical conjugate-based hydrogel embedded with primary Human articular chondrocytes and Fibroblast Growth Factor 18 variant (FGF-18v) for enhanced chondrocyte differentiation and matrix deposition. **Methods.** Chondrocytes were obtained from patients undergoing total knee replacement, embedded in the hydrogel (500000 cells per 150 µl hydrogel) and cultured for 7 days in chondrogenic medium. At day 7 cell viability was measured by Live/Dead assay and confocal microscopy. Additionally, chondrocytes were cultured in the hydrogel for 14 days in the presence of 1 and 10 ng/mL FGF-18v. As controls cells were cultured without growth factor or in the presence of 10 ng/mL of FGF2. On day 14, IHC was performed for Type II Collagen and Aggrecan, and gene expression levels were evaluated for COL2A1 and MMP1.**Results.** Chondrocytes embedded in the FBG:HA hydrogel were shown to have a high viability at 7 days of culture. Confocal microscopy showed cell spreading and presence of focal adhesions. Gene expression studies revealed a 2-fold increase in type II collagen expression and a 70 % reduction in the expression of MMP1, in response to FGF-18v but not by FGF-2. Immunohistochemistry for type II collagen confirmed over production of collagen II by cells cultured with 10 ng/ml FGF-18v. GAG quantification did not show significant differences between FGF-18 treated cells and controls.

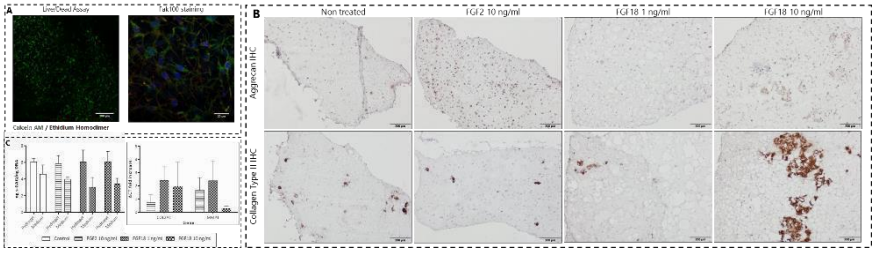

Figure 1. A) Cell viability and morphology of OA chondrocytes cultured in FBG:HA hydrogel for 7 days. B) Aggrecan and Type II Collagen IHC for OA chondrocytes cultured for 7 days in FBG:HA hydrogel without growth factors, 10 ng/mL FGF2, 1 ng/mL FGF18 and 10 ng/mL FGF18 (from left to right). C) DMMB assay and PCR for COL2A1 and MMP1 genes.

Conclusions. We report a significant effect FGF-18v on FBG:HA hydrogel 3D culture of chondrocytes demonstrating an anabolic effect on cartilaginous matrix production. This approach could bring new insights into the development of therapies for the treatment of cartilage lesions and Osteoarthritis.

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P613 Osteochondral tissue regeneration with biofunctional hydrogel scaffolds based on MSCs embedded in an Elastin-Like Recombinamers matrix

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Musculoskeletal disorders include a massive number of severe diseases, syndromes and injuries generally painful, that progress with time to degenerative states such as osteoarthritis. One of the most common solution for critical cases is total joint replacement by prosthesis. There is a great interest in developing new therapies to achieve an optimal osteochondral tissue repair such as biomaterial-based regenerative therapies, in which Elastin-Like Recombinamers (ELRs) play an essential role.

ELRs are synthesised by recombinant DNA techniques and they are able to self-assemble into different structures, of which hydrogels are the most promising for tissue regeneration [1]. Moreover, according to the elastin-like nature of the hydrogel and to the high percentage of elastin present in the native chondral matrix, ELR-based hydrogels, are likely to be similar to hyaline cartilage [2]. The hyaline articular cartilage is a highly specialized tissue characterized by its unique mechanical properties and is formed by a matrix in which chondrocytes are embedded. In this study, injectable hydrogel-forming ELRs containing RGD cell-adhesion sequences have been bioproduced in *E. coli* and further investigate by physicochemical characterization of the materials by SDS-PAGE, MALDI-TOF and DSC to determine gelation temperature (Tt) proving the hydrogel formation at physiological temperature. *In vitro* studies, cell viability experiments have been performed with chondrocytes and hMSCs through LIVE/DEAD and Alamar Blue assays showing a great cytocompatibility of ELRs. Furthermore, histological analysis such as H/E staining, collagen staining and GAG staining of the Hydrogels section further revealed the cells' capacity to develop their own ECM.

Regarding *in vivo* studies, fourteen New Zealand rabbits have been used for the generation of a critical knee defect that was filled with a cold solution (below Tt) of a mixture of ELR with hMSCs. Regeneration was evaluated after 4 months macroscopically, by MRI and computed tomography (CT) and microscopically by histological staining of sections from regenerated tissue. Tomographic and magnetic resonance images of extracted treated knees showed almost complete regeneration of the injured osteochondral area. Histological analysis of the injured section have shown *de novo* formation of hyaline cartilage, which is able to maintain the biomechanical characteristics of articular cartilage in comparison with the generally regenerated fibro cartilage. Finally, ELRs have shown to provide an optimal environment for the ability of the hMSCs to differentiate either into chondrocytes, or into Osteoblasts.

To conclude, this study shows a new horizon for biomaterial-based regenerative therapies in order to achieve an optimal osteochondral tissue repair.

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P614 Injured articular cartilage releases both migratory/homing factors and a purinergic priming factor which are mandatory for homing of mesenchymal stem cells to cartilage lesions

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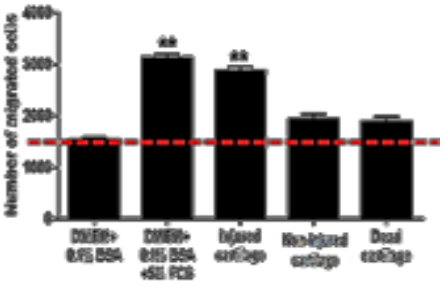
Introduction: Classically, articular cartilage is considered to have a poor repair capacity and traumatic cartilage injuries are a predisposing factor for the development of osteoarthritis. However, cartilage injuries in very young animals can spontaneously repair in vivo [1]. The mechanisms of this repair are unknown however, mesenchymal stem cells (MSCs) are known to migrate to the site of injured tissues. The aim of this study was to identify whether mesenchymal stem cells (MSCs) in the joint tissues respond to traumatic injury of articular cartilage and to determine the mechanisms by which this occurred.

Methods: Bone marrow-derived MSCs (BM-MSCs) and synovial fluid-derived MSCs (SF-MSCs) were isolated from bovine metacarpophalangeal joints and their numbers expanded by monolayer culture. An injured cartilage-conditioned medium (IJCM) was produced by injuring fresh articular cartilage with a scalpel and incubating the injured cartilage pieces for 24h with Dulbecco's Modified culture medium (DMEM) +0.1%BSA and then removing the medium and storing it at - 20^{O}C. Cell migration ability of MSCs was determined using classical cell migration assays (Transwell™, scratch and cell exclusion zone assays).

Results: BM-MSCs and SF-MSCs showed significant levels of migration (P<0.001) to freshly injured articular cartilage compared to non-injured or dead cartilage (Figure shows SF-MSC migration). IJCM significantly stimulated BM-and SF-MSC migration shown by reduction in wound width of 75%±5.7% (P<0.001) compared to 14%±1.2% in control (DMEM+0.1% BSA) in 'scratch' migration assays which require injuring the cell layer with a pipette tip to create a 'wound'. This migration was significantly (P<0.01) inhibited by neutralizing antibodies to TGFβ3 and CXCL12, and the CXCR4 receptor inhibitor AMD3100. No MSC migration was observed with IJCM in cell exclusion zone migration assays (no 'wound' is created in this assay protocol). However, migration in response to IJCM was observed in this system when a cell injury was created (by scraping off cells) close to the cell exclusion zone. Creating the 'scratch' wound caused release of rapidly metabolized ATP from the injured cells (189 pM at 30min and 24 pM at 4h after injury) determined by an ATP-bioluminescence assay. Addition, of stable ATP-ɣS or UTP-ɣS (250μM) with IJCM stimulated MSC migration in cell exclusion zone migration assays, suggesting a role for a putative P2 receptor. Addition of the P2 purine receptor antagonist, suramin, totally inhibited IJCM-stimulated MSC migration in the 'scratch'assay.



Conclusions: We suggest that in traumatic injury ATP, TGFβ$_3$ and CXCL12 were released from articular cartilage which together stimulated MSC homing to the site of injury. ATP transiently acted to prime the MSCs while TGFβ$_3$ and CXCL12 stimulated cell homing.

Acknowledgements: We are grateful to the University of Sheffield and JRI Orthopaedics for funding this research.

P615 Biomimetic, biofunctionalised, polymer implants to promote in situ repair of traumatic and early osteoarthritic cartilage defects

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Introduction: Osteoarthritis (OA) is a complex global disease characterized by progressive articular cartilage degeneration. It causes painful stiff joints, joint deformity and loss of joint mobility which can have a substantial impact on quality of life. Due to its progressive nature and disabling impact, OA has a high economic burden on health and social care systems. We are developing a biomimetic, implantable medical device which is bio-functionalised in a similar conformational and biochemical context to native tissues e.g. articular cartilage to promote stem cell homing and retention in the implant and subsequent tissue regeneration. The aim of this study was to assess the efficacy of this technology on the regeneration of articular cartilage *in vitro* and *in vivo.*

Methods: Poly-L-lactic acid (PLLA) was electrospun into random-fibre scaffolds (median fibre diameter 4.2 µm) which were plasma polymerized using allylamine. The surface-modified scaffolds were treated with heparin and ng amounts of chondrogenic and stem-cell homing factors. The bio-functionalized PLLA was assessed *in vitro* for its ability to support long-term cell viability (5 weeks) and chondrogenesis by bone-marrow MSCs and primary chondrocytes in absence of other added growth factors or FCS. Cell viability was assessed with PrestoBlue® and extracellular matrix (ECM) formation determined by measuring glycosaminoglycan content. The bio-functionalised scaffolds were assessed for *in vivo* activity by implantation into surgically-created 6mm chondral lesions in the medial condyles of sheep. MSCs were released from the subchondral bone by microfracture immediately before implantation of the MD. At 4 and 16 weeks, the treated joints were retrieved and cartilage regeneration assessed macroscopically and histologically.

Results: *In vitro,* functionalisation of the PLLA scaffold with a combination of TGFβ3 and CXCL12 promoted MSC attachment and ingress throughout the implant, and chondrogenic differentiation. Viability of the cells within the construct was maintained in absence of added chondrogenic factors or serum for at least 5-6 weeks. The MSCs underwent chondrogenesis and produced significantly more ECM ($P \geq 0.05$) than non-functionalised or partially functionalised MDs. The functionalised scaffolds also promoted chondrocyte attachment, long-term cell viability and ECM formation under the same experimental conditions. Some combinations of biological factors (e.g. $TGF\beta_1$+CXCL12) supported MSC chondrogenesis but inhibited ECM formation by primary chondrocytes. *In vivo* implantation, of TGFβ3 and CXCL12 functionalised MDs showed biological efficacy with regeneration of neo-cartilage with hyaline features (determined histologically) occurring at 4 weeks with the bio-functionalised MD but not in the empty defects or control non- functionalised MD.

Conclusions: Bio-functionalisation of PLLA scaffolds with ng quantities of TGFβ3 and CXCL12 promoted MSC migration and retention in the scaffolds and chondrogenesis with neo-cartilage tissue regeneration *in vitro* and *in vivo.*

Acknowledgements: We are grateful to the Dunhill Medical Trust & Medical Research Council for funding this research.

P616 Nano-ghosts delivery system and Link-N peptide, enhanced anti-inflammatory activity: a new approaches for the treatment of osteoarthritis

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Osteoarthritis is the most common joints' degenerative disease, characterized by cartilage deterioration and break down, severe pain, and rigidity. Treatments of osteoarthritis involve NSAIDs, visco-supplement and, lastly, surgery. Recently LinkN peptide, naturally expressed as N-terminal part of Link protein family, has been studied as candidate to treat osteoarthritis because it was shown to promote cartilage regeneration by its chondrocytes stimulating activity. In addition, synthetic LinkN was also shown to be able to promote cartilage regeneration and stimulate the production of extracellular matrix. Nonetheless, the LinkN clinical effect is diminished by its low stability, and therefore, the formulation of LinkN-drug carriers can largely improve its safety and efficacy.

Recently, we designed a novel kind of nanoparticles, termed Nano-ghosts (NGs), derived from the cytoplasmic membrane of mesenchymal stem cells (MSCs). Owing to their retention of MSCs' surface mechanism, the NGs were shown to target different types of tumors and are expected to similarly target other sites of inflammation, including the inflamed joints and the subchondral bone. Therefore, using LinkN-NGs should maximize its efficacy while reducing sideeffects.

Our goal in the present study, is to demonstrate that the NGs' can be loaded with LinkN to target the cartilage, to stimulate its regeneration and prevent the inflammatory process. The first phase of the project, involves the encapsulation of the peptide into the NGs using different approaches, showed that the peptide can be effectively loaded without affecting both NG's and LinkN properties. Furthermore, the targeting ability of NGs and their delivery capabilities have been studied showing that LinkN-NGs can reduce IL1ß induced inflammation in an in vitro model. Our on-going studies focuses on understanding how the LinkN interact with the IL1ß pathway during the inflammation condition, and move to ex vivo and in vivo models.

"This project has received funding from the European Union's Horizon 2020 research and innovation programme under Marie Sklodowska-Curie grant agreement No 642414"

P617 Histological evaluation of fibrochondrocyte and articular chondrocyte micro-aggregates for modular tissue engineering of fibrocartilage

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For the engineering of meniscus fibrocartilage, the choice of cell type and specific stimuli have a major impact on the engineered construct. Fibrochondrocytes (FC) and articular chondrocytes (AC) are possible cell sources, but one of the main problems is their dedifferentiation during expansion in monolayer. This study aims to generate 3D micro-aggregates with a phenotype similar to the avascular part of the meniscus. Micro-aggregates of FC and AC are compared, and the effect of the addition of IGF-I to the chondrogenic culture medium (with TGF-β) is evaluated.

Porcine FC and AC were expanded in monolayer until passage 2. A high-throughput agarose micro-well system, with 2865 pores of 200 µm diameter was used. After seeding 0,5 x 10^6 cells per well, uniform FC and AC micro-aggregates of approximately 175 cells were obtained. These micro-aggregates were cultivated at 5% O_2 up to day 21 in chondrogenic medium containing TGF-β1 or TGF-β1 and IGF-I. The morphology, viability and quality of the micro-aggregates were evaluated by live/dead staining and (immuno)histochemistry.

Both cell types formed round, compact aggregates from day 3 and remained viable up to day 21. AC aggregates were larger than FC aggregates with a mean diameter of 138 µm versus 105 µm on day 21. Immunohistochemical results showed that collagen I was expressed in both AC and FC aggregates and increased over time. However, only AC aggregates were positively stained for collagen II, whereas FC aggregates failed to express collagen II. Alcian blue staining showed a higher amount of sulphated glycosaminoglycans in AC aggregates compared to the FC aggregates. A silver staining showed reticular fibers in both AC and FC aggregates with an increased amount of fibers over time. AC and FC aggregates cultured in chondrogenic medium (with TGF-β) did not show remarkable differences when compared to aggregate cultures supplemented with IGF-I.

Histological evaluation suggest that micro-aggregates of AC possess a more desirable phenotype than FC aggregates as modular building blocks for tissue engineering of a larger scale construct. These findings will be verified by quantitative biochemical analysis and RT-qPCR.

P618 Influence of an anabolic loading period on global chondrocyte gene expression in osteochondral tissue engineering constructs

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Aims: Molecular pathways triggered by physiological loading are poorly defined. Aim was a genome-wide identification of mechano-regulated genes and candidate pathways in human chondrocytes and characterization of time evolution and re-inducibility of the response to a single anabolic loading episode.

Methods: Osteochondral constructs consisting of a chondrocyte-seeded collagen-scaffold connected to β-tricalcium-phosphate were pre-cultured for 35 days and subjected to dynamic compression (25% strain, 1 Hz, 9x10 minutes over 3h) before microarray-profiling was performed. Proteoglycan synthesis was determined by ^{35}S-sulfate-incorporation over 24 hours. Protein alterations were determined by Western blotting.

Results: Cell viability and hardness of constructs were unaltered by dynamic compression while proteoglycan synthesis was significantly stimulated (1.45-fold, p=0.016). Among 115 significantly regulated genes, 114 were up-regulated, 48 of them > two-fold. AP-1-relevant transcription factors FOSB and FOS strongly increased in line with elevated ERK1/2-phosphorylation and raising MAP3K4 expression. Proteoglycan-synthesizing enzymes CHSY1 and GALNT4 were stimulated and genes associated with the MAPK-, TGF-β-, calcium-, retinoic-acid-, Wnt- and Notch-signaling pathway were significantly altered. SOX9, BMP4 and BMP6 levels rose significantly also after multiple loading episodes at daily intervals up to the 14th cycle. Apparently unaltered canonical Smad-signaling and a rapid decline of SOX9 to baseline may explain why COL2A1 and ACAN expression remained unchanged after loading.

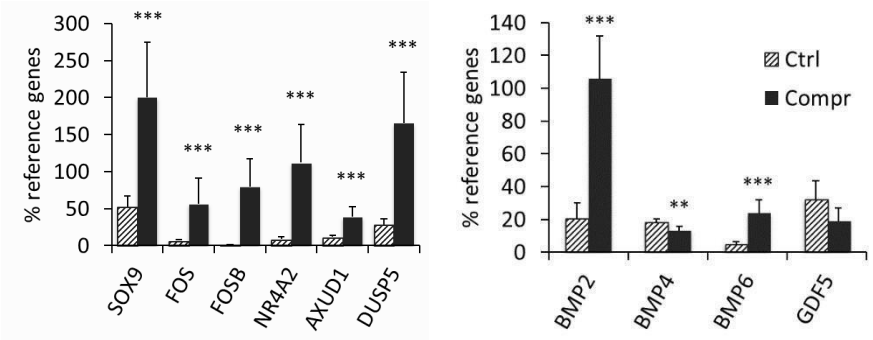

Fig. 1: Mechano-regulation of members of signaling pathways relevant for chondrogenesis and cartilage homeostasis after dynamic compression. qPCR, n=8, Mann-Whitney Test.

Impact: This study associates novel genes with mechanoregulation in chondrocytes and suggests that more pathways than so far anticipated apparently work together in a complex network of stimulators and feedback-regulators. This database of mechanosensitive factors responding to anabolic loading may now be used to optimize loading protocols for the generation of high-level cartilage replacement tissue.

P619 Electrospinning of functionalized poly(ε-caprolactone) for sustained drug delivery

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Introduction:

Electrospun scaffolds are promising materials for tissue engineering applications. They provide a mechanically stable environment that can be tailored in terms of biochemical and mechanical properties. They can, for example, reinforce a hydrogel which is otherwise too weak to subsist *in vivo*. Polymeric scaffolds can also be physically loaded with small molecule drugs, but this results in a very fast elution profile (hours). In this study, we developed a functionalized polyester-based material allowing release of molecules over several weeks. Our method allows simultaneous modification with several drugs that can, for example, target two main requirements of cartilage engineering, namely to synthesize extracellular matrix production in the presence of an inflamed environment.

Methods:

Prior to electrospinning, we functionalized poly(ε-caprolactone) (PCL) with dexamethasone and kartogenin, in the presence of 4-dimethylaminopyridine and N,N'-diisopropylcarbodiimide. For cross-linking of molecules sensitive to organic solvents (ie: proteins), maleimide and vinylsulfone functionalization protocols were developed to enable cross-linking *post* electrospinning, by Michael addition. The resulting materials were blended with unfunctionalized PCL and electrospun using a commercial setup.

Results:

The drug elution profile was quantified by HPLC over one month and the cross-linked molecules were released in a continuous manner, upon slow hydrolysis of the ester bonds. In contrast, the physically-loaded drug was completely eluted after a few hours only (Fig. 1).

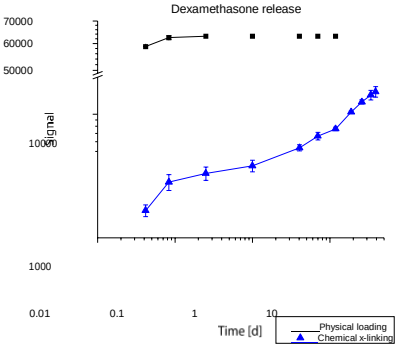

Figure 11 – Elution profile of dexamethasone-

The effects of the free drugs could be shown on bovine/human chondrocytes. Dexamethasone drastically reduced inflammation markers (COX-2 and IL-6) in a IL-1β induced environment, measured by western blot. Kartogenin induced an upregulation of collagen type II after weeks, quantified by immunostaining.

Bovine serum albumin-Texas red (BSA-TR) was used as a model protein drug. Under near physiological conditions, both PCL-maleimide and PCL-vinylsulfone could be functionalized by reacting with free cysteines on the BSA-(Fig. 2).

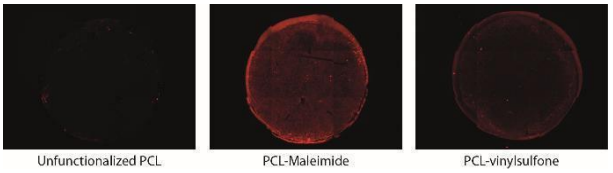

Figure 12 – Fluorescent imaging of TR electrospun membranes, following Michael

P620 Combined effect of hypoxia and matrix elasticity during chondrogenic differentiation of human mesenchymal stem cells

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Cartilage tissue engineering (TE) has the potential to repair acute chondral defects, which may prevent patient pain, immobility and the onset of osteoarthritis. One important aspect of cartilage TE is appropriately controlling progenitor cell differentiation. Both physical and biochemical stimuli are known to play important roles in regulating chondrogenesis. In particular, the articular chondrocyte phenotype is known to be regulated by mechanical stiffness *in vitro* and local oxygen concentration *in vivo* and *in vitro*. Cellular response to substrate rigidity is, at least in part, mediated by the Rho/ROCK signalling pathway, which regulates cytoskeleton organization and actin polymerization. Activation of Rho/ROCK signaling by stiff substrates inhibits chondrogenesis *in vitro*, while softer substrates favor differentiation of mesenchymal chondroprogenitor cells down the chondrogenic lineage. Similarly, the hypoxia inducible factor (HIF) signalling cascade mediates hypoxia's intracellular effects. It plays important roles in the chondrogenic differentiation of human marrow stromal cells (hMSC) and prevents chondrocyte hypertrophy *in vivo*. Nevertheless, potential interactions between these pathways during the *in vitro* chondrogenic differentiation of hMSC remain relatively unexplored. Here, we investigated the interplay between the HIF pathway and the cellular response to substrate rigidity. We hypothesized that by combining hypoxia with soft substrates, we could enhance the chondrogenic differentiation of hMSC to a greater extent than that due to each factor alone. Bone marrow-derived hMSC were chondrogenically differentiated on soft (0.5 kPa) and stiff (40 kPa) polyacrylamide hydrogels under normoxic (20% O2) and hypoxic (2% O2) conditions. Gene expression analyses and immunostaining for cartilage-like extracellular matrix components were then used to assess differentiation. To investigate the interplay between Rho/ROCK signalling and hypoxia, hMSC were differentiated in the presence and absence of ROCK inhibitor Y-27632 under normoxic and hypoxic conditions. Our results show that soft substrates upregulate expression of key genes necessary for chondrogenic differentiation of hMSC. We also observed that hMSC cultured on soft substrates under hypoxic conditions adopt more rounded morphologies compared to those on soft substrates under normoxic conditions. These observations suggest that the combination of hypoxia and soft substrates may enhance the chondrogenic differentiation of hMSC. These results may have important implications for cartilage TE as they suggest that combining hypoxia and soft matrices may be an effective strategy to drive the differentiation of hMSC down the chondrogenic lineage *in vitro*.

P621 Advancing osteochondral tissue regeneration by ordered differentiation of human periosteal cells

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The periosteum contains skeletal progenitor cells which participate in endochondral bone formation during fracture repair (1,2). Moreover, clinical trials have also shown good outcomes following implantation of periosteum flaps to damaged areas of the joint surface (3). When differentiated *in vitro* through exogenous supplementation with growth factors (GFs), human Periosteum-Derived Cells (hPDCs) can replicate critical steps of cartilage morphogenesis that occurs *in vivo*. The generation of multi-layered, scaffold-free cartilage constructs for osteochondral repair, with a stable and a transient form of cartilage has not yet been demonstrated. In this work hPDC derived cartilage intermediates were formed using high density culture in combination with optimised developmentally inspired differentiation conditions (including TFG-β1, GDF5 and BMP2). *In vitro* derived tissues were histologically analysed by alcian blue, COL1, COL2 and IHH immunostaining, and gene expression (qPCR). *Following* 28 days of differentiation the cartilage intermediates displayed enhanced deposition of matrix glycosaminoglycans (GAGs), collagen type-II and type-I and IHH expression. Analysis of early and late chondrogenic markers indicated enhanced expression of SOX9, COL2A1, Col10A1, and MEF2C along with the BMP antagonist NOGGIN, which is known to be expressed at various stages of skeletogenesis. To assess cartilage formation *in vivo,* hPDC derived cartilage intermediates were implanted subcutaneously in the nude mouse and in osteochondral defects in nude rats. Following 2,4 and 8 weeks, the ectopic explants were retrieved and analysed by micro computed tomography and histology for collagen type I and type II, and human osteocalcin. At 8 weeks, ectopically implants displayed either a stable cartilage phenotype with expression of collagen type II and a partially mineralized matrix, or transient cartilage leading to bone formation. Staining with a human specific osteocalcin antibody revealed a contribution of the implanted cells to the newly formed tissues. In the osteochondral defect model, the implants successfully engrafted and led to the development of subchondral bone and immature stable cartilage at the joint surface displaying a GAG-rich, non-mineralised matrix and expressing collagen type II and lubricin.

In summary, hPDCs which have been appropriately and efficiently preconditioned towards a cartilage intermediate, respond to microenvironmental cues leading to stable cartilage and bone formation and contribute to the restoration of full thickness osteochondral joint surface defects.

P624 Nasal chondrocytes are potential autologous cell-transplant candidates for treating degenerative disc disease

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Intervertebral disc (IVD) degeneration is one of the main causes for chronic back pain. Injection of autologous stem cells is still an experimental treatment for disc degeneration showing limited success so far. This is attributed to a low survival rate of the injected cells due to the harsh environment within the disc, which is hypoxic, acidic, low in nutrients, and possibly inflamed in a degenerative state. Studies performed in animal models have reported that juvenile chondrocytes display a better cell survival and production of extracellular matrix than stem cells, possibly due to chondrocytes being more accustomed to an avascular environment. Recently, it has been shown that adult human nasal septum chondrocytes (i.e., cells that can be easily available in an autologous setting, under minimally invasive conditions) have an increased rate of proliferation and synthesis of proteoglycan (GAG) and collagen in contrast to articular chondrocytes (ACs). This study was aimed at assessing whether human nasal chondrocytes (NCs) could be an opportune cell source for autologous cell transplantation therapy in the treatment of IVD degeneration.

Human bone marrow stromal cells (MSCs), ACs, and NCs were isolated from biopsies and expanded in cell culture for 2 passages. Thereafter, cells were cultured in either normoxic or hypoxic (2% O_2)(Hypo) conditions for 4 weeks in 3D pellet culture The chondrogenic media with either regular (4.5 g/l) or low (1g/l) glucose (LowGlu) levels were complemented either with or without the growth factor TGFβ1. The cell survival and the capability to form IVD-like tissue were evaluated by means of histological and biochemical analysis.

Quantification of DNA shows that the cell number of MSC decreased by more than 25% in the absence of TGFβ1 and was barely retained in the presence of the growth factor independent of environmental condition. ACs behaved similar to MSCs with the exception that the cell number increased in hypoxic conditions with the addition of TGFβ1, but independent of the glucose concentration. Surprisingly, the number of NC slightly increased (>12%) in all conditions without TGFβ1. Furthermore, the supplement of TGFβ1 increased the cell number by at least 48% in any of environmental conditions.

Histological Safranin O staining and biochemical analysis showed for all three cell sources that TGFβ1 was necessary for an adequate production of GAG. The reduction of glucose decreased the level of GAG in pellets formed by MSCs in both hypoxia and normoxia. In contrast, GAG production of ACs was unaffected by changes in glucose concentration, however hypoxic conditions influenced ACs to synthesize more GAG. Interestingly, NCs do not favor hypoxic conditions for GAG production, nonetheless in combination with decreased glucose levels they show a trend to produce the most GAG (35 pgr) compared to ACs and MSCs.

Our findings indicate that compared to MSCs and ACs, NCs are more prone to survive and synthesize cartilaginous extracellular matrix *in vitro* in conditions resembling those of the IVD (i.e., low oxygen and low glucose concentration) and are therefore an excellent candidate for a cell based therapy of degenerative disc disease.

P625 Platelet derivatives stimulate cartilage repair activating progenitor cells and maintaining human hyaline cartilage phenotype by Chondromodulin-I induction

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Objective: Regeneration of damaged hyaline cartilage still remains a tremendous clinical challenge; new trends in cartilage regeneration often contemplate the use of platelet derivatives (PRP or PL) for the effect on the proliferation of human articular chondrocytes preserving their somatic differentiation potential and inducing the cartilage repair. Our study identify new pathway induced by platelet derivatives that are able to regulate articular cartilagehomeostasis.

Materials and Methods: From human articular cartilage biopsy adult human articular chondrocytes (HAC) and chondro-progenitor cells (CPC) were isolated and expanded “in vitro”. The CPC population was obtained by PL (platelet lyset) treatment of human cartilage chips: after 10 days in culture the cells migrate and proliferate. Both cell populations were tested “in vitro” for clonogenic capability, for phenotype and gene expression of typical chondrogenic markers and for differentiation potential.

Results: Our data strongly suggested that fully differentiated chondrocytes possess “reserved stemness,” which are reactivated during in vitro expansion only in presence of PL, gradually displaying multipotent stem/progenitor cell characteristics. At the same time PRP is able to activate and recruit CPCs positive for nestin, gremlin-1 and sox-9 genes normally expressed in dividing cells during the early stage of development.

“In vivo”, cartilage matrix formation was assessed by histology after subcutaneous transplantation of HAC/CPC isolated cell culture; cells were seeded on PGA-HA scaffolds with or without PL and implanted into immunocompromised mice. The addition of platelet derivatives to cartilage grafts resulted in robustly formation of hyaline-like cartilage that showed the expression of type II collagen and Chondromodulin-1 (ChM1), a specific anti-angiogenic factor strongly present in avascular cartilage. Moreover, type X collagen and VEGF expression were not observed in the tissue graft, supporting the maintenance of hyaline cartilage phenotype and no hypertrophy tissue transition.

Conclusions: The combination of articular chondrocytes or precursors, platelet derivatives and PGA-HA scaffold can synergistically promote cartilage formation. At the same time PL is able to drive cartilage regenerative response by activating and recruiting CPCs positive for nestin and other early genes. Nestin is an intermediate filament protein expressed in dividing cells during the early stage of development and it is normally used as a marker of proliferating and migrating adult stem cell in multiple tissues. This protein is up regulated in response to injury and pathological processes.

The newly tissue formed maintains the cartilage phenotype by the overexpression of ChM1 mediated by PL treatment. ChM1 maintains cartilage homeostasis and inhibits endochondral ossification, suggesting the importance of ChM1 in governing stable chondrocyte phenotype. More studies are in progress to investigate the role of platelet derivatives on hypoxia environment and hypoxia-inducible factors (HIFs) modulation as critical markers on the maintaining of the articular chondrocyte phenotype.

P626 Preclinical study of a novel cell-free biphasic scaffold for osteochondral lesions in a sheep model

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Introduction: Several scaffolds have been developed for osteochondral lesions, which usually repair forming fibrocartilage, with poor biomechanical properties.

Objective: The aim of this new project is to develop and in vivo test a novel configuration of a cell-free biphasic scaffold for osteochondral lesions.

Materials and Methods: A critical osteochondral defect was generated in the medial femoral condyle of 36 skeletally mature sheep. Six defects were left untreated (CNTRL), thirty lesions were divided into three groups: ten lesions treated with a biphasic scaffold made of collagen type I and small cylinders of Magnesium hydroxyapatite embedded in collagen type I (HMG); ten lesions treated with a biphasic substituted formed by collagen type I and Wallastonite (BWS); ten lesions treated with a scaffold made of collagen type I and small cylinders of Wallastonite embedded in collagen type I (HWS). The animals were sacrificed after 3 and 6 months and the samples were analyzed by CT and MRI, macroscopic and histological evaluation.

Results: At 3 months, the ICRS macroscopic assessment showed a significant difference between HMG and CTRL. The CT and MRI scans displayed a reparative tissue especially in the HMG group. Histological evaluation at 3 months displayed a cartilaginous tissue in the HMG group. The significant difference between HMG and CTRL was only partially confirmed after 6 months by CT, MRI and histology.

Conclusions: Our study demonstrated that these novel biphasic scaffolds can be applied for one-stage procedures for osteochondral defects. Moreover, we proved the superior reparative potential of the HMG scaffold at the earlier time point.

P627 A cartilage lesion in a soccer players knee - treated with MACI followed by histological evaluation

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Introduction: Traumatic lesions of the articular cartilage represent relevant risk factors for the development of osteoarthritis. Because of the highly limited regenerative potency of the hyaline articular cartilage, it is important to have treatment-options which are capable to regenerate a cartilage-like tissue, to avoid or delay the development of a posttraumatic osteoarthritis. One of these procedures is the autologous chondrocyte implantation (ACI), which has been introduced and established by Brittberg *et al.* and which was further optimized within the last 15 years. Matrix assisted chondrocyte transplantation (MACI) is such a further development of the classical ACI and represents an option to treat traumatic lesions of the hyaline cartilage, mostly in the knee, especially in an Outerbridge III/ IV-situation or in defects which have a size larger than 3 cm^2 . MACI requires a two-time surgical intervention.

Materials and methods: A 28-year old soccer player received MACI (Novocart 3D, Tetec AG, Reutlingen, Germany) after traumatic cartilage lesion of the lateral femur condyle. Due to clinical reasons, a second look intervention four months after MACI has been performed and a biopsy of the regenerated tissue has been taken by using a 2 mm Jamshidi needle. The quality of the regenerated tissue was analysed histologically and immunhistologically.

Results: Histological HE-staining showed a completely avascular cartilageneous tissue with a homogenic extracellular matrix and cells with a round, chondrocytic phenotype. In the Alcian-blue staining, a blue-colored extracellular matrix was noticeable, which indicates a high content of mucopolysaccharides and glykosaminoglykanes. The immunohistological staining with the anti-collagen II antibody revealed a strong homogeneous positivity within the regenerated tissue.

Conclusion: Histological and immunhistological examination showed a tissue which fulfills the most important criteria we demand on hyaline articular cartilage. This proves, that modern ACI-techniques are an adequate and effective treatment-option for lesions of the hyaline articular cartilage.

P628 Influence of genipin-based crosslinking treatments on collagen scaffold properties for cartilage regeneration

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Trauma, excessive body weight or degenerative pathology result in gradual deterioration of the cartilage, leading to debilitating joint pain, functional impairment and, in some cases, arthritis onset. Unfortunately, the repair of chondral defects is one of the most challenging orthopedic clinical problems, since articular cartilage has poor healing potential. To address the unmet need for cartilage regeneration, many materials have been developed. Among them, collagen is one of the most frequently used materials due to its biocompatibility, biodegradability, low immunogenicity and cell adhesive properties. The goal of this study is the generation of a collagen-based scaffold for articular cartilage repair. To this end, a new fabrication protocol was developed in order to improve the poor mechanical properties of collagen. In detail, genipin was added in the collagen slurry prior to freeze-drying and dehydrothermal crosslinking treatments (group A). Two control groups were generated after freeze-drying of collagenslurry: dehydrothermal crosslinked (group B), dehydrothermal crosslinked followed by chemical crosslinking in genipin solution (group C). Interestingly, the chemical treatments with genipin significantly increased the scaffolds stiffness (Young modulus (KPa): group A=4.5±0.9, group B=1.5±0.2, group C=13.3±2.). The morphological analysis showed homogeneous and isotropic porosity with a uniform pore size (around 110 m for group A, 120 m for group B and 100 m for group C). Subsequently, human bone marrow derived mesenchymal stromal cells (MSCs) were seeded on collagen scaffolds. Cell-scaffold interactions, evaluated up to 7 days of culture, displayed high cell viability (live and dead staining), fibroblastic-like morphology and a homogeneous cell distribution around the pores edges (actin- nuclei staining). Finally, culture under chondrogenic conditions for 3-weeks promoted MSCs proliferation (cy quant) and chondral matrix deposition (Safranin-O and type II collagen staining). Furthermore, cells commitment towards chondrogenic lineage was assessed by RT-PCR (Sox9, aggrecan and type II collagen). Overall the reported results proved the efficiency of the fabrication protocol scaffold and the potential use of our scaffold for cartilage tissue engineering application.

(P629)

P629 Cartilage regeneration on saponin loaded gellan gum scaffolds

Ha Yan Jeon, Il Nam Choi, Yoo Sshin Jeon, Jong Ho Park, Eun Yeong Shin, Joo Hee Choi, Jeong Eun Song, Nirmalya Tripathy, Gilson Khang

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INTRODUCTION: Saponin is water-soluble so it is fabricated with gellan gum solution. Saponin, found in many plants like clematis chinensis osbeck, has long been used in traditional chinese medicine to treat joint diseases including osteoarthritis. Also its property is anti-inflammatory, antioxidant and anti-cancer. Gellan gum is a linear and anionic polysaccharide with the repeating unit consisting of a-l-rhamnose, b-d-glucose, and b-d-glucuronate. Also, gellan gum is colorless and has heat, acid and enzyme resistance properties. Gellan gum which is processed well and

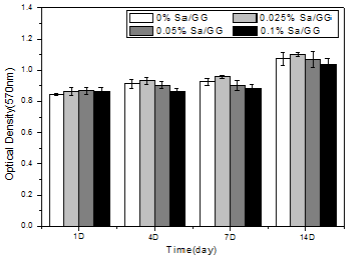

Fig. 1. Cell viability of saponin/gellan gum scaffolds after 1, 4, 7, 14days.

erial for tissue engineering scaffold [1]. 0.025% saponin od morphology, cell proliferation, sGAG content and mRNA saponin/gellan gum scaffold has possibility of applications ineering.

prepared by heating and stirring with DW and staying to be an gum scaffolds, gellan gum solution was blended with /gellan gum). The morphological and structural properties performed using SEM, compressive strength, FTIR, MTT

RESULTS: In vitro, saponin/gellan gum scaffold has more cell viability compared to the other saponin/gellan gum scaffolds is higher than on 0.025% saponin/gellan gum scaffold was oups. There is no significant difference of

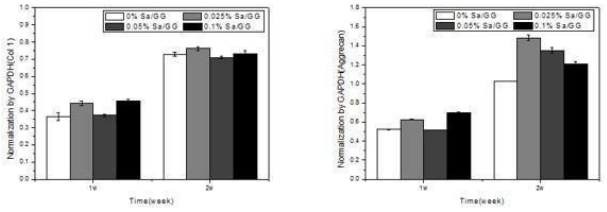

Fig.2: mRNA expression of chondrocytes cultured in saponin/gellan gum scaffolds after 7, 14days.

DISCUSSION & CONCLUSIONS

We have designed a biocompatible, biodegradable saponin/gellan gum scaffold to incorporate specific functionality such as enhancing proliferation of chondrocyte. *In vitro* results showed that the saponin/gellan gum scaffold efficiently maintains cellular migration, growth, cell phenotype, formation of cell junctions and gene expression required for functional chodrocytes. As a result, saponin/gellan gum scaffold has possibility as a tissue engineering scaffold so it can be used for cartilage regeneration.

ACKNOWLEDGEMENTS: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health &Welfare, Republic of Korea (grant number : HI15C2996).

P630 Enhancement of chondrogenic differentiation using gellan gum hesperidin hybrid scaffold

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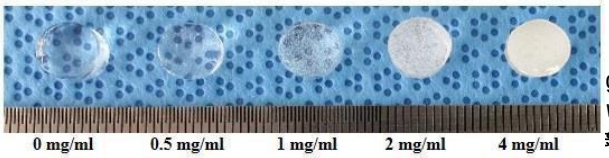
INTRODUCTION: Gellan Gum is a complex polysaccharide, composed of sodium gluconate, rhamnose, glucose at a ratio of 1:1:2, and can be obtained after purification, drying and pulverization processes. Colourless and transparent gellan gum has outstanding physiochemical at acid, heat and it can be easily tailored using pH or ion addition. Gellan gum is a water-insoluble material so dissolves by utilizing heat. Gellan gum has been applied various field of clinical practices and it became recognized polymer that spotlight in various fields owing to its rheological and structural characteristics. Gellan gum is widely used in tissue engineering owing to its biocompatibility and outstanding physicochemical properties, such as ability to form various form like film, hydrogel, microsphere, micro-capsulation and sponge. In this study, We have fabricated gellan gum scaffold followed by plating chondrocyte cells to gellan gum scaffold.

METHODS: Gellan gum scaffolds with Hesperidin 0, 0.5, 1, 2, 4 mg/mL were produced with DW, $CaCl_2$. All samples characteristic were studied by FTIR, compressive strength and SEM. Also, chondrogenic differentiation of rabbit cartilage cells cultured in scaffold was evaluated by ALP assay, MTT assay, RT-PCR, SEM.



RESULTS: Gellan gum scaffolds were fabricated with increasing Hesperidin content in the gellan gum scaffold. Large amount of Hesperidin is not necessary for high compressive strength. *In vitro* studies showed that cell proliferation and differentiation in Hesperidin scaffold (Fig. 4).

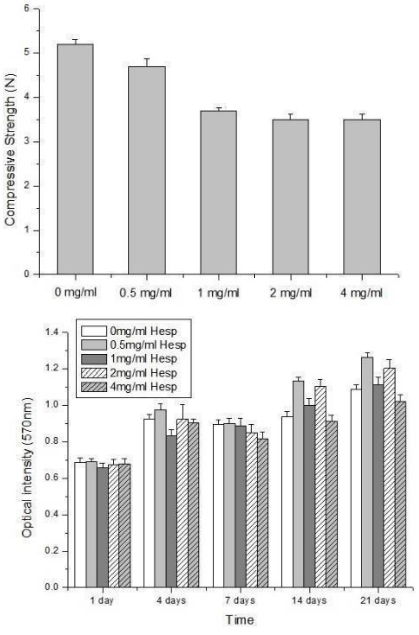


Fig. 1: Compressive strength of the gellan gum scaffolds containing Hesperidin.

Fig. 2: Cell viability of chondrocyte in gellan gum scaffolds containing Hesperidin, analysed by MTT assay.

DISCUSSION & CONCLUSIONS: This study showed the highest extracellular matrix secretion and cell proliferation in gellan gum scaffold with 1mg/mL Hesperidin.

(P631)

P631 Differential effects of platelet-derived products on ECM turnover by OA chondrocytes in collagen hydrogels

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Introduction:

Articular cartilage extracellular matrix (ECM) plays a vital role in maintaining the dynamic behavior of chondrocytes through cell-matrix interactions. Chondrocytes retain the cartilage matrix homeostasis in, matrix remodeling, response to mechanical loading, balancing the osmotic environment and tensile properties. This activity is compromised in osteoarthritic (OA) cartilage. Among several treatment strategies available such as viscosupplementation or intra-articular injections of autologous platelet-rich plasma (PRP), the latter offers better symptomatic relief according to recent studies. However, the molecular mechanisms underlying in supplementation of platelet-derived products on the OA matrix environment has not been elucidated. In our current study, we investigated the matrix turnover potential and redifferentiation of OA chondrocytes when supplemented by platelet-derived products.

Methods:

Chondrocytes harvested from OA patients undergoing total knee replacement were embedded in collagen type I hydrogels and cultured in the presence of 10% human blood derivatives: PRP, serum from platelet-rich fibrin (SPRF) or serum obtained by human blood donations. The cultures were kept under normoxic (20% O_2) or hypoxic (4% O_2) conditions. Constructs were evaluated at 7 and 14 days for cell proliferation by DNA quantification, matrix turnover gene expression by RT-qPCR and the glycosaminoglycan content by Dimethylmethylene Blue assay.

Results:

Increase in cell proliferation was observed on both serum- and SPRF-treated constructs accompanied by a fibrochondrocytic morphological phenotype. PRP, however, did not enhance proliferation from the initial seeding density and induced a round shaped chondrocyte morphological phenotype. Glycosaminoglycan content was accumulated under all conditions. PRP increased the cartilaginous matrix COL2A1 gene expression together with SOX9and MMP13 under both normoxic and hypoxic conditions. In contrast, serum and SPRF retained the de-differentiated chondrocyte phenotype with an expression of COL1A1 and inhibition of

P633 Successful cartilage regeneration by stem cells and cartilage fragments covered by amniotic membrane in an equine osteochondral defect model

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Osteoarthritis (OA) – the most common cause of osteochondral defects – has an incidence of approximately 10% in men and 13% in women aged over 60. This is likely to increase due to aging and obesity. Cartilage tissue has limited healing capacity. Current treatments include autologous chondrocyte transplantation (ACT), matrix-associated ACT (MACT) as well as *in situ*- induction of cartilage repair by microfracture. Therapy of cartilage defects with these methods is unsatisfying. ACT and MACT are costly and comprise the disadvantages of two separate operations. Microfracture is limited to smaller defects and known to produce fibrous cartilage. Furthermore, transplanted additives need a fixation to prevent detachment from the defect site. Currently used materials are either xenografts or autologous periosteal membranes. The former are intricate and expensive in production. The latter may cause morbidity at the extraction site and can lead to hypergranulation and pain at the transplantation site. Therefore, a clear need exists for new approaches to develop a sustainable therapy for cartilage defects and to stimulate the regeneration of hyaline cartilage.

In our study, we investigated the usability of equine amniotic membrane (AM) for the therapy of cartilage defects in equine knee joints. AM may contribute to cartilage regeneration as transplant fixation, scaffold for effective cell growth, and natural source for mesenchymal stem cells (MSCs) and growth factors. The equine osteochondral defect model consisted of one oval defect in critical size (2.36 cm^2) on the lateral condyle of the femur. Five groups (N = 6) were included, each with AM cover of the defect. Beside the control group with no further provision, four groups were combined with either I. Microfracture, II. Microfracture/cartilage fragments/fibrin glue, III. Adipose MSCs/cartilage fragments/fibrin glue, or IV. Bone marrow MSCs/cartilage fragments/fibrin glue. 6 weeks post-surgery arthroscopy was performed. 12 months post- surgery tissue was harvested including native cartilage for comparison. Analyses comprised macroscopic imaging, biomechanics, gene expression and histology.

Arthroscopy showed smooth integration of the AM into the defect bed. No adverse reaction was observed during the entire study. Macroscopic analysis showed all treated defects were significantly more covered with newly formed cartilage in contrast to no filling, with best results for two defects of the bone marrow MSCs group. With adipose MSCs the stiffness was comparable to native cartilage and Aggrecan, SOX9 as well as Versican expression was comparably higher. Histological results proved full regeneration of hyaline cartilage in the bone marrow MSCs group.

Summing up, the study proved the usability of AM in the therapy of cartilage defects.

P634 Spatial organization of chondrocytes influences cartilage repair in an ex vivo osteochondral plug model

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Introduction: Cell delivery is one of the main challenges in tissue regeneration. In focal cartilage lesions, chondrocyte implantation usually involves cell delivery in the defect depth, with or without a protective biomaterial on top, inducing regeneration from the deep zone of the cartilage towards the surface. Novel approaches using hydrogels allow homogeneous 3D cell distribution into a biomaterial matrix. Elucidating the impact of the spatial organization of transplanted cell on the extent and quality of the regenerative process in damaged cartilage is fundamental to improve the outcome of restoration techniques. The aim of this study is to explore the capability of chondrocytes to repair a focal defect as a function of their spatial distribution in a hydrogel, using an *ex vivo* osteochondral (OC) plug model. **Methods:** Healthy equine OC plugs where harvested from fetlock joints and kept alive in an *ex vivo* bioreactor that allows to feed the bone and cartilage layers with separate media [1]. Cylindrical full-thickness cartilage defects (4 mm diameter) were induced, and filled with varying allogeneic cell placements: i) mimicking MACI, with cells in the depth of the defect and topped with a cell-free hydrogel, ii) homogeneously distributed in a 3D hydrogel environment, and iii) a combination of i) and ii). A gelatin methacryloyl-based hydrogel (gelMA) was used as carrier matrix for cell encapsulation and as defect filler. The neo-cartilage in the defect, the native tissue, the interface between the two, and the subchondral bone were analysed via histology. Biochemical quantification (GAGs and DNA) and genetic profiling to detect the origin of the repair tissue were performed. **Results and Discussion:** OC plugs were histologically comparable to healthy tissue up to 57 days of culture, and native cells contributed to new GAG synthesis in non-treated, control plugs. Clear differences in the engineered neo-cartilage were found between the cells delivered at the defect depth or distributed in the hydrogel. In the first case, a thick layer of GAG and collagen type II-rich tissue adhered to the subchondral bone, but no repair was found in the gel or towards the superficial layer. Notably, a good integration was observed at the interface between the native cartilage and the gel. In the second strategy, the whole volume of the defect and the gel contained GAG and collagen type II positive matrix, produced by the encapsulated cells. Combining the two strategies resulted in well- integrated repair tissue throughout the defect volume, which is stably bound to the subchondral layer, suggesting a beneficial cohesion and mechanical stability of the graft. Moreover, gels cultured in the OC plugs showed higher values for GAGs and collagen type II, compared to controls placed in a tissue culture plate, suggesting that the plug matrix and the cells in it influence repair tissue formation, possibly through the release of bioactive factors. **Conclusions:** The OC plug model offers a platform for testing regenerative therapies in a set-up better resembling the *in vivo* environment. Cell-laden gelMA showed promising neo-cartilage formation, and enhanced integration when combined with cell co-delivery in the deep zone of the defect. These results provide important clues to improve the current approaches for cell-driven cartilage regeneration.

P635 Optimization of an injectable, growth-factor loaded hyaluronan hydrogel for cartilage repair

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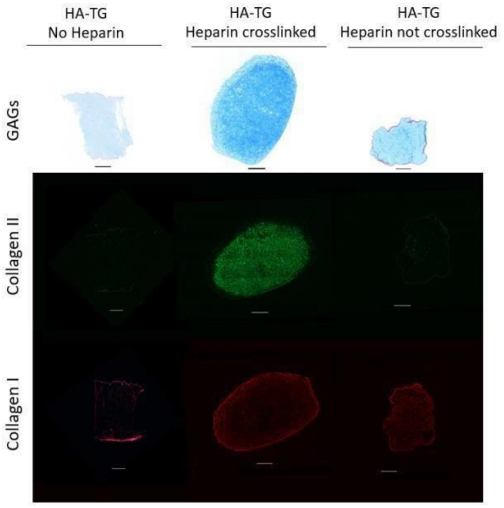
Articular cartilage focal lesions represent a therapeutic challenge due to the poor intrinsic healing capacity of the tissue. Surgical procedures, the current standard of care, have debatable long-term benefits and tissue engineering comes as a possible alternative. An enzymatically crosslinked hyaluronan hydrogel (HA-TG) was developed, which displays excellent chondrogenic properties (1). It however does not retain active molecules such as growth factors, which is needed for a one-step, injectable treatment of cartilage lesions. We hypothesized that the addition of covalently bound heparin to the HA-TG network (2), would support a sustained release of TGF- β1, a potent chondrogenic factor, and therefore promote matrix deposition. Human chondroprogenitor cells were thus encapsulated in HA-TG-heparin and TGF-β1 was loaded before gelation. The constructs were cultured in serum free media for 21 days, without supplementation of TGF-β1. Histological stainings showed that the burst release of TGF- β1 prevented the deposition of matrix components. On the contrary, a high deposition of collagen II and glycosaminoglycans (GAGs) was observed when heparin was covalently crosslinked. Collagen I, which is normally absent from healthy hyaline cartilage, was also present but in low amounts. Unbound heparin, however, only led to collagen I production and very low amounts of collagen II and GAGs (see figure, scale bar = 500 µm). This correlated with the results of an ELISA showing that covalently crosslinked heparin allowed a sustained release of TGF- β1. Ongoing experiments aim to determine the optimal growth factor concentration in an ex-vivo model based on histology and gene expression, as well as the best heparin concentration, using ELISA.

(1) Broguiere, Cavalli et al. (2016); (2) Sakiyama-Elbert and Hubell (2000)



P637 Strategies to enhance chondrogenic differentiation of dental pulp stem cells

Alessia Longoni, Inge E.M. Hooijdonk, Gerhard K.P. Bittermann, A.J.W.P. Rosenberg, Debby Gawlitta

UMC utrecht, utrecht, The Netherlands

Introduction: Dental pulp stem cells (DPSCs) represent an interesting cell source for craniofacial bone tissue regeneration due to their neural crest origin. It has been proven that neural crest-derived bones remodel and heal through recruitment of progenitor cells of the same embryonic origin[1]. Further, DPSCs are harvested with a minimally invasive procedure, display a high proliferation rate and are capable of undergoing multilineage differentiation[2]. Thus, DPSCs could be potentially used to develop a cartilaginous template that, upon implantation, could be remodelled into new bone tissue, mimicking the endochondral ossification process. However, DPSC chondrogenic differentiation capacity has not been extensively explored. Thus far, limited chondrogenic potential was observed when DPSCs were subjected to chondrogenic conditions that are common for multipotent stromal cells (MSCs). Our hypothesis is that DPSC differentiation may require a protocol that takes their different embryological origin into account. Therefore, the aim of this study is to test several strategies to define the optimal conditions to fully exploit DPSC chondrogenic potential. Once a protocol for chondrogenic differentiation of DPSCs is established, the potential for endochondral bone regeneration can be evaluated.

Material and Methods: Human DPSCs from the pulp tissue of third molars were isolated from five patients following informed consent. DPSCs were differentiated in aggregates of $2*10^5$ cells in chondroinductive medium (CM) supplemented with 10 ng/ml of TGFβ1. Controls were maintained in CM without TGFβ1. Four different amounts of DPSCs per pellet were tested to define the optimal cell number. Further, to tailor the CM, several growth factors associated with chondrogenesis were supplemented singularly or in combination. To take into account the DPSC's embryological origin, BMP2 and BMP7, involved in Meckel's cartilage development, were included. Chondrogenesis was assessed after 4 weeks of culture, by the DMMB assay, toluidine blue and collagen types I and II (immuno)staining. Hypertrophy and mineralization were assessed via collagen type X and Von Kossa (immuno)staining, respectively.

Results and Discussion: All chondrogenically stimulated DPSCs deposited GAGs, in a donor dependent manner. However, when CM for MSCs was used, pellets were small (<1mm) and showed a fibrous morphology, along with more collagen type I than II deposition. From all assessed cell numbers per pellet, $5*10^5$ cells exhibited the highest absolute amount of GAGs. Further, differences in GAG deposition were observed when different growth factors were supplemented singularly or in combination, however, no condition outperformed the DPSC chondrogenesis of the control condition.

Conclusions: DPSC chondrogenic differentiation capacity was confirmed, though limited. Further improvement of chondrogenesis may be achieved by adapting expansion protocols as well. Thus, chondrogenic potential of DPSCs might be enhanced if tailored culturing conditions would be applied from the moment of isolation, and not only during the differentiation stage.

[1] Leucht P et al, *Development* 2008 [2] Zhang W, *Tissue Eng.* 2006

P639 Use of alginate-agarose scaffolds for cartilage regeneration

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INTRODUCTION: Clinical management of large-size cartilage lesions is difficult due to the limited regenerative ability of the cartilage. Different biomaterials have been used to develop tissue engineering substitutes for cartilage repair including alginate. Although alginate hydrogels represent an ideal environment for chondrocyte culture, their biomechanical properties make difficult the *in vivo* implantation. Agarose has been used in combination with other polymers including fibrin in order to increase its consistency. In this study, we explored the usefulness of the use of agarose in combination with alginate to generate a chondrocyte-friendly scaffold consistent enough to be used for *in vivo* articular regeneration.

METHODS: Human articular chondrocytes were isolated and cultured in proliferation medium containing DMEM, 50 µg/mL ascorbic acid, 10% FBS, non-essential aminoacids, and antibiotics. After three passages, the cells were cultured with chondral differentiation medium containing DMEM, 1% ITS, 1% FBS and 10 ng/ml TGF beta for up to 4 weeks. Chondrocytes differentiation expression markers were studied by immunofluorescence using antibodies against type I and II collagens and aggrecan as well as by real time RT-PCR. Morphology changes were evaluated by fluorescence microscopy using rhodamine-phalloidin. Low-melting fusion point agarose (0.5- 2%) was mixed with alginate (1-3%) and polymerized together in 24 well culture plates. Human primary articular chondrocytes were isolated and cultured in the 3-D agarose-alginate scaffolds with chondral differentiation medium for up to 4 weeks. Chondrocytes differentiation expression markers as well as morphology changes were evaluated as above.

RESULTS: After isolation, chondrocytes were cultured for no more than four passages obtaining a minimum of 20 million cells of each donor. As observed by contrast phase microscopy, a rapid de-differentiation characterized by an acquisition of a fibroblast-like shape and a down-regulation of the expression of collagen II and aggrecan was observed. Re-differentiation of cultures was then induced using chondral differentiation cultured medium. Changes in cell morphology (the cells became rounded and started forming aggregates) as well as an increase in the expression of aggrecan first and type II collagen later were observed two weeks after changing the cell culture medium becoming significant after 3 weeks. In the absence of cells, agarose significantly increased the consistence of alginate hydrogels. As we observed, the addition of as few as a 0.5% of agarose to 1% alginate hydrogels was enough to facilitate the handling of scaffolds. Among all the combinations tested, 1% agarose + 3% alginate was the best taking into account the consistence of the scaffolds as well as the cell nesting and differentiation observed.

DISCUSSION & CONCLUSIONS: Agarose significantly increased the consistence of alginate hydrogels with a minimal effect concerning chondrocyte nesting or differentiation. These combined hydrogels containing cultured and differentiated chondrocytes could be useful to be used for *in vivo* cartilage regeneration.

P640 Human dental pulp stem cells cultured in alginate hydrogels for cartilage regeneration

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INTRODUCTION: Dental Pulp Stem Cells (DPSCs), have demonstrated a high capacity to reconstitute various dental and non-dental tissues and the inherent angiogenic, neurogenic, and immunomodulatory properties of their secretome have been subject of study. These mesenchymal stem cells have been differentiated *in vitro* to odontoblasts, osteoblasts/ osteocytes, adipocytes and neural cells among others. Although they have also shown potentiality to be differentiated in chondrocytes, their role in cartilage regeneration is poorly studied. In this study we have analysed the regenerative potential of DPSCs cultured in alginate scaffolds in an *in vivo* model of articular cartilage damage. DPSCs effectiveness has been also compared with rabbit primary articular chondrocytes.

METHODS: Rabbit articular chondrocytes were isolated and cultured in medium containing DMEM, 50 µg/mL ascorbic acid, 10% FBS, non-essential aminoacids, and antibiotics. DPSCs were isolated from human third molars and cultured in medium containing αMEM, 10% FBS, 2mM glutamine and antibiotics. 3% alginate scaffolds were performed in the presence or absence of DPSCs or chondrocytes at 2×10^6 cells/mL. For *in vivo* evaluation of cartilage regeneration, a full-depth chondral defect along with subchondral bone injury was originated in the knee joint of rabbits, where the alginate scaffold was implanted. The following experimental groups were included: control (lesion group), alginate, alginate + chondrocytes and alginate + DPSCs. Morphological evolution of cartilage repair was studied 3 months after implantation by histological techniques.

RESULTS: Macroscopic analysis of control animals revealed a distortion of articular surface with the formation of fibrotic tissue as was confirmed by histological studies. In alginate scaffolds implanted animals a discrete layer of fibrotic tissue containing non-organized chondrocytes was observed. Rabbit primary chondrocytes in combination with alginate hydrogels induced the formation of a thick layer of fibrotic tissue with an abnormal chondrocyte cell proliferation which showed a disorganized pattern. Conversely, in animals with implants of alginate + DPSCs, a lower bulging of the articular surface was observed at the macroscopic level. The microscopic study revealed the formation of a thick layer of cartilage with organized isogenic chondrocytes groups.

DISCUSSION & CONCLUSIONS: Alginate has proved effective in preserving chondrocyte phenotype *in vitro*. Although this hydrogel has similarities with chondral matrix, it is not able to induce the regeneration of native articular cartilage architecture after an injury. The use of primary rabbit chondrocytes is not advantageous maybe because the de-differentiation inherent to cell culture. In contrast, animals treated with scaffolds containing DPSCs showed a marked articular cartilage regeneration which may be due in part to the anti-inflammatory properties reported for DPSCs. Results presented here support the use of DPSCs for cartilage regeneration *in vivo*.

ACKNOWLEDGEMENTS: This work was supported by grants MAT2016-76039-C4-2-R and CIBER-BBN.

P641 ROLE OF MATRILIN-3 IN CHONDROGENESIS OF MESENCHYMAL STEM CELLS

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Mesenchymal stem cells (MSCs) have shown therapeutic promise in many experimental and clinical models of cartilage disease. However, a commonly reported feature of MSC transplantation is predominant fibrocartilage regeneration. This is due to the fact that, MSCs are environmentally responsive and they secrete bioactive factors in response to local cues such as pro-inflammatory molecules, hypertrophic chondrocytes, and bone with high mineral density at the transplanted site. Matrilin-3, an extracellular matrix component present in the cartilage can also exert chondrocyte protective effects. Therefore, using matrilin-3, the priming strategies to enhance MSCs bioactive factor secretions which are favorable for cartilage regeneration were investigated. Matrilin-3 priming dose and duration to MSCs were optimized based on collagen II and aggrecan mRNA expression. Priming with matrilin-3 has not altered the proliferation as well as does not induce cytotoxicity to MSCs. In addition, MSCs retained the similar expression levels of CD90, CD73, CD105, CD11b, CD34, CD45, and HLA-DR even after matrilin-3 priming. Cell cycle analysis showed that the priming has increased proportion of cells at S-phase. Cytokine array results suggested that, matrilin-3 priming has enhanced the bioactive factor secretion such as anti-inflammatory (Interleukin-10, IL-10), endogenous stem cell mobilization (Chemokine (C-C motif) ligand 5, CCL5) and cartilage specific tissue remodeling and regenerative (tissue inhibitor metalloproteinase inhibitor 2, TIMP2; matrix metalloproteinase-1, MMP1) factors compared to non-primed MSCs. In the indirect co-culture system, primed MSCs has increased chondroitin sulfate and collagen II content along with N-cadherin in degenerated human nucleus pulposus cells compared with non-primed MSCs. This study highlights the strategy of matrilin-3 priming to improve MSCs secretome for targeted cartilage therapeutic efficiency. Future preclinical studies will be performed to validate the therapeutic efficacy on rabbit model of degenerative disc disease.

P642 The importance of after seeding treatment during re-swelling time of the scaffold after compression release-induced suction seeding technique

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The cell seeding in a biomaterial is an important process for tissue engineering applications that helps to control and modulate tissue formation. Intuitively, seeding of cells within a three dimensional biomaterial may seem to be straightforward thanks to the available void volume inside highly porous scaffolds. However, this is not always the case especially for a thick scaffold presenting low permeability, being tortuous and with hydrophobic properties. These scaffolds are useful in functional tissue engineering where a load bearing biomaterial with mechanical properties close to host tissue is required. For instance, in cartilage tissue engineering tough scaffolds with low permeability are required for load bearing after transplantation while maintaining cells differentiation. In general, uniform and effective cell seeding of these types of scaffolds is still a challenge.

An efficient and uniform distribution of the cells is essential to obtain a normalized initial condition either for in vitro experiments such as mechanobiological studies or for clinical applications. Only in this situation, a homogeneous artificial tissue could be formed since there is no chance to modify cells distribution and density after an ineffective cell seeding. Among different seeding methods, the compression release induced suction has been reported as a promising approach in particular for cartilage tissue engineering. Its effectiveness may significantly vary depending on different factors such as scaffold geometry or microstructure. The relative importance of these factors in the efficiency and uniformity of seeding is not well established. We observed even by implementing optimized compression release induced suction loading regime, the cell penetration through thickness is not satisfying for scaffolds showing low permeability. We showed that a practical after seeding treatment (AST) such as slow rotation is helpful to boost penetration of cells inside the core of the scaffold to reach a homogenized distribution. We could explain the AST working mechanism by hypothesising that the cells penetration in the scaffold is due to the slow fluid movement inside the tortuous pores of the scaffold during scaffold re-swelling time. After several loading-unloading cycles, the hydrated scaffold lose some fluid inside the pores. By giving time for re-swelling and also providing dynamic movement of the cells suspension around the scaffold by an appropriate AST, we can increase cells penetration inside the scaffold. The optimum CIS loading regime followed by the proposed step of AST led to effective medium infiltration and cells penetration not only for permeable scaffolds but also for scaffold presenting low permeability. We also investigated the influence of contributing factors such as scaffold permeability, thickness and coating on seeding efficiency and distribution using factorial design of experiments. Our results indicated that the cell seeding efficiency and uniformity is reduced while reducing permeability of the scaffolds. We observed, scaffold coating with fibronectin increased the cell seeding efficiency since it promotes binding site for cells attachment. Our results also showed that increasing scaffold thickness in the range of knee articular cartilage (e.g.,
1.5 mm to 3 mm) does not have a significant influence on cells distribution inside the scaffolds.

For instance

Consider that a scaffold mimicking the native cartilage structure should present a structural heterogeneity, therefore the authors should discuss how the results of the simulation model could be affected by the scale-dependent pore distribution and structural heterogeneity features of the scaffold.

P643 How to get cells in a dense cartilage matrix?

Sylvia Nürnberger[1,2], Cornelia Schneider[1], Bernhard Rieder[3], Andreas Teuschl[3], Johannes Zehetner[4], Gerjo van Osch[5], Heinz Redl[1], Susanne Wolbank[1]

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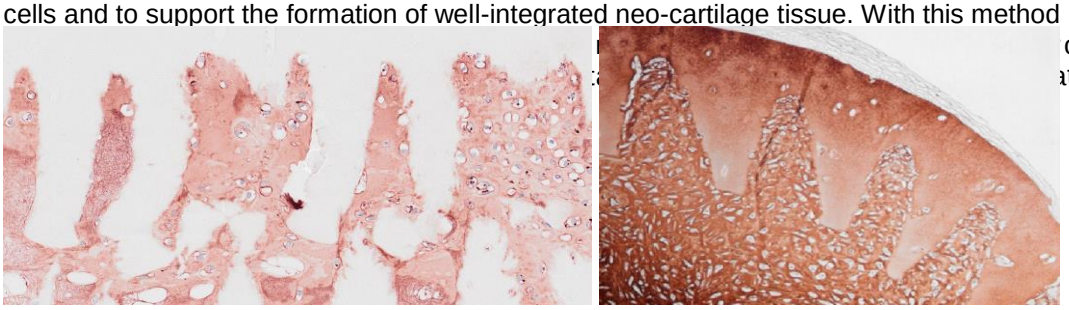
Decellularized cartilage scaffolds have been considered to be a promising approach for cartilage regeneration. In case of articular cartilage however, repopulation was impossible so far because of the matrix density. In the current study we present an approach to facilitate cell invasion by laser incision strategies.

The cartilage was previously decelluarlized and optionally went through a GAG-depletion process. Two types of lasers (femtosecond, CO2) were tested to produce holes, furrows and grids into the cartilage surface. Scaffolds were then seeded with chondrocytes, ASCs or co- cultures in order to test seeding strategies and neo-cartilage integration. After initial *in vitro* experiments, *in situ* cultivation (in osteochodral plugs) and *in vivo* implantation (osteochondral plugs implanted subcutaneously into nude mice) were performed to analyse the tissue development in a cartilage environment and under systemic conditions.

Cartilage laser incision could be achieved in highly defined and regular manner in terms of dimension and distance. Furrows and grids turned out to be the best patterns for high reseeding efficacy and in terms of surface area for cell attachment. Incisions in a distance of 100 µm were preferred since this roughly corresponds to chondrocyte distance in native tissue which also results in constructs stable during handling as well as long term cultivation. Seeded cells adhered well to the surface, especially in GAG-depleted scaffolds, deposited matrix and filled up the laser- space. When differentiated, the newly formed matrix formed a continuous transition to the cartilage scaffold. Mechanical testing against commercial scaffold controls showed a compression modulus much closer to that of native cartilage.

Laser-incisions to open up the cartilage matrix was proven to allow repopulating the material with cells and to support the formation of well-integrated neo-cartilage tissue. With this method



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Different patterns of incisions into decellularized and GAG-depleted articular cartilage produced with two different laser techniques. Immunostaining of newly synthesized collagen type II formed from chondrocytes (left) and general collagen type II in a ASCs-construct (right) showing well differentiated neo-cartilage filling up the gaps in the scaffold.

P644 Progenitor cells in auricular cartilage demonstrate promising cartilage regenerative potential in 3D hydrogel culture

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Introduction: The reconstruction of auricular deformities is a very challenging surgical procedure that could benefit from a tissue engineering approach. Nevertheless, a major obstacle is presented by the acquisition of sufficient amounts of autologous cells to create a cartilage construct the size of the human ear. Extensively expanded chondrocytes are unable to retain their phenotype, while bone marrow-derived mesenchymal stromal cells (MSC) show endochondral terminal differentiation by formation of a calcified matrix. The identification of tissue-specific progenitor cells in auricular cartilage, which can be expanded to high numbers without loss of cartilage phenotype, has great prospects for cartilage regeneration of larger constructs. This study investigates the largely unexplored potential of auricular progenitor cells for cartilage tissue engineering in 3D hydrogels.

Methods: Auricular chondroprogenitor cells (AuCPC) were isolated from the cartilage of the equine external ear using a fibronectin adhesion assay. These AuCPC, as well as auricular chondrocytes (AuCH) and MSC, were assessed for multipotency through the expression of putative stem cells markers and a trilineage differentiation assay. Furthermore, cells were encapsulated in 10% w/v gelatin methacrylate (gelMA) hydrogel to create cylindrical structures (diameter = 6 mm, height = 2 mm) that were cultured in chondrogenic differentiation medium. Cartilage regenerative performance was evaluated after 28 and 56 days of culture by means of gene expression profiles, biochemical and mechanical analysis, and histology and immunohistochemistry.

Results: AuCPC demonstrated the capacity to differentiate towards cartilage, bone and adipose tissue, confirming their stem cell-like multipotency. In addition, the cells expressed surface markers analogous to MSC. After 56 days of 3D culture in gelMA, relative mRNA expression of collagen type I, collagen type II and aggrecan by AuCPC was comparable to AuCH and MSC, whereas interestingly its elastin expression was the highest and collagen type X expression the lowest among cell types. Quantification of glycosaminoglycan (GAG) and DNA content demonstrated increased cartilage matrix formation in all cell types, which corresponded with the mechanical properties as indicated by an increasing compressive Young's modulus over time. Histological stainings verified GAG deposition and the production of collage type I and II in all cell types.

Discussion: The results from this study indicate that a multipotent progenitor population present in auricular cartilage has a promising cartilage regenerative potential. The auricular progenitor cells produce a neocartilage with properties comparable to the tissue produced by auricular chondrocytes and MSC, yet interestingly the high expression of elastin mRNA suggests a direction towards elastic-type cartilage. Moreover, due to their proliferative stem cell properties, the cells can yield high cell numbers from only a small cartilage harvest, improving the feasibility of using an autologous cell source. The potent ability to generate neocartilage in 3D hydrogel culture combined with their proliferative properties ranks auricular progenitor cells as a promising cell source for the tissue engineering of large auricular cartilageconstructs.

P645 Platelet activated scaffolds guide cartilage rib regeneration during tissue development

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Poland syndrome is a congenital disease, characterized by deformation and aplasia of pectoral muscles, ribs and arts. The disease etiology is still unknown, furthermore in some young patients, is possible to find rib cage abnormalities, with partial or total lack of some ribs and consequent unprotection of internal organs. In clinical practice, the technique currently used provides the implant of absorbable polylactic acid (PLA)-bars, which are continuously replaced during the body growth, submitting the patients to incessant and exhausting surgery interventions. In this study, we designed a scaffold suitable for cell delivery and we developed a rabbit model for the repair of costal defect. Our study is a highly innovative approach, which can promote the regeneration of cartilage defects in growing tissues.

We developed a “ready to use” hyaluronan based scaffold, biologically activated by addition of Platelet Rich Plasma (PRP). We set up a surgical procedure in a rabbit model for the reconstruction of a small piece of rib cartilage (1,5-2 cm). The cartilage defect was created in the seventh rib, and the PRP-biomaterials are implanted in the lesion area in combination or without (control) rabbit chondrocytes to obtain a model of allogenic graft. The platelet derivatives can support the cells proliferation and the maintenance of chondrocytes potential (Pereira 2013), therefore neocartilage formation. This system of allogeneic cell transplantation is supported by our recent studies showing that human allogeneic chondrocytes inhibit the immune response of T cells and the differentiation of peripheral blood monocytes cells (PBMCs) in antigen presenting cells (APCs).

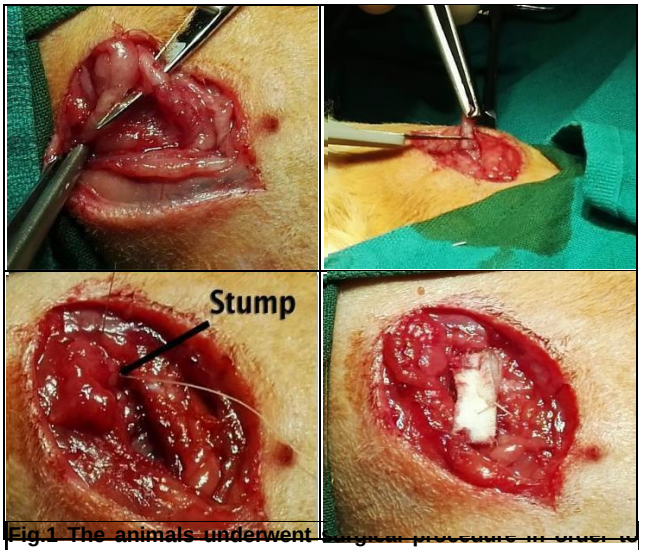

Fig.1 The animals underwent surgical procedure in order to practice a critical chondro-costal defect, which is replaced with biomaterials, fixed to the defect stumps.

After surgery, Rx was performed at 15, 30, 60 and 90 days. The implanted constructs were recovered after 1,5 and 3 months to evaluate by resin histology the rib cartilage reconstruction. Our study represents a preclinical animal study to test safety and efficacy of allogenic platelet derived products to use for cell-based regeneration in a tissue during the development and growth. This approach can be translated in a large spectrum of congenital or acquired thoracic malformations.

P646 Development of an in vitro model mimicking early osteoarthritis using mesenchymal stem cells undergoing chondrogenic differentiation

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Osteoarthritis is a degenerative disease that is mainly caused by aging, although in younger patients (aged 25 – 50) it can be a consequence of sports-related injuries or trauma. This results in early osteoarthritis with subsquent changes in cartilage extracellular matrix. Cell-based tissue engineering strategies using mesenchymal stem cells (MSCs) are thought to be an ideal cell type for the treatment of early osteoarthritc defects. Our group demonstrated in a clinical study, that interleukin-1 (IL-1) was expressed in cartilage plugs from patients with early osteoarthritis. *In vitro* studies have shown that IL- inhibits cartilage formation in chondrocytes or MSCs undergoing chondrogenesis. However, these studies show complete inhibition of tissue formation, whereas in the context of early osteoarthritis, cartilage extracellular matrix remains around the defect site. Thus, the present study sought to develop a model mimicking early osteoarthritis using using MSCs.

PROTOCOL: Human MSCs (Male donors; aged 18-60 years) were isolated from bone marrow and expanded in culture for one passage. 2 x 10^5 MSCs were aliquoted into wells of a 96-well cell culture plate in the presence of 10ng/ml TGF- 1 or in combination with IL-1 administered at a range of concentrations (0.1, 0.5, 1 and 10ng/ml) and centrifuged to form pellets. Pellets were removed from culture on Days 7, 14 and 21. Pellets were evaluated using histological (DMMB staining, collagen type I, II, MMP-13 and TGF- receptor II), pellet area and collagen type II ELISA.

RESULTS: Chondrogenic pellets in the presence of IL-1 demonstrated a dose dependant inhibition in chondrogenesis. Concentrations equal or greater than 1ng/ml IL-1 showed a reduction in pellet area and no positive staining for collagen type I, II (including ELISA analysis) and DMMB. However, at concentrations lower than 1ng/ml, despite a slight reduction in pellet area, positive staining for collagen type I, II and DMMB was observed. Furthermore, MMP-13 matrix staining was increased and TGF- receptor II stained cells was decreased in pellets at IL-1 concentrations.

CONCLUSION: A dose dependant catabolic response in cartilage extracellular matrix formation was demonstrated for IL-1 treated MSCs undergoing chondrogenesis. At concentrations equal or greater than 1ng/ml IL-1 , MMP13 expression was observed in the matrix, indicative of osteoarthritis. This was related to reduced expression of TGF- receptor II under these conditions that is required for TGF- induced chondrogensis. However, at concentrations below 1ng/ml IL-1 , a reduced catabolic reponse in extracellular matrix components was observed, whilst showing a moderate expression in MMP-13 and the presence of cellular TGF- receptor II expression. Therefore, this latter model may be used to develop pro-chondrogenic strategies for the treatment of early osteoarthritic defects.

P647 Articular cartilage regeneration in osteoarthritis rat model Karthikeyan Rajagopal, Sanjay K Chilbule, Noel Walter, Vivek Dutt, Vrisha Madhuri *Christian Medical College, Vellore, Tamilnadu, India*

Introduction:

Mechanical injury to the articular cartilage leads to upregulation of detrimental factors such as bone morphogenetic protein, vascular endothelial growth factor resulting causes osteoarthritis (OA). We tested if the OA progression can be inhibited by intraarticular injection of Bone Morphogenetic Protein (BMP) pathway and Vascular endothelial growth factor (VEGF) antagonists and regenerate the articular cartilage using Wnt agonist which is vital for early chondrogenesis.

Materials and methods:

Rat knee osteoarthritis (OA) was created by surgical excision of the medial meniscus and medial collateral ligament. Two groups of twenty each of Sprague Dawley rats for controls (surgery alone) and tests (surgery + intraarticular injection of drug combination every two weeks) were used. Ten animals from each group were sacrificed at four weeks and eight weeks. Modified Mankin score was used to evaluate the histological outcome.

Results:

Surgical osteoarthritis knee model was successfully created with significant higher modified Mankin scores for operated knees, both in short (p=0.0001) and long term (P= 0.001). MicroCT confirmed the appearance of osteophyte, subchondral cyst and subchondral sclerosis in the defect. During short term follow-up, modified Mankin score was less in the test animals (4.9±0.8) as compared to controls (5.6±1.07) (P= 0.17) but the trend was reversed in the long term follow-up (P=0.13). Subgroup analysis revealed that the lateral condyle (p=0.03) and anterior compartment of the knee joint (p=0.03) had significantly higher scores in the long term.

Impact of investigation:

Combined approach of using BMP antagonist, Wnt agonist, and VEGF antagonist was ineffective in stopping the progression of osteoarthritis in a rat surgical OA knee model. This could also be attributed to multiple injection. Sustained delivery of drugs using scaffold and testing their effect individually may ameliorate the disease progression.

P648 Chitosan-gelatin nanocomposite scaffold for articular cartilage regeneration

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A scaffold mimicking the structure and function of articular cartilage is a pre-requisite for Articular Cartilage (AC) repair. In this study, we tested the suitability of a composite chitosan-gelatin scaffold incorporated with nanoclay (CG-NC) for cartilage regeneration. We hypothesize that the composite scaffolds will provide cue for extracellular matrix secretion and have better mechanical strength by the addition of laponite. Articular chondrocytes were isolated from the rabbit knees and cultured on CG-NC scaffolds for three weeks. Tissue engineered cartilage (TEC) was assessed by biochemical and gene expression analysis. Regenerative potential of TEC was tested on rabbit osteochondral defect model, while cell-free scaffolds on the opposite knee defect served as control. Sixteen weeks post implantation, defects were assessed for their gross appearance and histology. Biochemical analysis shown that glycosaminoglycan/DNA content was increased with days of culture from 100ng on 14th day to 140ng on 21st day of culture. Real time PCR also confirmed that expression of cartilage specific gene COL2A1 and SOX 9 on 21st day culture with 2 folds less collagen type 1 expression than the day 14th culture. O'Driscoll scores (mean ± S.D) revealed that the quality of regenerated cartilage was good in three (18.0 ±3.6) out of the five transplanted rabbits as compared to their control (8.3 ± 0.57). The mean score of the two test rabbits which had a poor outcome was 7.0, similar to the control defect, and was correlated with delay in surgical procedure. Results indicate that CG-NC scaffolds act as a suitable vehicle for chondrocytes and facilitated AC regeneration indicating the potential of the CG-NC scaffolding system for AC tissue engineering applications.

Impact of the investigation

Our animal study shows that the tissue engineered constructs has an ability to repair the osteochondral defect. Further tweaking in the fabrication of scaffold to mimic collagen orientation of native cartilage may accelerate the healing. This strategy should further be tested in a large animal model prior to clinical studies.

P651 Graphene oxide is an effective growth factor delivery vehicle inducing human MSC chondrogenesis

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The use of graphene (G) and its oxides (GO) to form cell substrates and guide appropriate cell function is a relatively new topic. Formats of 2D G/GO-coated surfaces, 3D chemical-vapour deposited foams and GO dispersions within cell pellets have generated interesting results particularly in the area of directing stem cell differentiation into specific lineages. Here we report the use of GO flakes dispersed in a collagen hydrogel to deliver the chondrogenic factor TGF-β3 to encapsulated human mesenchymal stem cells (hMSCs) to direct appropriate differentiation.

GO flakes with TGF-β3 pre-adsorbed on their surface were dispersed in a collagen hydrogel in which with hMSCs were simultaneously encapsulated. Interestingly, the flakes adsorbed >99 % TGF-β3 with <5 % released over 28 days. The conformation of adsorbed TGF-β3 mimicked its native (active) conformation and this conformation was retained for longer compared to free TGF-β3 in culture medium. For hMSCS encapsulated in GO-containing gels cell viability was excellent (>99 %)indicating the system's excellent biocompatibility. Cellular uptake of the nano-flakes was minimal. TGF-β3 pre-adsorbed on GO was able to efficiently guide cell differentiation into a chondrocytic phenotype. The expression of chondrogenic genes in gels with TGF-β3 pre-adsorbed on GO, together with deposition of cartilage-specific extracellular matrix exceeded that observed in cultures without GO and in cultures where the growth factor was supplied exogenously in the media

The results demonstrate that GO acts as an efficient growth factor delivery carrier within a scaffold/ hydrogel. The ability of GO to ensure local delivery of growth factors/cytokines without release makes it attractive for tissue engineering strategies, in particular where regionally-specific MSC differentiation is required, for example in osteochondral tissue engineering.

P652 Results of a composite chitosan-based scaffold for the osteochondral tissue regeneration in animal models

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OBJECTIVE

Osteochondral defects are a challenging issue, since the native hyaline cartilage and the underlying subchondral bone structure cannot be regenerated by any available treatment. Chitosan is a promising biomaterial for both cartilage healing and bone formation induction. Aim of this study was to evaluate the in vivo regenerative potential of hybrid MgHA/(col+chit) based scaffold.

DESIGN

The hybrid MgHA/(col+chit) based scaffold was tested in a medium-size animal model (12 rabbits at 8 weeks) to investigate its ability to support bone tissues regeneration, and in a large-size animal model (6 sheep at 6 months) to ascertain its osteochondral regenerative potential. Results were evaluated macroscopically and by microtomography, histology, histomorphometry, and immunohistochemical analysis.

RESULTS

In the rabbit model, the scaffold showed poorer performance compared with a commercially available product for bone regeneration. In the sheep model, all the macroscopic, micro-CT, histological and immunohistochemical analysis did not show significant differences between experimental group and control. ($p>0.05$), with no evidence of cartilage and subchondral bone formation. Moreover, the persistence of scaffold material and lack of integration with connective tissue around the scaffolds was observed.

CONCLUSION

This hybrid hydroxyapatite collagen/chitosan scaffold failed to provide both bone and cartilage regeneration. The results of both rabbit and sheep models raised some concerns on the osteochondral application of chitosan, particularly regarding bone tissue, as any treatment of the surface chondral lesion is likely to fail without support from an intact subchondral bed. Further studies are needed to explore the best formulation of chitosan based composites for osteochondral treatment.

P653 Cartilage differentiation of hBM-MSCs on human wharton's jelly-derived scaffolds

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Introduction: Since articular cartilage is an avascular tissue, damaged articular cartilage has limited self-regeneration capacity. Current methods for human Mesenchymal Stem Cell (hMSCs) differentiation into cartilage results in tissues with a lower quality as compared to native articular cartilage. To overcome these problems extracellular matrix (ECM) based tissue engineering (TE) strategies have been developed. Decellularized biological scaffolds have the potential to provide the appropriate signals, in order to support cellular retention, migration, proliferation and differentiation. Given the high amount of collagen, hyaluronic acid (HA) and glycosaminoglycan (GAG) in umbilical cord, this tissue can be considered as an abundant natural biomaterial for TE applications.

The aim of this study was to prepare scaffolds for cartilage TE derived from human Wharton's jelly and to culture hMSC within them under static conditions. It is hypothesized that chondrogenic differentiation can be induced in bone marrow derived hMSCs through the loading on tissue specific scaffolds.

Material and Method: Human umbilical cords were collected from full-term births with informed consent of the mother after either cesarean section. After removing the three main vessels, the human umbilical cord was decellularized by incubation in SDS followed by incubation in PBS. Acetic acid and Pepsin were used as a digestive solution to make Pre-Gel from Wharton's jelly. 6 mm diameter porous scaffolds were fabricated. 5×10^6 MSCs seeded on the scaffolds and kept in chondrogenic medium for 21 days. To evaluate chondrogenesis, polymerase chain reaction and histology were then performed. Gelatin based scaffolds was used as control group.

Results: Histological findings from H&E and DAPI staining confirmed that the decellularization process was successful as there were no visible cell nuclei. Real time-PCR results demonstrated that hMSCs in tissue scaffolds showed an increased expression of Col II, ACAN and Sox9 markers compared with control group, whereas there were no significant differences between expression of Col I, Col X and Runx2 between tissue and gelatin scaffolds. The fast green safranin O staining indicated a high level of GAGs in tissue scaffold compared to the gelatin scaffold.

Discussion: It is confirmed that ECM derived scaffolds are able to provide a native environment for the cells and promote cell proliferation and differentiation. However, some limitations regarding availability of human cartilage make ECM-derived cartilage still challenging for therapeutic application. To address this issue, Wharton's jelly which has very low number of cells and high amount of ECM components can be taken into the consideration as an appropriate substitute. Here, it was demonstrated that enhanced chondrogenic differentiation, can be achieved through the use of ECM materials of Wharton's jelly.

P655 The role of autophagy during chondrodifferentiation of human mesenchymal stem cells

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INTRODUCTION: Cartilage lesions due to injury heal poorly, leading to early onset of osteoarthritis, chronic joint pain and disability. Mesenchymal stem cells (MSCs) in combination with polymeric carriers can be used to induce differentiation of the cells towards chondrogenesis. Autophagy is a cellular mechanism, required for differentiation of adult stem cells. Influencing autophagy during differentiation of MSC to chondrocytes may alter cell fate. Therefore, we investigated the effect of rapamycin, an autophagy activator, during differentiation of MSC in 3D microtissues to chondrocytes.

METHODS: Cartilaginous microtissues were prepared by mixing droplets of human MSCs in chitosan (sChi) with droplets of oxidized alginate (oxAlg). Microtissues were induced in chondrocyte differentiation media in presence and absence of rapamycin for 10, 14 and 21 days. Changes in gene and protein expression for collagen type II and X and autophagy markers Atg5 and LC3 were investigated by real-time-PCR and immunofluorescence.

RESULTS: Upon chondroinduction, down regulation of key autophagy gene Atg5 and Beclin1 was observed in the non-stimulated group. We observed an increased expression of collagen 2 and hypertrophic marker collagen X starting at day 10 with highest expression on day 21. Rapamycin treated group showed increased Atg5 gene expression with the highest peak at day 14. Treated microtissues exhibited better cell morphology and viability with high expression of Atg5 and punctated immunostaining pattern for LC3 confirming accumulation of autophagosomes.

CONCLUSIONS: Our study demonstrates that autophagy plays an important role in chondrodifferentiation of MSC. Administration of rapamycin could serve as novel therapeutic approach for treating cartilage injuries for future clinical applications.

P656 Biomechanical and histological evaluation of spontaneous cartilage regeneration in two different defect models in rabbits

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Background

The regeneration capacities of cartilage are limited, which is one of the major reasons for posttraumatic osteoarthritis. This animal study aimed to clarify, whether this tissue has natural healing capacities dependent on the lesion type.

Methods

To compare different kinds of spontaneous restoration of cartilage, two types of cartilage defect models of the medial femoral condyle in rabbits with (drill) and without (scratch) opening of the subchondral bone layer were employed. Besides macroscopic and histological evaluation, the regenerated tissue was examined biomechanically analyzing thickness, instant and shear modulus.

Results

After 6 weeks, all defects with an open subchondral layer showed regenerated tissue reaching an average macroscopic ICRS grading of 2.00±0.63. In contrast, none of the full-thickness lesions was healed (4.00±0.00). However, in case of a patella dislocation the regeneration was comparable to the defects with opened subchondral bone (1.25±0.50). Similar results were found regarding the filled lesion area. Histologically, the newly formed tissue of both groups with repair showed hypertrophy and less intense staining for glycosaminoglycans than normal cartilage. Correlating, the biomechanical testing showed a statistically significant increase in thickness. However, the repair tissue seen after full-thickness lesions with patella dislocation and drilled osteochondral defects had inferior biomechanical stress resistance with less instant and shear moduli. Furthermore, the thickness and the biomechanical parameter differed between the medial and the lateral femoral condyle.

Discussion

Cartilage can be repaired spontaneously, if the defect also affects the subchondral bone layer. However, regeneration is also possible in pure cartilage lesions in case of decreased biomechanical stress for the injured area. The regeneration is associated to hypertrophy and diminished biomechanical stress resistance. Because of the significant difference in thickness, instant and shear moduli comparing medial and lateral femoral condyles, both anatomical regions should be evaluated separately.

P657 Artificial tissue cutter: creation of standardized chondral defects in an osteochondral model

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Introduction: Cartilage treatment strategy is dependent on the lesion severity. Development and evaluation of cartilage treatment strategies in different defect geometries in pre-clinical models have to address defects ranging from fissures and cartilage only lesions to large defects involving subchondral bone. Aim of this study was to modify our osteochondral model (Schwab et al. 2016) by creating standardized cartilage defects with an automated device. Matrix assisted cell free and cell loaded treatments were compared to investigate the influence of surrounding tissue on cartilage regeneration in *ex vivo* model.

Methods: Artificial Tissue Cutter (ARTcut®) is a computerized numerical controlled device for automated wounding of soft and hard tissues or tissue engineered products. Sensor controlled drilling allows creation of reproducible defects with standardized geometry. In this study, chondral defects, 4mm in diameter and 1mm in depth, were created with ARTcut® in cartilage of porcine osteochondral explants (8mm diameter x 5mm height). Lesions were left untreated, filled with cell free or chondrocyte loaded collagen I hydrogel isolated form rat tail and cultured for 4 weeks in *ex vivo* culture platform with tissue specific media. Live-dead staining was performed to investigate cell viability (day 0 & day 28). Cartilage regeneration was evaluated by (immune-) histological stainings and quantification of proteoglycan (GAG) content inhydrogels.

Results: Implementation of ARTcut® allows creation of chondral defects with a defined depth, measured from the surface of superficial layer of the explant. Live-dead staining did not show evidence of necrotic tissue formation due to drilling. Instead, invasion of cells into cell free hydrogel was observed after 4 weeks *ex vivo* culture. These cells originated from cartilage of explant but showed elongated morphology which will be further analyzed for cartilage phenotypic markers. Chondrocytes in cell loaded approach synthesized cartilage matrix, shown by Safranin- O staining and immunohistological staining for collagen II and aggrecan and absence of collagen I and X. GAG/DNA content increased at day 28 to 5-fold (21.7 µg/µg) compared to day 0 (4.38 µg/µg).

Impact: ARTcut® represents an innovative device for creation of cartilage defects with varying geometries in osteochondral model to mimic different lesion severities and thus stages in degenerative joint disease. Comparison on chondral and osteochondral defects allows to study influence of opening subchondral bone on cartilage regeneration in *ex vivo* model.

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P658 Mechanical and image analysis of porous PGSm particles cultured *in vitro* as scaffolds for cartilage repair

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Introduction

Research into cartilage regeneration has proven to be complicated in repairing a seemingly simple tissue. Tissue engineered approaches explore the use of natural and synthetic materials as biomaterial scaffolds. Natural scaffolds have excellent tissue compatibility; however often lack mechanical strength, have unstable degradation rates and have limited reproducibility. Synthetic materials overcome these drawbacks; however they often have limited tissue compatibility. PGSm (poly(glycerol sebacate methacylate)) is a novel formulation of the soft biomaterial PGS. The aim of this study is to analyze the use of PGSm porous particles, seeded with MSC derived chondrocytes for cartilage repair; and to explore various methods of mechanical and image analysis.

Methods

PGSm was synthesised and methodologies developed to produce porous PGSm particles (100-500 µm). Synthesis and production techniques can be varied to produce particles with a tuned diameter size, pore size, level of porosity and Young's modulus. Primary bovine chondrocytes were seeded and cultured on PGSm particles for 3 weeks. Cell viability assays were taken throughout and GAG assays (glycosaminoglycans) were performed at 3 weeks. Cell/scaffold constructs were immunofluorescent labelled with DAPI, Phalloidin and Collagen-II and imaged on a confocal microscope. Samples were cryosectioned and stained with picosirius red, toluidine blue, and H&E . Samples underwent various fixatives including an osmium tetroxide fix to determine the best fixation strategy; 2D and 3D images were obtained using Scanning Electron Microscopy and Micro-CT. Compressive mechanical analysis was performed on both the porous PGSm material and the porous PGSm particles seeded with cells.

Results

Cell viability results showed that the particles are a viable scaffold for chondrocyte culture. The chondrocytes laid down an extensive network of collagenous extracellular matrix and formed atissue disk over 3 weeks. GAG assays confirmed the presence of sulphated GAGs. Histology stains showed cells and collagen throughout the porous particles. The confocal image (Fig.1), shows porous particles (green), DAPI (blue) and Phalloidin (red.) Anti Collagen-II antibody confirmed the presence of collagen-II. Tissue scaffold compressive modulus values were calculated.

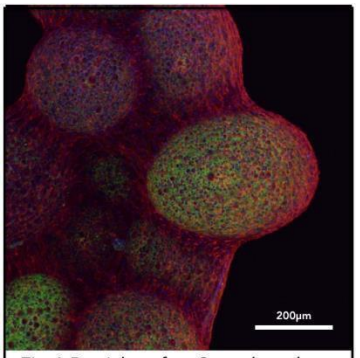

Fig.1 Particles after 3 weeks culture

Conclusion

PGSm particles are promising scaffolds for cartilage repair, they are biocompatible, have an appropriate modulus, are tuneable and are reproducible. Interestingly in only 3 weeks the particles merged together with collagen fibres forming a complete particle disk.

P660 Fabrication of tendon and cartilage tissue constructs with commercially available polymers

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Tissue engineering holds a great potential in tendon and cartilage regenerative medicine strategies, since the traditional methods of repair for cartilage and tendon injuries have disadvantages that can deter their long-term effects. To achieve production of cartilaginous tissue, scaffolds must provide a desirable 3D environment. where tenocytes and chondrocytes will deposit their tissue-specific extracellular matrix. The electrospinning process has been used to produce nanofibrous scaffolds for various musculoskeletal tissues including cartilage tendon and ligament. In this work two commercially available polymer materials, a) BIOSYN® :poly(glycolide -co- dioxanone -co- trimethylene carbonate) and b) MAXON®: poly (glycolide- trimethylene carbonate) were used to fabricate electrospun scaffolds. The structural, mechanical and thermal properties were assessed with electronic microscopy, uniaxial mechanical testing and differential scanning calorimetry (DSC) respectively. Human chondrocytes and tenocytes were expanded up to passage 3 in DMEM media, supplemented with 10% fetal bovine serum and 1% penicillin / streptomycin. 50,000 cells / cm^2 were subsequently cultured for up to 14 days. Cell viability and metabolic activity was assessed using Live/Dead® and alamarBlue® assays respectively. Cell morphometric analysis was carried out using DAPI and Rhodamine conjugated Phalloidin and subsequent image analysis (ImageJ). Extracellular matrix deposition was assessed with immucytochemisty. 5546

Polymer	Fibre Diameter (nm)	Stress at Break (Mpa)	Strain at Break (%)	Modulus at 2 % Strain (Mpa)
Biosyn®	1436 ± 530	2,62 ± 1,47	126 ± 47,44	0,04 ± 0,03
Maxon®	1796 ± 384	2,25 ± 0,95	111 ± 24,59	0,04 ± 0,03

Figure 1: Biophysical characterisation of electrospun scaffolds.

Keywords: Electrospinning, cartilage repair, tissue engineering

P661 Chondrogenic induction of mesenchymal stem cells by the combination of kartogenin and 3D tri-copolymer scaffolds in a self-designed bioreactor system

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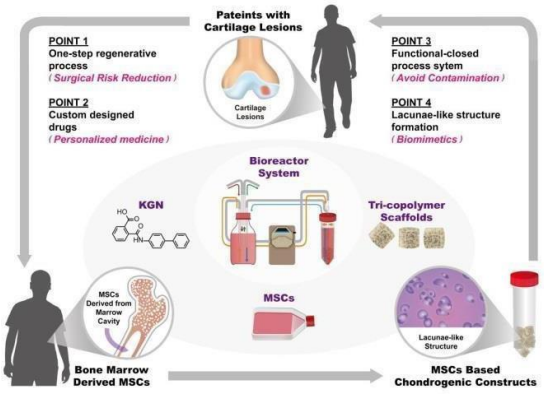
Cartilage tissue may be damaged because of ageing, trauma, or excessive loading. Nonetheless, unlike tissues with sufficient blood flow, cartilage tissue possessed limited intrinsic healing potential because of insufficient nutrient supply. Consequently, tissue engineering for cartilage repair is a rapidly growing field for clinicians to achieve fully differentiated cartilage tissue substitutes to be administered in repairing surgeries. As a result, the need for investigating not only a reliable system for cartilage tissue manufacturing but also affordable for most patients is crucial.

In this study, we optimized the chondrogenic process in mesenchymal stem cells (MSCs) by combining a small molecule kartogenin (KGN), which has been demonstrated to promote chondrocyte differentiation both in vitro, and in two osteoarthritis animal models, and a three dimensional (3D) tri-copolymer scaffold composed of gelatin, hyaluronic acid and chondroitin-6-sulfate. After cultured in homemade perfusion bioreactor system, *Acan*, *Sox9* and *Col2a1* gene expression were significantly up-regulated in 3D culture condition. The lacunae-like structure showed active deposition of type II collagen and aggrecan deposition. Based on the current results, we expect that this research provide an example for small molecule regulated practice and thus provide a new combination for cartilage tissue engineering.



P662 An automated bioreactor to determine the effects of post-operative rehabilitation protocols on articular chondrocytes

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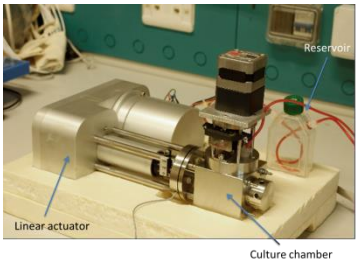
The incidence of cartilage lesions is constantly on the rise due to the population ageing and to a more active lifestyle. The inefficient natural recovery from these lesions is due to the poor self-healing ability of articular cartilage is responsible and can eventually lead to the onset of osteoarthritis. For this reason, a number of surgical approaches has been developed to restore the articular cartilage. During the post-surgery rehabilitation period, the timing and the intensity of physical workout is crucial to guide the patient in the healing process. It is known that the mechanical stimulation of chondrocytes and extracellular matrix through joint movement regulates cartilage homeostasis. In this context, we developed a bioreactor that provides the in vivo-like mechanical stimuli of hydrostatic pressure (HP) and interstitial perfusion in order to determine the effects of different rehabilitation strategies at the cellular level.



The bioreactor is fully automated and programmable and it is able to generate up to 10 MPaof cyclic hydrostatic pressure (HP) and from 30 to 1000 µm/sec of interstitial perfusion. We tested different stimulation patterns on primary human articular chondrocytes seeded in a collagen scaffold. Cycles of stimulation of 20 min/hour with a frequency of 1 Hz for 4 hour/day were applied to the constructs for 1 week to simulate walking with crutches (HP of 3MPa), getting up from the chair (9MPa) and a gradual increase of physical workout (3-6-9 MPa). We also maintained chondrocytes without stimulation and perfusion as a static control to mimic patient immobilization. We found a comparable metabolic activity in chondrocytes cultured with the low (3 MPa) and the incremental (3-6-9 MPa) HP regimen and in static conditions. However, metabolic activity significantly decreased in chondrocytes subjected to 9 MPa HP. The amount of glycosaminoglycans (GAGs) produced per single cell did not present significant differences among all the tested conditions. The gene expression analysis showed instead a favourable trend in chondrocytes cultured under incremental HP. In particular, an increased expression of SOX9, ACAN, and COL2A1 was found in chondrocytes subjected to incremental HP compared to static controls and cells subjected to 3 MPa and 9 MPa loading conditions. Notably, the chondrocytes subjected to 9 MPa showed the lowest levels of SOX9, ACAN, and COL2A1 suggesting a negative effect of high HP stimulation on chondrocyte phenotype. This observation was corroborated by the high expression of MMP1 and MMP13 determined in chondrocytes stimulated with 9 MPa HP. Interestingly, we found that all the tested HP regimen were able to reduce the expression of COL1A1, indicating that HP limits the production of fibrocartilage. In conclusion, our bioreactor system can be used to mimic a physiological-like environment to generate a model of cartilage formation and to evaluate the effects of different mechanical stimulation patterns at the cellular level.

P663 Micro/Nano-technology for physeal growth plate engineering

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Physeal growth plate is a complex cartilaginous tissue with various linear zones that is responsible for the longitudinal growth of long in children and adolescents. However, it is vulnerable to injury which can lead to growth plate arrest. Cartilage tissue engineering provides a potential solution for reconstruction of growth plate. The challenge will be to identify the optimal culture conditions for the different zones of growth plate. The project provides a non-invasive molecular beacon (MB)-based nanosensor platform to monitor target specific messenger RNA (mRNA) expressed during chondrogenic differentiation of human mesenchymal stem cells (MSCs) in 3D agarose hydrogel. These nanosensors were fabricated through co-encapsulation of biodegradable poly(lactic-co-glycolic acid) nanoparticles (PLGA NPs) and uptake by MSCs. Sustainable release of these MBs from the PLGA NPs allows longitudinal monitoring of gene expression through fluorescence means, at the same time offers its potential as screening platform to obtain optimal culture conditions for different zones of growth plate. Validation of the platform was done using quantitative reverse transcription polymerase chain reaction (qRT-PCR). With the optimal culture conditions, growth factors can be delivered through the use of magnetic microbubbles (MMBs). Growth factors on MMBs can be release at specific time points through stable oscillation under ultrasound simulation. Delivery using MMBs also improves the penetration of released growth factors. The magnetic property of MMBs enables localisation of the MMBs, thus offers a possibility for engineering multi-zones growth plate.

P664 Influence of matrix properties of cartilage bioimplants on human chondrogenic progenitor cells

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INTRODUCTION: Since cartilage tissue has no intrinsic regeneration capacity appropriate replacement materials are needed for cartilage defect treatment[1]. A decellularised carti-lage matrix derived from porcine nasal septum already showed very promising qualities[2]. The aim of this study was to investigate the influence of two treatment steps on matrix properties and the effect of matrix quality on human chondrogenic progenitor cells (CPC).

METHODS: Scaffolds from porcine nasal septum were decellularised by an intensified chemical multistep procedure by varying two steps: first treatment with sodium hydroxide (1 M NaOH 1,5 h, 3 h, 6 h) and second with guanidine hydrochloride (GuHCl 1 M, 2 M, 4 M; 4 d)[2]. The amount of denatured collagen (w_D) and the content of sulfated glycosaminoglycans (w_{GAG}) was determined (Table1). Also scaffolds were seeded with primary human CPC derived from nasal septum and articular cartilage and cultivated in a modified chondrogenic medium up to 28 d[3]. Cell growth, ECM synthesis and migration were evaluated histologically. **RESULTS**: An extended treatment with NaOH tends to result in increasing w_D whereas w_{GAG} was not affected. At higher concentrations of GuHCl lower w_D was detected while w_{GAG} slightly increased. *In vitro* seeding with primary human CPC showed good chondroconductive properties of the scaffolds. During cultivation a homogeneous cell layer and synthesis of ECM could be observed and the layer thickness increased up to 250 µm. Improved or even noticeable cell migration was not detected.

Table 2: Amounts of denatured collagen (w_D) and GAG (w_{GAG}) of different variations.

		1 M NaOH			GuHCl		
	native	1,5 h	3 h	6 h	1 M	2 M	4 M
w_D [%]	12,14 ± 0,62	56,61 ± 0,10	66,24 ± 1,03	87,92 ± 4,26	56,61 ± 0,10	60,80 ± 1,66	47,16 ± 0,43
w_{GAG} [%]	19,19 ± 3,08	1,40 ± 0,28	1,45 ± 0,26	1,41 ± 0,15	1,40 ± 0,28	1,87 ± 0,36	2,22 ± 0,45

DISCUSSION & CONCLUSIONS: W_{GAG} was significantly reduced whereas NaOH and GuHCl variations showed narrow differences. The slightly higher content of GAG at higher GuHCl concentrations may be caused by agglomerating GuHCl and therefore being less efficient[4]. As expected the amount of denatured collagen increased with extended NaOH treatment due to strong hydrolytic effects of 1 M NaOH. *In vitro* seeding with primary human nasal and articular CPC proved the scaffolds to provide good properties for cell adhesion and synthesis of new ECM components, even at high w_D values. To achieve improvement of cell migration further modifications of the decellularisation procedure will be tested. **R ACKNOWLEDGEMENTS**: The authors wish to thank the DFG (German Research Foundation, BU461/36-1, RO 2207/5-1, BR 919/10-1) for funding this project.

P665 Generation of three-dimensional (3D) engineered articular cartilage using Human ESCs

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Tissue engineering is a promising approach for treatment of articular cartilage injuries using a combination of cells, scaffolds and biological molecules. Human embryonic stem cells (hESCs) are attractive to tissue engineers due to their pluripotency and self-renewal properties. We previously developed a defined differentiation protocol to generate chondrogenic cells from hESCs. Chondrogenic cells generated in this 14 day 2D protocol were able to repair osteochondral defects in rats when cells were encapsulated within fibrin gels. However, fibrin gels alone do not sustain cartilage formation in vitro from these cells in chondrogenic medium.

Three-dimensional (3D) scaffolds, including hydrogels, sponges or 3D-printed scaffolds have been commonly used in the field of tissue engineering. In this study, we aimed to produce 3D tissue-engineered cartilage from fibrin gels modified with hyaluronic acid (HA), which is an important component of native cartilage, and from 3D-printed polycaprolactone (PCL) scaffolds fabricated by 3D printing with or without plasma treatment to improve hydrophilicity. To produce 3D constructs, chondrogenic cells were encapsulated within fibrin gels modified with HA or were directly seeded onto the surface of plasma-treated PCL scaffolds for 3D culture. PCL scaffolds treated with fibronectin were found optimal for cell survival. The biological response of cells, in terms of the expression of chondrogenic genes, i.e. SOX9, COL2 and ACAN, in the two different types of scaffolds were investigated. Results revealed that chondrogenic cells in fibrin gels modified with HA showed a modest increase in expression of chondrogenic genes, when compared to the low expression in unmodified fibrin. hESC- chondrogenic cells in plasma-treated PCL scaffolds assembled tissue matrix and retained relatively high chondrogenic gene expressions. Thus, PCL scaffolds are a useful support for hESC-chondrogenesis and may be useful in tissue engineering approaches to generate cartilage from these cells.

P666 Biomimetically surface modified fibres for cartilage regeneration

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Electrospinning technique let to form fibres with nanometric diameter, thanks to which they are highly similar to the net of polymeric fibres occurring in extracellular matrix (ECM). Selection of appropriate materials for their forming or surface modification can additionally improve their biomedical properties [1]. Polyelectrolytes, occuring naturally in cartilage ECM, help to maintain appropriate amount of water for chondrocytes proliferation and activity [1]. Chitosan is an example of a semi-crystalline polysaccharide, which is commonly used in biomedical applications. The protonated amino groups are responsible for the formation of polycations, which subsequently form compounds with natural and synthetic anions [1, 2]. In this study polycaprolactone/ chitosan (PCL/CHT) fibres were formed. Thanks to appropriate selection of high voltage polarity on the spinning nozzle we influenced the electrospinning efficiency of PCL/CHT blends and additionally the fibres' surface chemistry [3, 4]. Further, chondroitin sulphate (CS), the second polysaccharide, was attached to the fibre surface by layer-by-layer technique (LbL). The aim of this research was to study the effect of surface modification of fibres on their properties and cell response.

SEM analysis indicates the effect of the polarity applied during electrospinning on PCL/CHT fibre diameter distribution and morphology. At the same time surface modification did not affect on this properties. XPS data revealed increasing amount of nitrogen and sulphur on the fibres surface after LbL procedure. The atomic concentration of these elements on the fibre surface increased with repeating of LbL process. Contact angle data indicated the correlation of applied polarity with surface composition of PCL/CHT fibres. For all blends prepared with negative charge on the spinning nozzle, significant increase of wettability is observed as compared to fibres formed with positive polarity. Surface modification in all cases decrease the contact angle. Mechanical tests of fibre mats also indicate significant effect of polarity on the properties of PCL/CHT nonwovens, as well as surface modification. In order to study the effect of polarity applied during electrospinning on surface modification of PCL/CHT fibres on cell proliferation *in vitro* conditions, MTT assays were conducted. Data revealed that charge polarity during electrospinning may significantly influence cells' proliferation on PCL/CHT fibres. An increase or decrease of cells' proliferation, depending on PCL to CHT ratio in the fibres, was observed. The LbL modification as well had various effect on cell proliferation on each PCL/CHT fibres depend on fibres composition. All described changes in cell proliferation occurred in the range of high biocompatibility of the materials. The effect of LbL modification on cell morphology was analysed by EM imaging and cytoskeleton staining.

Acknowledgment:

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P667 Novel *ex-vivo* osteochondral defect model in a joint bioreactor system for articular cartilage repair studies

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Background: Although several treatments for cartilage repair have been developed and used in clinical practice, little is known about the mechanisms that are involved in the formation of repair tissue after these treatments, since they often lead to fibrocartilagineous tissue. Current research aims to improve the functional outcome of these treatments. For this purpose an *ex-vivo* model allowing the investigation of the interplay between mechanical and biological mechanisms involved in cartilage repair can be of great value.

The aim of this study was 1) to generate osteochondral explants in a reproducible manner, 2) to create accurate and reproducible defects and 3) to adapt and validate a custom loading system that recapitulates articulating motion *in-vivo* using a living osteochondral plug-on-nickel based alloy articulating interface.

Methods: Osteochondral plugs (diameter=8 mm) were harvested from bovine stifle joints using a punching machine and diamond trephine drill. The bone part of each plug was trimmed to obtain a final height of 5 mm. Adjustable instruments were designed to guarantee always flat plugs and to create defined defects (diameter=4 mm) in articular cartilage (depth=2 mm) and subchondral bone (depth=3 mm). A nickel based alloy indenter with 16 cm radius was used as articulating interface to apply dynamic compressive and shear loading, allowing roughly 70% contact surface area on the articular cartilage. The indenters were rotated at a frequency of 0.5Hz, stroke of 6.5° and loaded at an approximately contact pressure of 50 N and 5 N. Cell viability of the explants was assessed after harvest and after 7 days of culture by cutting fresh frozen plugs and performing lactate dehydrogenase (LDH)/ethidium homodimer assay.

Results and Discussion: Four osteochondral plugs were harvested per joint (Fig. 1 Upper panel) and full thickness chondral or osteochondral defects were reproducibly generated in each plug (Fig. 1 Lower panel). Cells within both cartilage and bone regions remained viable throughout the culture period, as assessed by LDH staining (Fig. 2). A zone of cell death was observed in the outermost cell layer at the cut edges of the cartilage and the edges of the defect for all samples.

Compression and shear-induction did not wear the cartilage in comparison to the free swelling controls, as assessed by safranin-O/fast green staining (Fig. 3). These results provided confidence that this new testing system will be adequate to screen new biomaterials and regenerative therapies and study the cell responses under relevant mechanical stimuli for cartilage repair.

Figure 13. Chondral and osteochondral defect model

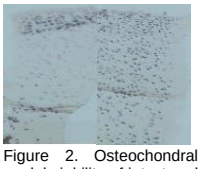

Figure 2. Osteochondral model viability of intact and defect plugs after 7 days of culture

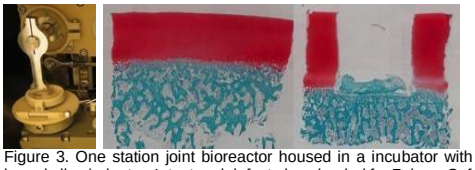

Figure 3. One station joint bioreactor housed in a incubator with Nickel based alloy indenter. Intact and defect plugs loaded for 7 days. Saf O Fast Green staining.

P668 rAAV-mediated *sox9* overexpression induces chondrogenesis in human bone marrow aspirates seeded in woven poly(ε-caprolactone) scaffolds

Jagadeesh Kumar Venkatesan[1], Frank Moutos[3], Ana Rey-Rico[1], Janina Frisch[1], Gertrud Schmitt[1], Farshid Guilak[2], Henning Madry[1], Magali Cucchiarini[1]

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Articular cartilage supports joint loading and motion but displays a limited intrinsic ability for self-healing. Implantation of genetically modified bone marrow aspirates is a promising approach to treat cartilage defects, and may be more readily translated to the clinics than the use of isolated cells. Here, we tested the benefits of applying an rAAV *sox9* construct to human bone marrow aspirates seeded in 3D woven poly(-caprolactone) (PCL) scaffolds to stimulate the chondrogenic differentiation processes as a future approach to enhance cartilage repair. Successful overexpression of *sox9* was noted in *sox9*-treated aspirates compared with control conditions over the period of evaluation (Fig. 1). Chondrogenic differentiation was evidenced in the aspirates on day 21 especially with *sox9* as noted by toluidine blue staining and type-II collagen immunostaining (Fig. 2). Application of *sox9* significantly increased the proteoglycan contents of the aspirates *versus* control treatments (up to 2.2-fold) while no effects were noted on the DNA contents. Immunoreactivity to type-I and -X collagen was less intense with *sox9* (Fig. 2). The findings were corroborated by results of a real-time RT-PCR analysis showing enhanced chondrogenic differentiation with *sox9* relative to control treatments (up to 4-fold higher COL2A1 and ACAN expression, $p \leq 0.001$) and reduced hypertrophic differentiation (up to 15-fold lower COL1A1 and COL10A1 expression, $p \leq 0.001$), probably due to increased SOX9 levels (up to 6-fold higher, $p \leq 0.001$) (Fig. 3). Genetic modification of human bone marrow aspirates via rAAV *sox9* with seeding in woven PCL scaffolds activates chondrogenic differentiation processes while delaying hypertrophy. Marrow aspirates were readily seeded in 3D woven scaffolds, providing immediate functional properties that mimic those of the hyaline cartilage. Combined cell-, gene-, and tissue engineering-based therapy is of promising value to develop novel, effective options for cartilage repair.

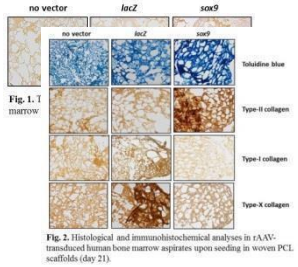

Fig. 1. T
marrow

Fig. 2. Histological and immunohistochemical analyses in rAAV-transduced human bone marrow aspirates upon seeding in woven PCL scaffolds (day 21).

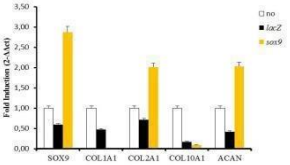

Fig. 3. Real-time RT-PCR analysis in rAAV-transduced human bone marrow aspirates upon seeding in woven PCL scaffolds (day 21).

Data are given as mean (SD). Statistically significant compared

with *no vector and $^{+}$*lacZ*.

P669 Organized tetra-copolymer scaffold regulated the human synovium-derived stem cells for cartilage tissue engineering: an in vitro study

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INTRODUCTION: Cartilage tissue engineering is a potential approach for the treatment of degenerative joint diseases. Current scaffolds still have several problems such as inflammatory reactions to the implanted materials, poor cell distribution due to unequal pore size, and toxic degradation products [1]. In accordance with the providing a relative suitable environment for chondrogenesis, we consider to use similar constituents of the extracellular matrix (ECM) of hyaline cartilage, collagen/gelatin/hyaluronic acid/chondroitin sulfate tetra-copolymer for fabricating new scaffold by using the microfluidic technology [2]. Because of the multi-lineage differentiation potential and highly inherited chondrogenesis possibility, human synovium-derived stem cells were used in this study, seeded in the organized tetra-copolymer scaffold, and analyzed the *in vitro* chondrogenesis efficiency

METHODS: A microfluidic device was used to prepare a collagen/gelatin/hyaluronic acid/chondroitin sulfate tetra-copolymer scaffold with an organized hexagonal structure. The physical and mechanical properties of the highly organized scaffold were further characterized. Human synovium-derived stem cells were harvested from the knee joint during total knee arthroplasty. The cells were expanded, seeded into the tetra-copolymer scaffolds and cultured in the chondrogenic medium. The cell viability (WST-1), cell toxicity (LDH), glycosaminoglycan (GAG), DNA quantification for cell proliferation, gene expression (real time PCR) analysis, and histological inspections were further analyzed for evaluating the efficacy of chondrogenesis.

RESULTS: Histological analysis revealed that implanted cells distributed uniformly, maintained functional phenotype and secreted components of ECM. Real time PCR revealed the differentiated chondrocytes can keep phenotype, highly express aggrecan and collagen type II. Alcian blue staining revealed that GAG was secreted. These findings demonstrated the tetra-copolymer scaffold is effective in chondrocyte culture.

DISCUSSION & CONCLUSIONS: Our new scaffold had high efficiency for cell growth, and the differentiated chondrocytes within this scaffold maintained normal phenotype with adequate ECM production. In summary, this highly organized tetra-copolymer scaffold provided a suitable environment for enhancing chondrogenic differentiation of human synovium-derived stem cells in this *in intro* study.

P671 Macro-porous micro-carriers as a vehicle for chondroprogenitor expansion and scaffold formation for the rapid biofabrication of osteochondral implants

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INTRODUCTION**:** To date, there has been limited and conflicting success in tissue engineering approaches for the repair of cartilage defects. We have attempted to address these shortcomings by using a native cell source, articular cartilage-derived chondroprogenitors [1], in order to generate neo-cartilage for tissue transplantation. In order to derive processes that are amenable to scale-up and rapid biofabrication of osteochondral constructs we investigated if culture-expansion of chondroprogenitors on collagen-based macroporous microcarriers (Cultispher®-G) could be extended so that they could also act as scaffolds for cartilage production. Our preliminary analyses of chondroprogenitor growth on unmodified microcarriers showed that the crosslinked surface could impair cellular attachment and subsequent differentiation capacity. Therefore, in this study we examined the growth and differentiation of articular cartilage-derived chondroprogenitors on uncoated and gelatin and soluble collagen coated microcarriers.

METHODS**:** Micro carriers were prepared as manufactures protocol and pre-treated with 20% foetal bovine serum (FBS), 2% gelatin or 20 μg/mL collagen (Type 1 Rat tail) for 24 hours prior to seeding. The micro-carriers were then seeded at a ratio of 20 cells per micro-carriers and allowed to settle for 20 mins and then placed on a slow speed incubator roller for 20 mins roll and 20 minutes static for 24 hrs. After 24 hours had elapsed each condition were placed in ultra-low cell adhesion T_{25} culture flasks and either cultured with basal media containing 10% FBS, DMEM high-glucose, 1% L-glutamine, 1% non-essential amino acids, 1% HEPES and 1% Penicillin, Amphotericin B and Streptomycin, with and without 5ng/mL FGF-2 and 1ng/mL TGFβ1 (Experimental groups: FBS, COL, GEL, FBSFT, COLFT and GELFT). Media change was undertaken every two days with a cell count utilising Presto Blue assay during the cellular expansion phase. Once cellular expansion reached expansion threshold, pellet fabrication (2000 microcariers per pellet) and scaffold disc fabrication (10,000 microcarriers per disk) were undertaken and culture media switched to chondrogenic media and cultured for 21 days with a media replenishment every 3 days. On Day 21 scaffolds were fixed and processed for histological analysis using toluidine blue and biochemical analyses.

RESULTS**:** Chondroprogenitors cultured on coated and uncoated microcarriers in the presence or absence of FGF2 and TGFβ1. All microcarrier combinations showed a similar increase in cellular proliferation which was pronounced between days 4 and 12, after which growth plateaued. At plateau, total cell number was highest in microcarriers exposed to growth factors. Following cell expansion, microcarriers were pelleted or placed in transwell supports and cultured for 21 days in chondrogenic medium to induce differentiation. All constructs displayed extracellular matrix accumulation within the microcarriers and extensive deposition between microcarriers. Pre-treatment of the microcarriers with collagen or gelatin and pre-culture without TGFβ1 and FGF2 led to increased matrix production compared to the growth factor expanded chondroprogenitors when analysed using DMMB assay.

DISCUSSION & CONCLUSIONS**:** In this study, we show proof of concept data for the dual use of porous microcarriers as a surface for chondroprogenitor expansion and furthermore as a subsequent scaffold for the production of neo-cartilaginous constructs. We found that whilst coating microcarriers had little effect on cellular proliferation, it had a significant effect on differentiation of chondroprogenitors, this may be due to the degree of crosslinking present in microcarriers inhibiting chondrogenesis either direct or indirect interference of cell function. The technique allows scalable production of cartilage discs to cover small to large joints by increasing the number of microcarriers that are initially seeded. Further work on maturing the cartilage constructs is required in order to produce stiff and durable implants, and this can be achieved either by improving chondrogenesis medium or inducing in vitro maturation [2].

P673 Fishing nucleus pulposus progenitor cells from bovine intervertebral discs using three different sorting methods

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Introduction: The nucleus pulposus (NP) harbours a small fraction of precursor cells, positive for Angiopoietin-1 receptor Tie2, that were recently isolated by fluorescence-activated cell sorting (FACS). These autochthonous NP progenitor cells (NPPC) might be of high interest for IVD regeneration [1]. As FACS sorting can be cumbersome and even destructive for cells other cell sorting protocols using magnetic beads or selection by bead size might be good alternatives for NPPC isolation.

Materials and Methods: Bovine nucleus pulposus (NP) tissue was harvested from 1-year old animals and digested using a two-step protocol with pronase and collagenase type 2 overnight [2]. Subsequently, one out of three sorting methods was applied using the same primary antibody against Tie2 (bioss, USA): 1) FACS, (FACSaria III, BD Biosciences, Belgium), 2) Magnetic-activated cell sorting (MACS) (Miltenyi Biotec, Inc.) and 3) size-based sorting using pluriBead® technology (pluriSelect, inc.) where a construct between the primary antibody and the plurBead is formed. After incubation with NP cells followed by filtration and separation of the bead.

Tie2+ and Tie2- cells sorted by FACS and pluriSelect were subjected to differentiation assays, i.e. adipogenesis and osteogenesis. Colony forming unit (CFU) assay was carried out by resuspending 3,000 cells (Tie2+ and Tie2-) in Methocult (Stem cell Technologies) and cultured for 7 days. For differentiation cells were cultured in control medium (α-MEM with 5%FCS), osteogenic and adipogenic medium, all supplemented with 2.5ng/mL FGF2 [3] for 21 days.

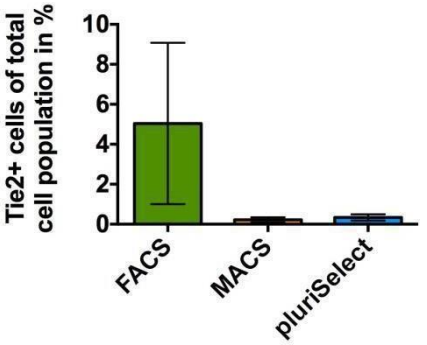

Figure 1: *Cell yield as percentage from total number of sorted cells ± SEM; FACS n=9, MACS n=2, pluriSelect n=2.*

Results: Isolation of NPPC was possible with all three methods investigated. However, Tie2+ cell yield differed widely among them (Fig 1). For MACS and FACS CFU assay showed higher colony numbers in the Tie2+ selected cells. Further, differentiation assays confirmed multipotency of FACS selected Tie2+ cells by fat droplet count and alizarin red.

Discussion: The tested sorting methods are potential ways to isolate NPPC. pluriSelect™ technology offers the benefit of separation of the bead. What makes it together with FACS a more desirable candidate for further treatment approaches. Despite low cell yield pluriSelect™ makes future research in finding ways to culture and expand NPPC a step closer due to its cost-efficiency and ease of handling.

P674 The Role of Surface Topography on Cellular Dynamics of Peri-Implant Angiogenesis and Osteogenesis

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Metallic endosseous implants are a mainline medical treatment in dentistry and orthopedic surgery, but optimizing the establishment of the bone-implant interface remains a challenge.

Peri-implant wound healing is a multistage regeneration process involving neovascularization and osteoconduction. The latter has been defined as migration and recruitment of osteogenic cells to the implant surface. Nano-topographically complex implant surfaces have been shown to increase early implant stability and decrease healing time. However further studies are required to uncover the regulatory effect of the complex surfaces on the two mentioned healing steps at the cellular level. We hypothesize that Perivascular Mesenchymal cells (PMC) are the progenitors of osteogenic cells, and the implant surface topography influences the pattern of peri-implant osteogenesis through controlling the rate and the architecture of microvascular network development following implantation. We established a platform for intravital tracking and 3D quantification of neovascularization and perivascular cell dynamics. Two-photon fluorescence microscopy was performed from day 3 up to day 43 post-surgery. Perivascular Mesenchymal cells were endogenously labelled with tdTomato in our mouse model, the neovasculature and the Ti-implant were visualized with green fluorescein and Second Harmonic Generation (SHG) respectively. The lateral surface of the Ti implant was either machined or modified with Nanotubes.

Preliminary results showed that the rate of the vascularization was slower around the machined implant surface. The nascent vessel fragments were spatially closer to the nano-surface with significantly higher branching level in the early time points. PMCs appeared at the wound site from day 3 post-implantation, some of them changed morphology and became stabilized on the vessels after day 15, others became osteocytes in the newly formed bone. Comparative analysis of two implant surface types showed that the population of the PMCs was significantly higher in the proximity of the implant surface in the nano-treated group between days 3 and 15. Our results provide evidence that PMCs are brought into the wound site in parallel with the invasion of the site by capillaries. The trafficking of these osteoprogenitor cells in the peri-implant wound microenvironment is controlled by the rate and pattern of the microvascularization, which is enhanced by nano-structurally complex implant surfaces. Reengineering endosseous implants based on the findings of this project would possibly help overcome the delayed bone healing associated with diseased conditions and ultimately improve clinical outcomes.

P675 Covalent immobilization of stromal cell derived factor 1α-derived peptide and heparin on poly(L-lactide-co-ε-caprolactone) copolymers for in situ tissue regeneration applications

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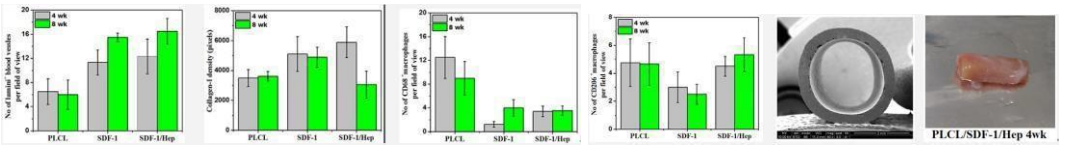
Aim: The aim of this study was to develop stromal cell-derived factor-1α-derived peptide (SDF-1αP) and heparin conjugated poly(L-lactide-co-ε-caprolactone) (PLCL) copolymer for *in situ* blood vessel regeneration applications. SDF-1α has been shown to accelerate *in situ* tissue repair by endogenous stem/progenitor cell mobilization and by enriching inflammatory response. However, being a large protein, SDF-1α can be easily degraded by matrix metalloproteinases (MMPs) and is tedious to be tethered with polymeric materials. Heparin can prevent thrombosis and accelerate vascularization.

Materials & Methods: Star-shaped and linear PLCL were prepared by ring-opening polymerization. SDF-1αP (Peptron, Korea) was conjugated with the hydroxyl groups of eight-armed PLCL copolymers (M_n, 67 kDa) using carbonyldiimidazole (CDI) coupling chemistry. Heparin (Sigma Aldrich, Korea) was tethered with star-shaped PLCL using DCC/DMAP coupling methodology. Bilayer vascular grafts were fabricated using electrospinning. SDF-1αP and heparin content were determined using HPLCL-based amino acid analysis and toluidine blue assay respectively. The biocompatibility of electrospun grafts was assessed after subcutaneous implantation in Sprague-Dawley rats for up to 8 weeks. Explanted grafts were characterized by histological and immunohistochemical analysis.

Results & Discussion: Amino acid analysis and toluidine blue assay revealed that SDF-1αP and heparin were successfully tethered to star-shaped copolymer, and their content were 4.87 nmol and, 1.02 µg per milligram, respectively. SEM micrographs revealed nanofibrous and microfibrous morphology in the inner side (thickness, 20 µm) and outer side (thickness, 180 µm) of the grafts, respectively. Haematoxylin and Eosin staining showed more cellularization and tissue formation in SDF-1αP and Heparin/SDF-1αP groups than the control group. Similarly, SDF-1αP and Heparin/SDF-1αP groups also showed higher amount of laminin$^+$ blood vessels, α-SMA$^+$ cells, and collagen type 1 compared with the control group. Evaluation of inflammatory response showed lower numbers of CD68$^+$ (M1) macrophages in control groups compared to SDF-1αP and heparin/SDF-1αP groups, however, there was no significant difference between the numbers of CD206$^+$ (M2) macrophages probably because these events occur during the initial stages of implantation. Taken together, these results confirmed that the strategy adopted here can be used to make bioactive biomaterials for endogenous stem/progenitor cell mobilization and these materials can be fabricated into different shapes and structures as needed for tissue regeneration applications.



(P676)

P676 Injectable photocrosslinkable hyaluronic acid hydrogels incorporated with platelet lysate for dental pulp endogenous regenerative therapy

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The main functions of the dental pulp, namely dentin formation and tooth homeostasis maintenance, can be severely compromised by pulpitis or pulp necrosis. The restoration of dental pulp remains a challenge for dentists; nonetheless, it has been poorly addressed. An ideal system aiming pulp regeneration should enable the recruitment of relevant progenitor cells and promote revascularization of pulp cavity, and simultaneously regenerate the pulp and dentin-pulp complex.

Herein was proposed a photocrosslinkable hydrogel system based on hyaluronic acid (HA) and platelet lysate (PL). PL is a cocktail of growth factors (GFs) and cytokines involved in wound healing orchestration, obtained by the cryogenic processing of platelet concentrates. Soluble factors present in PL have great therapeutic potential. However, the short lifespan of GFs is well known and limits their applicability in regenerative medicine approaches. We envision that his versatile system could be injected into the endodontic cavity and crosslinked in situ for the further controlled release of the platelet-origin cytokines, aiming at stimulating endogenous regeneration.

Stable HA hydrogels incorporating PL were prepared after photocrosslinking of methacrylated HA (me-HA) solution, previously dissolved in PL, by excitation of the photoinitiator Irgacure 2959 with UV light. Human dental pulp stem cells (hDPSCs) isolated from dental pulp tissue extracted from permanent teeth were seeded on PL-laden or plain hydrogels at a cell density of $5x10^4$/sample. The metabolism as well as population of hDPCs was increased over time in the study group most likely as consequence of the adhesion sites provided by the fibrinogen present in PL, as well as the mitogenic properties of PL cytokines. Moreover, the expression of the osteogenesis transcription factor RUNX2, the extracellular matrix protein COLIA1 and ALP genes was enhanced in the cells seeded onto the hydrogels incorporated with PL. The ALP activity in cellular lysates, increased after 14 days in culture, corroborated the ALP gene expression results. In addition, calcium deposition was observed after 21 days, in the study group.

Overall, data demonstrated that the HA hydrogels incorporating PL increased the metabolism and stimulate the dentin matrix deposition by hDPSCs, providing clear evidence of the potential of the newly developed system in the regeneration of damaged pulp/dentin tissue. Further research will focus on the ability of the system to recruit pulp tissue progenitor cells and promote neo-vascularization in order to fully regenerate dental pulp tissue.

Acknowledgements: Maló Clinic, Porto, Dr Ana Ferro and Dr Bruno Queridinho, for the donation of the permanent teeth. RECOGNIZE project (UTAP-ICDT/CTM-BIO/0023/2014); FCT grants SFRH/BD/96593/2013 (R.A), SFRH/BPD/111729/2015 (M.R.) and IF/00685/2012 (M.E.G.)

P677 First characterization of human amniotic fluid stem cell extracellular vescicles as a powerful paracrine tool endowed with regenerative potential

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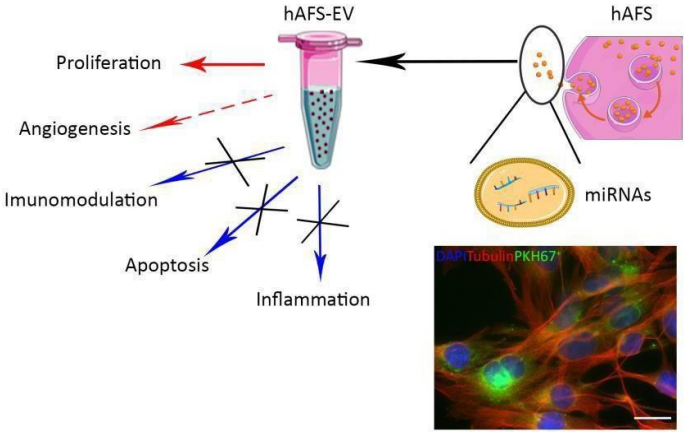
Several works suggest that stem cells might exert therapeutic beneficial effects due to their paracrine potential, rather than by trans-differentiation when transplanted in vivo. Growing interest has been driven to the characterisation of the stem cell secretome and of stem cell-derived extracellular microvesicles (EV), including exosomes, as mediators of such modulatory effect. Human amniotic fluid stem cells (hAFS) have been recently described as immature fetal mesenchymal progenitors with a distinct secretory profile and significant regenerative paracrine potential. In this study we describe the role of hAFS-derived EV (hAFS-EV) as mediators of regenerative paracrine effects.

c-KIT$^+$ hAFS were isolated from leftover samples of amniotic fluid from prenatal screening and stimulated to enhance EV release (24h under 1% O_2 preconditioning). EV were obtained by ultracentrifugation of the hAFS-conditioned medium (hAFS-CM) and characterised by transmission electron microscope, nanoparticle tracking analysis, western blot and flow cytometry. hAFS-EV were tested in vitro on murine myoblast C2C12 cells, human dermal fibroblasts (HDF), and peripheral mononuclear cells (hPBMC) demonstrating their role as biologically mediators of proliferative, pro-survival and immunomodulatory effects. The hAFS-EV anti-inflammatory and regenerative potential was confirmed in a mouse model of muscular atrophy, [HSA-Cre, Smn(F7/F7) mice] in which the transplantation of hAFS previously showed to increase muscle strenght and survival rate. In particular, our preconditioning strategy revealed a significant enrichment within the hAFS-EV of exosomes enhanced with regenerative microRNAs that could be directly transferred to the responder cells.

In conclusion, this is the first study showing that hAFS actively secrete EV in their conditioned medium with hypoxic preconditioning being a promising strategy to improve their exosomal content. Although preliminary, these encouraging findings suggest a novel translational approach based on exploiting the regenerative potential of hAFS-EV for future paracrine therapy.



P678 The human amniotic fluid stem cell secretome as a new paracrine source to unlock endogenous cardiac regeneration

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Cardiovascular disease (CD) and heart failure represent the main cause of mortality in the Western countries. CD pathogenesis is mainly related to inefficient cardioprotection, defective repair and lack of myocardial renewal following cardiac injury and/or ageing. However, recent work demonstrated that the adult mammalian heart is not completely devoid of regenerative capability since it has an endogenous restorative program mainly based on cardiac progenitor cells (CPC) activation and cardiomyocyte de-differentiation and proliferation. Nevertheless, these mechanisms are not efficiently active in the adult heart under pathological situations, such as myocardial infarction. A working strategy to enhance and restore in full the cardiac endogenous potential for repair and regeneration would open new frontiers in cardiac medicine. Human amniotic fluid-derived stem cells (hAFS) and their conditioned medium have recently been shown to exert beneficial effects on cardiac cells by significantly reducing the infarct size in an acute model of rat ischemia/reperfusion injury. Here, we sought to determine whether the whole of paracrine soluble factors and extracellular vesicles secreted by hAFS, namely the hAFS *secretome,* has the potential to efficiently reactivate the endogenous program of cardiac regeneration by acting on both resident cardiomyocytes and CPC.

c-kit positive hAFS were isolated from left over samples of amniotic fluid with normal karyotype collected during II trimester diagnostic amniocenteses, following informed written consent. Cells were cultured in serum-free medium for 24 hours under normoxia (20% O_2) or hypoxia (1% O_2) conditions to stimulate the release of paracrine factors in their conditioned medium (hAFS-CM). The paracrine potential of hAFS-CM to provide pro-survival and proliferative effects was evaluated *in vitro* on primary mouse and rat neonatal ventricular cardiomyocytes (NVCM) and on human cardiac progenitor cells (hCPC) isolated from discarded human cardiac fragments obtained during valve replacement procedure, following informed consent from patients. To assess the hAFS secretome proliferative potential, NVCM and hCPC cells were pre-treated with normoxic or hypoxic hAFS-CM (hAFS-CM_{Normo} and hAFS-CM_{Hypo}) for 40 and 18 hours respectively and evaluated by EdU staining followed by high throughput analysis, BrDU ELISA assay and Ki67 immunostaining. Target cells were also subjected to either oxidative (150 M and 600 M H_2O_2 solution for NVCM and hCPC, respectively) or hypoxic damage (1% O_2, only NVCM), for 4 hours and their viability measured by MTT. Proteomic and cytokine comparative analyses of hAFS-CM_{Hypo} versus hAFS-CM_{Normo} were performed to identify candidate molecules involved in specific cell-signaling pathways. Priming with either hAFS-CM_{Normo} or hAFS-CM_{Hypo} re-activated NVCM proliferation by 5.3- and 5.6-fold respectively ($p<0.001$). This effect was further confirmed on hCPC cells, showing a burst in cell proliferation by 1.3- and 1.5-fold ($p<0.01$) respectively, compared to untreated cells. Cell apoptosis was significantly counteracted by pre-incubation with both hAFS-CM_{Hypo} and hAFS-$CM_{Normo.}$ with significant increase of cell viability in NVCM (by 1.8- and 2-fold under oxidative damage and 1.3- and 1.5-fold under hypoxia, respectively, $p<0.0001$) and hCPC (by about 1.8- and 2-fold respectively, $p<0.001$). Preliminary results showed increased expression of ANGPT2, IGFBP3, IL8, PDGF-AB/BB, MIF and THROMBOSPONDIN-1 in hAFS-CM_{Hypo} compared to hAFS-CM_{Normo}. Proteomic comparative analysis confirmed significant enrichment of proteins related to cell-cycle, DNA replication and signal transduction in the hAFS-CM_{Hypo} compared to hAFS-CM_{Normo} ($p<0.05$). In conclusion, the hAFS *secretome* can mediate regenerative cardioprotective effect while triggering proliferation of both NVCM and hCPC. These encouraging findings support future stem cell-based paracrine therapy as working strategy to resurge in full the endogenous regenerative program from within the heart.

P679 The inflammatory phenotype of infrapatellar fat pad can be modulated by triamcinolone acetonide, probably via the residing macrophages

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Introduction – Joint injuries are common and often result in cartilage defects and inflammation of the joint. Joint inflammation however may prevent successful cartilage defect repair. The inflamed infrapatellar fat pad (IPFP) in the knee joint secretes factors that inhibit successful cartilage repair by inhibiting chondrogenic differentiation and stimulating degradation. Pro-inflammatory macrophages residing in the IPFP are suspected to contribute to this effect (1). To improve cartilage repair, restoration of the joint homeostasis is necessary. Therefore, we aim to reduce inflammation in the IPFP focusing on macrophages residing in the tissue to create an environment that allows cartilage repair.

Materials and methods – IPFP tissue was obtained as leftover material from patients with osteoarthritis who underwent a total knee arthroplasty. The IPFP was cut into small pieces and cultured in Dulbecco's Modified Eagle Medium with Glutamax (DMEM-HG) supplemented with 1% insulin-transferrin-selenium (ITS+) and one of the following medications: 0.1µM-100µM triamcinolone acetonide (TAA) (Sigma-Aldrich), 10µM celecoxib (Sigma-Aldrich), 50µM pravastatin (Sigma-Aldrich) or 100µM fenofibrate (Sigma-Aldrich). After 24 hours of culture, IPFP explants were snap frozen in liquid nitrogen for gene analysis of Tumor Necrosis Factor alpha (*TNFA*), Interleukin beta (*IL1B*), *IL6*, *IL10*, Cluster of differentiation 206 (Mannose receptor C, type 1, *MRC1*), and *CD163*, or immediately processed for flow cytometric analysis using conjugated antibodies against CD14-APC-H7, CD80-PECy7, CD86-PE, CD163-PerCP-Cy5.5, and CD206-FITC.

Results – Only TAA significantly reduced the gene expression of pro-inflammatory cytokines *TNFA*, *IL1B*, and *IL6* in IPFP explants and increased the expression *IL10*, *MRC1* and *CD163* as markers for anti-inflammatory processes. We therefore continued with TAA to examine whether TAA indeed modulates the phenotype of the macrophages residing in the IPFP. TAA did not influence the percentages of CD80+/CD14+ and CD86+/CD14+cells (regarded as pro-inflammatory macrophages). TAA significantly increased the percentages of CD163+/CD14+($p< 0.001$) cells (regarded as anti-inflammatory macrophages). Only with the 10 µM concentration of TAA, the percentage of CD206+/CD14+($p< 0.001$) within the IPFP significantly increased (regarded as tissue repair macrophages).

Discussion and Conclusion - The IPFP can become inflamed and secrete factors that influence the joint environment. Based on gene expression, TAA seems to decrease general IPFP inflammation, whereas celecoxib, pravastatin and fenofibrate had a less clear anti-inflammatory effects on IPFP. In conclusion, our data indicate that TAA can be used to specifically reduce IPFP inflammation through modulation of the pro-inflammatory macrophages residing in IPFP to an anti-inflammatory state. This might eventually improve the joint environment for better cartilage repair.

P680 The potential of porcine peritoneum tissue as an anti-adhesion barrier for flexor tendon regeneration

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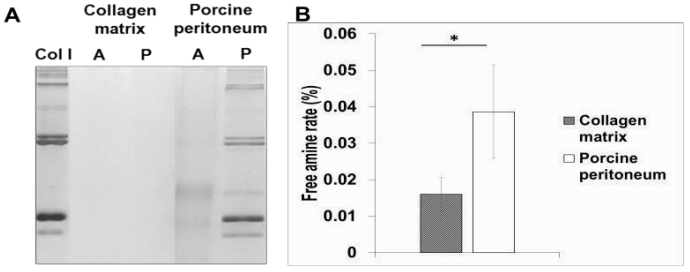
The formation of adhesions following surgery in tendon leads to the inhibition of tendon gliding and reduction in biomechanical properties. Adhesion formation occurs due to the interaction of two damaged tissues through inflammation and coagulation processes, thus, the most common approach after surgery is the use of mechanical barriers to isolate the tendon and to function as a substitute for the disrupted tendon sheath. However, current mechanical barriers remain ineffective in preventing tendon adhesions. Therefore, we investigated a xenograft derived from porcine peritoneum that includes a basal membrane as a potential solution to prevent the formation of adhesions during tendon healing.

The biochemical, biological, biomechanical and thermal characteristics of the xenograft were assessed relative to a commercially available collagen matrix for tendon healing applications. Higher solubility of the xenograft was observed using SDS-PAGE following acid/pepsin extraction, and free amine content showed a significant higher ratio of free amines in the xenograft, suggesting a lower crosslinking ratio, which was confirmed by a lower denaturation temperature (p<0.01). MMP degradation assays showed a significantly higher weight loss in the xenograft after 24h *in vitro* suggesting faster remodelling would occur *in vivo.* However, the xenograft exhibited higher biomechanical properties (p<0.01). Immunohistochemistry showed the presence of collagen type I and III, together with fibronectin in both materials; meanwhile, collagen type IV, laminin and elastin were only present in the xenograft. Adult dermal fibroblast cytocompatibility and adhesion on both sides of the xenograft were also assessed *in vitro*. Cells seeded on the basal membrane and connective tissue sides displayed similar proliferation and metabolic activity, furthermore lower adhesion and no significant cytotoxicity effects were observed relative to the control after 14 days *in vitro*. These results support the potential of the porcine xenograft as antiadhesion barrier for tendon healing.



C

Samples	Onset (ºC)	Peak (ºC)	Heat (J/g)
Collagen matrix	58.52 ± 0.47	62.04 ± 0.21	-1.20 ± 0.14
Porcine peritoneum	48.93 ± 0.85**	52.56 ± 0.66**	-6.67 ± 1.20**

Figure 1. (A) SDS-PAGE of acetic acid (A) and acetic acid + pepsin (P) extractions. **(B)** Free amine analysis assessed with ninhydrin assay (n=6). **(C)** DSC analysis. Data presented as mean ± standard deviation (n=5)* p>0.05, **p>0.01.

P681 Photosynthetic sutures with pro-regenerative features

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Tissue defects and surgical wounds are characterized by disruption of the vascular network leading to scarce oxygen, thus the wound healing process is impaired by hypoxia, poor regeneration capacity and high infection rates, which altogether promote tissue scarring rather than regeneration.

Some attempts have already been made to use sutures as carrier systems for active cells or molecules to help in the healing process, taking the advantage that they are in direct contact with the wound, by adding *e.g*. antibiotics or adipose-derived mesenchymal stem cells.

By exploiting the photosynthesis process, our team developed the first generation of photosynthetic biomaterials, which in the presence of light decrease tissue hypoxia by the constant supply of photosynthetic oxygen, allowing tissue oxygenation in the absence of environmental or perfused oxygen supply. This strategy was also implemented using genetically modified microalgae to engineer photosynthetic scaffolds that, in addition to oxygen, can provide other pro-regenerative molecules to the wounded tissue, for instance by constantly delivering growth factors, even avoiding concerns of gene therapy or limitations for their direct administration.

Following that example, in this project we developed bioactivated surgical sutures with the potential to improve wound healing, as they constantly release photosynthetic oxygen and growth factors. We demonstrate that biodegradable polyglactin sutures can be seeded with microalgae *Chlamydomonas reinhardtii*, which distribute evenly among and throughout the filaments, and remain viable in the suture, showing high biocompatibility and photosynthetic activity *in vitro*. Furthermore, the algae also resist the mechanical stress of suturing full skin, insomuch as most cells remained in the suture and maintained their metabolic activity. Additionally, growth factor delivery was evaluated *in vitro*. These results represent the first step towards engineering photosynthetic sutures with pro- regenerative features, contributing to the establishment of new therapeutic approaches for the treatment of skin or other tissues defects.

P682 Molecular and histological analysis of the corpus spongiosum

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Introduction & Objectives

Urethral reconstruction is performed in patients with urethral strictures or for correction of congenital disorders. In case of shortage of tissue, engineered tissue may enhance urethral reconstruction. As the corpus spongiosum (CS) is important in supporting the function of the urethra, tissue engineering of the urethra should be combined with reconstruction of a CS. For that purpose, detailed knowledge of the molecular composition of the CS, more specific of its extracellular matrix (ECM) is needed for scaffold designing. The objective of this study is to analyze the molecular composition of the CS and to investigate the use of the decellularized ECM as scaffold for tissue engineering.

Material and methods

Broad informed consent was obtained. During male-to-female sex reassignment surgery the CS including the urethra was harvested. This CS was further processed in several ways: first fixed and embedded in paraffin, then either fixed and processed for scanning electron microscopy (SEM) or decellularized using detergent solution. Decellularized tissue was either embedded in paraffin or processed for SEM or used as a scaffold to grow both mesenchymal stem cells as well as endothelial cells. (Immuno)histology was performed on paraffin tissue sections and on seeded scaffold.

Results

The CS can be divided in four layers, first an transition zone from urethra epithelium to a collagen rich layer, followed by a second, elastin rich layer. The third layer is formed of veins, arteries and vascular spaces and the last layer is the transition from this vascular rich region to the tunica albugina. In the decellularized CS the different components of the ECM were visible and distinguishable (elastin bundles, collagen both in bundles and filaments). ECM components as laminin were retained after the treatment by detergent solution. Both mesenchymal stem cells as well as endothelial cells showed good survival on the ECM.

Conclusion

This study provides novel and detailed information on the molecular composition of the CS, which is important for scaffold design for tissue engineering. Furthermore, as different cells types show good survival on the ECM of the CS, we will aim to develop a gel with similar molecular composition for use in 3D bio-printing.

P683 Alginate and hyaluronic acid as injectable hydrogels to apply BMP2-loaded microspheres induced differences in macrophage infiltration, vascularisation and bone formation

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Introduction: Biomaterial scaffolds functionalised to stimulate endogenous repair mechanisms incorporating osteogenic factors offer a potential alternative to bone-grafts. However, choosing the right material is critical to couple the key events needed for bone formation. In this study, we used collagen microspheres loaded with rhBMP-2, and included them in one of the two different hydrogels used. The goal of this study was to determine the most biocompatible injectable hydrogel system containing BMP2-loaded microspheres in terms of host reaction –macrophage lineage cell infiltration-, vascularisation and bone formation in a cell-free system.

Methods: 10-week-old male Sprague Dawley rats were used. Collagen type-I based recombinant peptide (RCP) microspheres developed by Fujifilm loaded with a constant concentration of rhBMP-2 (3.5 µg per injection) were incorporated in alginate SLG (Novamatrix, Sandvika, NO) or poly(N-isopropylacrylamide) hyaluronic acid hydrogels (AO Research Institute Davos, CH) and subcutaneously injected (total volume 200 µl, n=6 per condition). 1, 4 and 10 weeks after implantation, animals were euthanised. Implants were harvested and scanned using micro-CT to evaluate the formation of mineralised tissue. Implants were decalcified, paraffin embedded and processed for histology and immunohistochemistry. TRAP, CD68, iNOs, CD206, and CD163-positive cells were counted, along with the number of blood vessels, in a blinded fashion.

Results: Injectable alginate gel with microspheres containing rhBMP-2 promoted ectopic bone formation, but the HA did not. At 4 weeks mineralised tissue was observed by micro-CT in most of the implants. At 10 weeks the amount of calcified tissue had increased 5 fold compared to 4 week scans ($p < 0.05$) and bone formation had occurred in all of the alginate constructs on histology. However, in the HA formulations, no bone structure was found. Neovascularisation significantly increased over time when both, SLG ($p < 0.01$) and HA ($p < 0.001$) hydrogels were used. At week 1, cellular infiltration was visible in both of the implanted formulations. The number of CD68 positive cells decreased over time in the alginate implant. However, when HA was used as hydrogel, a delay in macrophage recruitment was observed, showing the peak in CD68 positive cells infiltration at 10 weeks ($p < 0.01$) compared to the infiltration observed at both, 1 and 4 weeks.

Discussion & conclusions: The use of rhBMP-2 loaded RCP microspheres contained within alginate hydrogel promoted subcutaneous ectopic bone formation without further need of adding cells. However, when HA was used as hydrogel, most gel disappeared within the first weeks and no bone formation was observed. Interestingly, cellular infiltration patterns (including macrophage subtypes) and tissue vascularisation had a different magnitude and kinetics between formulations, possibly resulting in the failure of bone formation in the HA constructs. For HA formulations, macrophage-osteoclast cell lineage infiltration increased over time, contrary to what happened with alginate one. These results demonstrate that the selection of the hydrogel, with consideration of host response is as important as growth factor dose and overall material biocompatibility for bone formation.

P684 Platelet-rich plasma treated tibial side 3rd grade rupture of medial collateral ligament

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Aims: Platelet-rich plasma (PRP) is widely used on tendon and ligament healing in sports medicine. Although the possible benefits of PRP in an acute ligament trauma, there has been no studies concerning ligament healing followed with imaging studies.

Methods: In the present case study, we demonstrate non-operatively treated grade 3 rupture of tibial side MCL injury using multiple PRP injections. The patient was followed with magnetic resonance imaging (MRI) and a detailed progression of Return – to – Play (RTP) was documented.

Results: The MRI showed scar formation in the rupture site at week 3 after injury. The RTP for the patient was in the week 6 after injury. The rehabilitation process was well tolerated during PRP injection program. Only mild and rapidly subsiding discomfort was exhibited after the injections. No adverse events occurred.

Impact of the investigation: The present case shows MRI documented healing of grade 3 MCL rupture using multiple PRP injections. These findings could indicate possible benefits of PRP treatment in the MCL ruptures, even in the ones which are gradus 3 and sited at tibial side.

P685 Attachment studies of peri-implantitis-associated pathogens to titanium and cobalt chromium and the development of an antimicrobial coating

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Objectives: Peri-implantitis is an infectious disease associated with inflammatory destruction of mucosal and osseous tissues leading to implant loosening and failure. It is historically believed that implants are sequentially colonised. A conditioning film composed of salivary proteins allows adhesion of aerobic commensal early colonisers. These microorganisms establish a favourable environment for the attachment of anaerobic pathogenic late colonisers commonly found in peri-implantitis.

The overall aim of this study is to investigate the attachment of pathogenic bacteria involved in peri-implantitis to titanium (Ti) and cobalt chromium (CoCr) surfaces, widely used in implantology, and to develop an antimicrobial coating in order to decrease bacterial attachment and viability.

Methods: Medical Ti alloy and CoCr laser sintered, polished surfaces (Renishaw PLC – Medical and Dental Products Division) were characterised by X ray and spectral analysis, as well as by roughness and contact angle measurements.

The growth of anaerobic peri-implantitis-associated pathogens was monitored under anaerobic conditions by optical density measurements and bacterial culture quantification every 4 h. Bacterial attachment to Ti and CoCr discs was studied using live-dead staining and confocal microscopy.

Preconditioning of metallic surfaces with long alkyl chained phosphonic acid (PA) was optimised to improve conjugation of antimicrobial compounds. Several conditions were tested: incubation time (1s, 1h, 2h, 3h, 6h, 24h), concentration (0.5, 1.0, 1.5 mM) and solvent (tetrahydrofuran, ethanol).

Results: Ti and CoCr presented the same surface roughness profiles with polishing treatment resulting in very smooth surfaces (0.129 µm +/- 0.067, mechanical Ra +/- sd). Contact angle measurements demonstrated differences between Ti and CoCr hydrophilicity with CoCr being more hydrophobic: 77º (+/- 9.200) versus 86º (+/- 8.700) respectively. Ti surfaces corresponded to the orthorhombic crystal structure of TiO_2, whilst CoCr surfaces were found to be faced-centered cubic. Preliminary attachment studies demonstrated adherence of *F. nucleatum* to non-coated Ti and CoCr surfaces without a conditioning film or early colonisers. Ti surfaces showed a much higher attachment than CoCr. PA attachment was time-dependent: an increase was observed up to 2h, followed by a plateau from 2 to 6h and a decrease in attachment at 24h.

Conclusions: Attachment of pathogens to metallic surfaces without preconditioning or successive colonisation indicates that late colonisers may be able to directly attach to dental implants and abutments and promote migration towards the gum, increasing the probability of chronic inflammation and tissue destruction.

PA binds with oxide metallic surfaces through mono-, bi- and tri-dentate P-O-Ti bonds, followed by the formation of numerous successive self-assembled monolayers. This is the first step of the antimicrobial coating development aiming to decrease bacterial load to lead to a healthier environment surrounding the implant and ultimately decrease failure rates.

P686 Fabrication, characterization and determination of biological activities of poly (ε-caprolactone)/chitosan-caffeic acid composite fibrous mat for wound dressing

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In the present study, we designed composite fibrous mats consisting of poly (ε-caprolactone) (PCL), chitosan (CH), or chitosan-caffeic acid conjugate (CCA) fabricated by an electrospinning technique for wound dressing application. The average diameters of PCL, PCL/CH, and PCL/CCA composite fibrous mats were 1.30 ± 1.07, 1.20 ± 1.22, and 0.94 ± 0.68 μm, respectively. Based on universal tensile machine (UTM) analysis, the PCL/CCA composite significantly increased tensile properties compared with the PCL and PCL/CH composites. Additionally, initial cell attachment and cell proliferation of the composites using neonatal human dermal fibroblast (NHDF-neo cells), as well as the anti-microbial effect against *Staphylococcus aureus*, was investigated. The PCL/CCA composite shows significantly higher initial cell attachment and cell proliferation than the PCL and PCL/CH composites, and a high anti-microbial effect was observed compared to the PCL and PCL/CH composites. Based on these results, the CCA is demonstrated to be good supplemental bioactive agent for wound dressing applications and skin tissue engineering.

P687 Adult astrocyte scratch wound model: evaluation of treatment for traumatic brain injury

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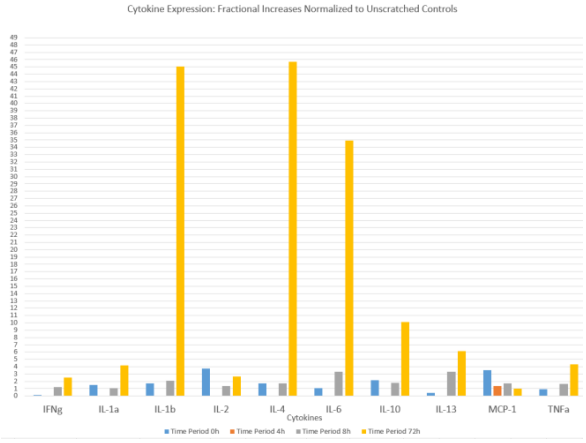
Traumatic Brain Injury (TBI) results in detrimental effects on the livelihoods of millions of people in the United States alone, resulting in the 138 deaths daily.[1] We are in the process of developing a tissue engineered collagen scaffold with pharmacological and cellular components, which we call the "Brain Patch." This Brain Patch is aimed at treating secondary injury due to TBI. The collagen patch is infused with antibacterial and anti-inflammatory chitosan nanoparticles, and mesenchymal stem cells. To test the effectiveness of the nanoparticles' antiinflammatory properties, we developed a scratch wound model that uniformly wounds a mixed culture of astrocytes and microglia.[2] We used this method to evaluate cultured adult rat astrocytes' responses to wounding. After performing a RayBiotech quantibody assay, we were able to evaluate the astrocytes' cytokine expression over 0, 4, 8, and 72 h time periods. Previous work focused on fetal rat astrocytes and imparted a range of scratch areas when wounding. Those results yielded that the smallest scratch area was effective in producing the largest cytokine response. Therefore, this experiment also utilized that scratch area. While previous work is limited to fetal cytokine response, this research focuses on adult astrocyte cytokine expression. This is apt as the Brain Patch is intended for adult subjects. To measure cytokine expression of scratched cells, we calculated fractional increases, normalized to unscratched adult controls (Fig. 1). Ratios above 1 signify upregulation. We see that down regulation of all cytokines occurs about 4 hours after scratching. Furthermore, at 72 hours after scratching, the cells significantly upregulate their cytokine expression. This contrasts previous work involving fetal astrocyte cytokine expression, in which IL-6 and IL-10 up and down regulation balances out over time, resulting in almost scarless tissue. [2] Thus, we can conclude that the 72 hour time point at this scratch area would be an optimalmoment to administer and evaluate the Brain Patch. Future work includes more trials, running statistical tests on this data, and investigating the biochemical mechanisms that drive these observed reactions. Figure 1. Results from the cytokine assay showing fractional increases in cytokine expression from controls, over four time periods.



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1. CDC., "Injury Prevention & Control: Traumatic Brain Injury & Concussion." Sept. 2016.

2. Marino, A., Dea, A., Egerter, R., Zosman, H., Orwin, E. "A Scratch Wound Model for Evaluation of Treatments for Traumatic Brain Injury." Poster Presentation at annual Biomedical Engineering Society Meeting, Tampa, Florida, 2015

P688 Anti-adipogenic factors in platelet rich plasma counter its pro-adipogenic effects, while serum from platelet rich fibrin supports stem cells adipogenic differentiation.

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Introduction :

Fat tissue, due to its high concentration of pluripotent stem cells, has a role in aesthetic medicine and reconstructive surgery for treating soft tissue defects. However, poor survival of the transplanted cells limits the usefulness of this material in regenerative medicine. Platelet rich plasma (PRP) derivatives are also commonly applied injectables in soft tissue regeneration, so one may hypothesize that it can improve the viability or adipogenic differentiation of mesenchymal stem cells (MSCs). However, plasma derivatives are notoriously heterogeneous in composition and we set out to investigate the effects of PRP and serum from platelet rich fibrin (SPRF) on the adipogenic lineage of MSCs.

Methods :

Metabolic activity and proliferation rate of human MSCs cultivated in media supplemented with three types of blood derivatives (fetal calf serum (FCS), PRP or SPRF) were determined using the XTT assay. Adipogenesis was evaluated by oil red staining, triglyceride content and expression of adipogenic genes. Composition of adipokines, as well as pro-and anti adipogenic cytokines was measured by dot-blot in blood derivatives.

Results :

Proliferation of MSCs was supported by both FCS and SPRF in a time-dependent manner, but surprisingly, PRP had a much weaker effect. Proliferation rate was presented as a fold change relative to metabolic activity on day 0 and after 2 days it was observed that in all groups the cell number was at least doubled, but without significant difference within the groups. After 5 days the rapid increase of cell growth was indicated only in FCS (5.4 fold change) and SPRF group (5.8 fold change) and there was a significant difference vs. serum free group (1.9 fold change) and PRP group (3.0 fold change) ($p<0.05$). Microscopic examination revealed changes in cell morphology and density after cultivation with PRP, whereas effects of SPRF and FCS were comparable. Lipogenesis was only observed in groups with adipogenic differentiation medium, with SPRF showing a significantly stronger adipogenic effect. This was confirmed by intensive accumulation of lysochrome dye in lipid droplets, higher triglyceride concentration and elevated expression of specific adipogenic genes : PPARG, FABP4 and ADIPOQ in SPRF versus PRP supplementation. Adipokines : adiponectin, leptin, lipocalin and pro-adipogenic cytokines : VEGF, PDGF, IGF-1, were at a similar level in SPRF and PRP, but adipogenesis inhibitors : EGF, IL-1, TGF-β, were only abundant in PRP.

Discussion & Conclusions:

Despite widespread use of PRP as a supporting agent in soft tissue repair, it was significantly less effective compared to serum derivatives. An explanation for this outcome can be the presence of adipogenic inhibitors in PRP. We therefore suggest that SPRF can be a more suitable blood product for supporting adipose tissue viability and quality during surgical procedures.

Acknowledgements

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P690 Building a tool box to assess the risk of undesired proliferation associated with regenerative medicine approaches during early drug discovery

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The term Regenerative Medicine has mostly been used to describe treatment paradigms based on cell and gene therapy and several guidelines describe regulatory expectations of preclinical activities needed to support progression into man. Regenerative therapies also encompass the approach to use drugs such as small molecules, biologics and nucleotide-based drugs to (re)establish normal organ function, for example by promoting cell proliferation, cell regeneration, change in cell fate, or conversion of cell type, to restore tissues and function. A potential safety concern associated with that could be uncontrolled proliferation, with downstream consequences on organ function or even neoplasia. The challenge is then: How can one best assess such risks early during drug discovery? Can one rely on currently established toxicological principles or is a new safety testing paradigm needed for drug-induced regenerative medicine?

We have started to build a tool-box of in vitro and in vivo assays to enable project decisions or trigger further mechanistic activities. By combining a thorough Target Safety Assessment, in vitro assays and in vivo analyses, assessed versus project specifics (target biology, patient population, indication, dosing, DMPK), we have been able to influence early discovery projects with information-driven decisions rather than carrying forward a theoretical risk. Specific examples include in vitro assessment of proliferation of target cell type compared with unwanted cell populations, including cells of the same organ, “off target” cells, and cells with high proliferative capacity such as stem cells. In vivo, assessments of unspecific proliferation have been performed in various tissues. Results show that relatively specific proliferation of target cells can be achieved.

P692 Dynamic biomaterials that direct cell migration via a chemoattractant gradient to enhance meniscal repair

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Dense connective tissue injury results in ineffectual repair in adults, due to the paucity of endogenous cells that colonize the wound site. We hypothesized that this hypocellularity at the wound site results from the dense and stiff extracellular matrix (ECM), which presents a biophysical barrier to cell migration, as well as the lack of directional cues. Using the knee meniscus as a model system, we investigated whether the provision of an exogenous platelet-derived growth factor-AB (PDGF-AB) chemoattractant gradient might recruit cells to the wound site to accelerate meniscal healing, and whether this effect would be enhanced with partial matrix degradation.

To carry out this study, we developed tri-component nanofibrous scaffolds that sequentially released a matrix-degrading enzyme and PDGF-AB in a localized and coordinated manner. To enable cell migration from the tissue into the nanofibrous network, a 'sacrificial' water-soluble poly(ethylene oxide) (PEO) fiber fraction first delivered a burst dose of collagenase to the wound interface. Afterwards, a second, slower-degrading fiber fraction composed of hydrolytically degradable hyaluronic acid (HA) released PDGF-AB over the course of 5 weeks to guide cells to the defect. A remaining population of stable poly(ε-caprolactone) (PCL) fibers acted as a physical template that provided mechanical integrity and instruction for organized ECM synthesis upon cell arrival at the wound site.

To test the effect of chemoattractant-directed migration on meniscal repair in an in vivo setting, a nude rat xenotransplant model was employed, where a scaffold was inserted within an adult meniscal explant and placed subcutaneously for up to 4 weeks. The defect was either left unfilled or filled with one of four scaffolds: a blank control scaffold, a collagenase-releasing scaffold, a PDGF-releasing scaffold, or a dual biofactor scaffold that sequentially released collagenase and PDGF. Although we investigated only one concentration and release rate for each biofactor, our data indicate that these complementary approaches independently increased interfacial cellularity. The highest cell densities at the wound interface were achieved by scaffolds sequentially releasing collagenase and PDGF-AB, likely by facilitating endogenous cell migration and proliferation after reducing the local density of collagen and other matrix constituents. Matrix deposition within the dual biofactor scaffolds, including type II collagen, and interfacial integration were also enhanced compared to control scaffolds. This versatile platform will enable one to maximize cell recruitment while minimizing damage to the tissue microenvironment, as well as deliver additional therapeutics, such as anabolic growth factors and anti-inflammatories, to promote matrix synthesis and inhibit matrix catabolism in the long-term. By combining multiple bioactive components that expedite cell colonization and wound integration in a spatiotemporal manner, these novel scaffolds will improve repair of the meniscus and other dense connective tissues.

P693 Human platelet-derivatives for clinical-grade expansion of mesenchymal stem cells from different tissue sources

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Background: Although fetal bovine serum (FBS) is still the most commonly used supplement for ex vivo expansion of adult human mesenchymal cells (MSCs), it is important to substitute animal-derived products for clinical applications, according to current Good Manufacturing Practices (cGMP). Human platelet-derivatives (PDs) represent optimal FBS-substitutes due to the nature and range of the released growth factors (GF).

Objectives: To optimize the preparation of platelet-derived supplements in terms of (a) GF concentrations, and (b) expansion and differentiation of human MSCs from different tissue sources. The overall aim was to optimize the preparation of a cGMP-compliant platelet GF concentrate for large-scale MSC expansion.

Methods: PDs were prepared as 'releasates' (PR) or 'lysates' (PL) depending on the method of GF release, i.e., via chemical activation with thrombin (1-10U/ml), or via mechanical lysis via freezing and thawing (1-3 cycles), respectively. PR and PL, prepared from pooled platelet concentrates (5-20 donors), were systematically tested and compared, in terms of (a) ELISA-based GF concentrations [platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)-β1, and vascular endothelial growth factor (VEGF)], and (b) in vitro proliferation, colony-formation, surface marker expression (flow cytometry), and multi-lineage differentiation assays of human gingiva-derived (GMSCs), bone marrow-derived (BMSCs) and adipose-derived MSCs (ASCs). Cells cultured in 10% FBS were used as the control. Experiments were performed in triplicates, using cells from at least three donors, and data were statisticallyanalysed.

Results: Four different protocols were selected and adapted for preparation of PR and PL. For in vitro experiments, PR/PL concentrations of 5% and 10% were tested. Higher concentrations of GFs (PDGF-BB, TGF-β1, VEGF) were identified in PL vs. PR. Moreover, proliferation of MSCs was higher in PL vs. PR; in some donors, proliferation was higher in 5% PL vs. 10% PL, suggesting that 5% may be the optimal concentration when using this method of PL preparation. Comparable surface marker expression (stromal phenotype) was observed in all MSCs expanded in PL vs. FBS. Significantly higher proliferation (population doubling-level) and colony-formation, and comparable differentiation potential of GMSCs, BMSCs and ASCs was observed in 5% PL vs. 10% FBS.

Conclusion: Human PL prepared from pooled platelet concentrates by a simple, economical and cGMP-compliant method, represents the optimal GF-supplement and FBS-substitute, while maintaining the stromal properties, i.e., phenotype, proliferation and differentiation potential, of MSCs from different tissue sources.

P695 Preliminary tests of murine myoblasts culture for the treatment of muscle injuries caused by bothrops asper venom

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We preliminarily evaluated the muscular regeneration potential of a myoblast culture on a murine model of necrosis produced by *Bothrops asper* venom. To carry out the experiments, myoblasts were isolated from skeletal muscle of Balb-c mice. Isolated cells were characterized by immunodetection with Desmin (purity), MyoD1 and Myf5 (early differentiation); sarcomeric α-actinin and myogenin (late differentiation). Skeletal muscle damage was induced by intramuscular injection of the venom. After 24 hours, cells were injected intramuscularly in the gastrocnemius in a PBS solution. After two weeks, the gastrocnemius muscle was dissected and processed for histological analysis. Residual CK was determined in muscle homogenates as a quantitative indicator of muscle regeneration. In all experiments, a control group of animals with vehicle or venom was included. Histology showed more regeneration areas in animals that received the cell treatment than those treated only with the venom. However, the content of residual CK data did not show quantitative differences in terms of muscle regeneration. We observed that the application of cells suspended in saline solution was not effective as their distribution in the damaged area was not sufficient. Money & Vandenburgh (2008) report that 90% of the injected cells in a damaged muscle area died from the induced ischemia conditions which limits the success of the treatment to reduced zones of damage. Therefore, we are currently assessing the regeneration potential of the myoblast culture embedded in a hydrogel and/or in preparations of platelet rich plasma (PRP). We hipothesize that these preparations would either provide a better distribution of the cultured cells and/or provide a source of growth factors that improve muscular regeneration by increasing intramuscular migration and myogenic capacity in the damaged muscle (Skuk & Tremblay, 2003). These experiments constitute the first steps in Costa Rica towards evidencing the potential of cellular therapies in the regeneration of skeletal muscle.

P696 Role of CD146 in mesenchymal stem cell homing and regeneration of the intervertebral disc: an in vitro and whole organ culture study

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Introduction: While homing of mesenchymal stem cells (MSCs) toward degenerative intervertebral discs (IVD) has been well described, little is known about the migrating MSC sub-population. CD146/MCAM is involved in regeneration of different musculoskeletal tissues. Therefore, the aims of this study were to compare (1) the migration of CD146+ versus CD146- MSCs towards conditioned medium (CM) from induced-degenerative IVDs, (2) the regenerative potential of CD146+ versus CD146- MSCs in vitro and ex-vivo.

Materials and methods: CM was collected from bovine caudal IVDs with endplates after loading in a bioreactor under degenerative conditions (high frequency, low glucose) for 7 days. Human MSCs from bone marrow aspirates were separated in CD146+ and CD146- populations through fluorescence activated cell sorting and used for migration assays toward CM (n=6 donors) and chondrogenesis in pellet cultures (n=3 donors). Glycosaminoglycan (GAG) (DMMB), DNA (Hoechst) and Safranin-O/Fast green staining were analysed. CD146+/- MSCs were seeded onto trypsin-treated bovine IVDs (n=3 donors). After 3 weeks, GAG synthesis rate was measured by Sulphur 35 incorporation.

Results and discussion: CD146+ MSCs had superior migration toward CM compared to CD146- MSCs (22.5±6.8 versus 15.7±6.6 % migrated cells; p<0.0001) (Fig. 1a). CD146- cell pellets had a higher (although not significant) GAG/DNA ratio compared to CD146+ cell pellets (120.7±40.0 and 93.7±44.3, respectively). The same trend could be observed in histological slides (Fig. 1b). The trypsin degenerated organ culture experiment confirmed these findings, with higher GAG synthesis rate in the annulus fibrosus (AF) of IVDs treated with CD146- cells compared CD146+ cells (Fig.1c). We therefore hypothesize that CD146+ cells possess a higher migration potential while CD146- MSCs might represent a more chondrogenic phenotype with limited migration potential.

Conclusion: CD146 distinguishes subpopulations of MSCs with distinct migration and regenerative potential in the degenerative IVD.

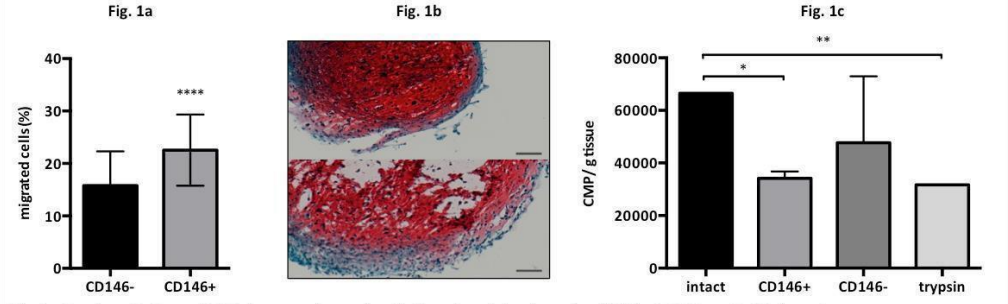

Fig. 1a: Number of migrated MSCs (trans-well assay) as % of total seeded cell number (****p<0.0001; n=6 MSC donors).
Fig. 1b: Safranin-O/Fast green stained Pellets; Upper CD146-, Lower CD146+. Note the more intens staining in the upper pellet. Scale bar: 100µm
Fig. 1c: CPM3 detection/ gramm of tissue (One-way ANOVA; n=3 MSC donors)

P698 Prepration of connective tissue growth factor siRNA /Low density lipoprotein nanocomplex and attenuation of scar formation by modulating ultra-structural collagen organization

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Nanocomplex of Connective Tissue Growth Factor (CTGF) small interfering RNA (CTGF siRNA) with cationic solid lipid nanocomplex, reconstituted from natural components of protein-free-low-density lipoprotein were prepared and its Inhibitory effect on CTGF expression and scar formation focusing on ultra-structural collagen organization were investigated. Various ratios of CTGF siRNA-PEG :LDL (1:1 to 1:30) were prepared and down regulation were evaluated by immunoblot analysis. Control and CTGFsiRNA modified cells populated collagen lattices were prepared and rates of contraction measured. Collagen organization in rabbit ear 8mm biopsy punch wound at 1day to 8wks post injury time were investigated by transmission electron microscopy and histology with Olympus System and TS-Auto software. CTGF expression was down regulated to 56% of control by CTGF siRNA-PEG/LDL (1:30) complex ($p<0.01$) and collagen lattice contraction was inhibited. CTGF siRNA-PEG/LDL (1:30) treated wound showed basket weave pattern of collagen arrangements with mean diameter of 188±47 nm (n=196). Mismatch siRNA treated wound contained a high frequency of parallel small diameter fibrils (mean 90±20nm, n=563). Down regulation of CTGF by siRNA-PEG/LDL nanocomplex modulated collagen fibril organization comparable to normal skin. This result suggests potential application of CTGF siRNA-PEG/LDL nanocomplex for scar-less therapy.

P699 Optimizing electroporation parameters for non-viral transfection of primary intervertebral disc cells for tissue engineering

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Introduction: Low back pain (LBP) is an increasing global health problem associated with intervertebral disc (IVD) trauma and degeneration. Current treatment options include surgical interventions with partial unsatisfactory outcomes reported such as failure to relieve LBP, non-unions, nerve injuries or adjacent segment disease. Cell-based therapy and tissue engineered IVD constructs supplemented with transfected disc cells that incorporate factors enhancing matrix synthesis represent an appealing approach to regenerate the IVD. Gene delivery approaches using transient non-viral gene therapy by electroporation is of a high clinical translational value since the incorporated DNA is lost after few cell generations, leaving the host's genome unmodified. Human primary cells isolated from clinical-relevant samples were generally found very hard to transfect compared to established cell lines. In this study we aimed for the identification of suitable parameters, i.e. voltage pulse, number and duration, for efficient transfection of human and bovine IVD cells for the Neon® Transfection System. For this we chose an overexpression plasmid containing the gene for growth and differentiation factor 6 (pGDF6).

Material & Methods: To demonstrate efficiency, primary human and bovine AF and NP cells (hAFC/hNPC (N=4) and bAFC/bNPC (N=5)) were transfected with the commercially available plasmid pCMV6-AC-GFP tagged with copepod turbo green fluorescent protein (tGFP). Flow cytometry was subsequently applied to quantify transfection efficiency. For the exemplary transfection with GDF6, h/bNPC and hAFC were transfected with pGDF6 with different parameter sets. After 7 and 14 days of transfection, cells were investigated for expression level of GDF6, GFP and different marker genes for the IVD phenotype (aggrecan [*ACAN*], collagen type 1 [*COL I*] and collagen type 2 [*COL II*])

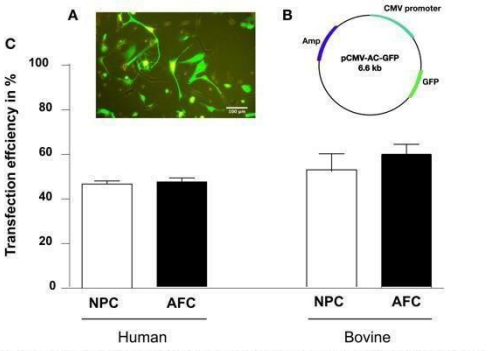

Figure 1: A. Example of pCMV-AC-GFP human nucleus pulposus cells 48 hours after transfection. **B.** Vector map of pCMV6-AC-GFP plasmid (Amp: Ampicillin resistant gene) with 6.6 kb region containing the cytomegalovirus (CMV) promoter. **C.** Percentage of transfection efficiency of human (N = 4) and bovine (N =5) NPC and AFC as quantified by flow cytometry. The percentages of transfection efficiencies are (mean ± SEM): hNPC 46.7 ± 1.4 %, hAFC: 47.1 ± 2.4 %, bNPC 52.44 ± 7.9 %, bAFC 59.6 ± 5.0 %.

Results: Our results showed that two pulses of 1400 V for 20 ms revealed good and reproducible results for both human and bovine IVD cells with efficiencies ≥ 47 %. The pGDF6 transfected cells showed a trend of GDF6 up-regulation after 7 days of transfection. Surprisingly no up-regulation of extracellular matrix protein, such as *ACAN, COL I* and *COL II* could be detected.

Conclusion: The presented parameters allow for successful human and bovine IVD cell transfection and provide an opportunity for subsequent regenerative medicine application.

P700 Human muscle precursor cells overexpressing PGC-1α support early myofiber formation for bioengineering of slow twitch sphincter muscle

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Muscle precursor cells (MPCs) are quiescent muscle cells capable of muscle fiber reconstruction. However, their quality and quantity gradually decline with age. Therefore, autologous MPC transplantation is envisioned for the treatment of muscle diseases, such as Stress Urinary Incontinence. The goal of this research was to explore the possibility of genetically modifying human MPCs to overexpress PGC-1α, a central factor in muscle exercise adaptation, in order to enhance skeletal muscle formation and quality for application for urinary sphincter regeneration.

hMPCs were harvested from *M. rectus abdominis* of patients. After genetically modifying the cells to overexpress PGC-1α (or GFP control), viability, proliferation and myogenic phenotype were evaluated *in vitro*. The expanded cells were suspended in a collagen carrier and *s.c*. injected bilaterally on the back of nude mice. One, two and four weeks later the bioengineered skeletal muscle tissues were harvested and further assessed by histology, WB and RTPCR.

We were able to confirm the sustained myogenic phenotype of the genetically modified hMPCs. Viability and proliferation potential were not significantly different compared to native cells *in vitro*. Fiber formation capacity and contractility were enhanced in PGC-1α modified hMPCs *in vitro.* Subcutaneously injected cell-collagen suspensions were harvested after 1, 2 and 4 weeks and histological analysis confirmed the earlier myotube formation in PGC-1α modified samples. Increased contractile protein levels were detected by WB, with a significant initial switch to slow type muscle fibers.

By genetically modifying hMPCs to overexpress PGC-1α we were able to enhance the early myotube formation *in vitro* and *in vivo*, thereby developing a novel strategy for improving sphincter muscle tissue engineering for future application.

P701 Nanoghosts targeted delivery of RNA interference therapy for the treatment of Osteoarthritis

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Osteoarthritis (OA) is the leading cause of worldwide joint disabilities. OA is characterized by articular cartilage loss, subchondral bone thickening and osteophyte formation. The clinical management of OA is largely palliative with an urgent need for effective disease modifying treatments. In recent years, RNA interference (RNAi) technologies have been suggested as a promising approach for treating many diseases including OA. Nonetheless, the clinical use of RNAi oligonucleotides is largely hindered by the lack of safe and effective vectors.

Recently, our lab has developed an innovative delivery platform, termed Nanoghosts (NGs) that is based on nano-vesicles reconstructed from the plasma membrane of mesenchymal stem cells (MSCs). The NGs were shown to retain MSCs' surface features and targeting capabilities towards sites of inflammation, abilities exploited to address OA with RNAi therapy.

Our goal is to functionalize NGs to increase their selectivity towards OA and establish protocols for efficient NGs loading with RNAi molecules. Moreover, we aim to prove NG system safety and efficacy in preclinical OA models. Chondrogenesis effect will be studied with RNAi-loaded NGs *in vitro* with 2D and 3D cultures of hMSCs, along with their effect over cell differentiation, susceptibility to inflammatory factors, and ECM remodeling capacity. The efficacy and translational potential of the system will be then assessed *in vivo* using acute and chronic rodent OA models.

Our preliminary data show that miRNAs are efficiently loaded in Nanoghosts, with an encapsulation efficiency of 20%, using electroporation. We also demonstrated NGs targeting towards hMSCs, their target for chondrogenic stimulation miRNA molecules. Ongoing studies are characterizing hMSCs miRNA uptake and 3D hMSCs pellet culture are establishing a positive control for miRNA chondrogenic *in vitro* chondrogenesis studies.

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P702 Pre-conditioning of dental pulp-derived cell spheroids with hypoxia and hypoxia mimetic agents

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Scaffold-free strategies, such as the transplantation of dental pulp-derived cell (DPC) spheroids, are a promising approach for pulp regeneration. Hypoxia pre-conditioning has been shown to enhance cell engraftment and pro-angiogenic capacity in a variety of regenerative approaches. It is however unknown if pre-conditioning interferes with spheroid formation of DPC. In the presented study we evaluated the impact of hypoxia and hypoxia mimetic agents on the formation and activity of DPC spheroids. DPC spheroids were generated on agarose wells. Cells were exposed to hypoxia and the hypoxia mimetic agents Dimethyloxallylglycine (DMOG), Desferrioxamine (DFO), L-mimosine (L-MIM) or combinations of hypoxia and hypoxia mimetic agents. Images were taken after seeding, 6 and 24 hours and spheroid size was measured. Cell viability was evaluated with the resazurin-based toxicity assay, the MTT assay and Live-Dead staining. Vascular endothelial growth factor (VEGF), Interleukin(IL)-8, Stromal cell-derived factor(SDF)-1, Angiogenin (ANG) and Angiopoietin-like 4 (ANGPTL 4) production by DPC spheroids was evaluated with the respective enzyme-linked immunosorbent assay. DPC spheroids formed under hypoxia, after treatment with hypoxia mimetic agents and under the combination of hypoxia and hypoxia mimetic agents. Cells remained vital and no significant differences in spheroid sizes were found compared to normoxic conditions. Hypoxia and hypoxia mimetic agents increased VEGF, IL-8 and ANGPTL 4 levels in DPC spheroids, but did not modulate SDF-1. ANG was increased by hypoxia mimetic agents, but not considerably modulated by hypoxia. Combinatory treatment with hypoxia and HMA did not further boost VEGF and IL-8 production. Overall our results show that pre-conditioning with hypoxia and hypoxia mimetic agents does not impair the process of spheroid formation, but increases the pro-angiogenic capacity. Future studies will reveal if these pre-conditioning strategies support regenerative approaches in endodontics.

P703 Evaluation of cell isolation methods for the generation of oral mucosa keratinocytes as an advanced therapies medical products

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Background: Advances in tissue engineering have permitted three-dimensional reconstruction of the human oral mucosa for in vitro and in vivo applications. Current regulation in this field is very complex and bioengineered tissues must be generated using good manufacturing practices (GMP) and pharmaceutical-grade products and reagents. However, very few works focused on the evaluation of methods and protocols fulfilling these quality requirements. In this work, we have carried out a comparative study of several products used for cell isolation and establishment of primary cultures of oral mucosa epithelial cells.

Methods: First, 5mm oral mucosa biopsies were obtained from Sprague-Dawley rats and treated with 2 routine-use cell dissociation solutions (0.05% trypsin and 0.5% trypsin) and 2 solutions specifically designed for use in GMP rooms (trypLE-select and trypLE-CTS enzymatic solutions). 5 cell dissociation cycles of 30 minutes each were used on each biopsy. For each cycle, dissociated cells were quantified and cell viability assays and characterization experiments were performed during three weeks of in vitro culture to determine cell viability, proliferation and cell profile of oral mucosa keratinocytes.

Results: Our results demonstrated that routine-use cell dissociation solutions were efficient for the establishment of primary keratinocytes cell cultures, with colonies appearing from the first week of culture. Viability of the cultured colonies was 70% for 0.5% trypsin, and 63% for 0.05% trypsin. When GMP-specific solutions were used, we found that both products succeed for the generation of primary cultures of human oral mucosa keratinocytes, although a higher number of oral mucosa fibroblasts surrounding the keratinocytes colonies was found. Cell viability was 69% for trypLE-select and 32% for trypLE-CTS.

Discussion and Conclusions: Our results show that the different solutions were associated to different levels of cell dissociation and viability, suggesting that bioprocessing artificial tissues under GMP conditions requires the adaptation of current protocols based on routine-use products. In this case, it is well known that human oral mucosa keratinocytes are difficult to isolate and culture, and generation of viable primary cultures of these cells is challenging. Although our preliminary results should be confirmed by future studies, results suggest that trypLE-select solutions could replace routine-use trypsin without affecting the efficiency of neither the isolation protocol nor the cell viability, although its specificity for epithelial cells is lower.

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P704 Clinical translation of a fibrin-agarose skin substitute generated by tissue engineering

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Introduction: The skin is the largest organ of the body, which plays essential role in maintaining the normal physiology and homeostasis. The skin is often affected by burns, neoplasm, infections, autoimmunological diseases and trauma. Over the recent years, several human skin substitutes were developed by tissue engineering for the treatment of severe skin conditions, especially for extensive and deep burns, and some of them showed very good results [1]. In the present work, we describe the successful clinical translation process of a human skin substitute generated in laboratory using fibrin-agarose nanostructured biomaterials.

Methods: A fibrin-agarose skin model was previously generated and evaluated ex vivo and in vivo in nude mice [2]. Results demonstrated that both the epithelial and the stromal layers of the bioengineered skin substitute were biomimetic and fully functional once grafted in vivo. These preclinical data allowed us to obtain approval by the Spanish Medicines Agency (AEMPS) for clinical compassionate use in a 29-year-old woman with deep burns affecting more than 70% of her body surface, with serious vital risk. A biopsy was obtained from the patient healthy skin and stromal fibroblasts and epithelial keratinocytes were isolated and expanded in culture in a GMP facility at the Unit of Cell Production and Tissue Engineering of the University Hospital Complex of Granada, Spain. Then, a skin substitute was generated with these cells using nanostructured fibrin-agarose hydrogels as scaffolds, which was autologously implanted in the patient.

Results: Ex vivo quality controls demonstrated that the bioengineered skin generated in the GMP facility had a well-structured dermal substitute with abundant fibroblasts covered by a stratified epithelial layer resembling the native skin. Sterility, karyotype and endotoxin analyses fulfilled all requirements for a medical product for clinical use. In vivo grafting was successful and the patient showed good clinical evolution, with epithelization of all affected areas. No complications were detected during the follow-up period.

Discussion: Clinical translation of novel tissue-engineering products is challenging. Development of bioengineered tissue substitutes must be accompanied by strict and thorough analyses and quality controls allowing approval by the national regulatory agencies. The positive results found for fibrin-agarose skin allow us to state that these advanced therapies products may be clinically useful for the treatment of severe burns.

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P705 Local depth-dependent nanoindentation measurements of human articular cartilage

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Scaffold design is one of the key factors for successful tissue engineering strategies. The emerging 3D printing technology allows a completely new scaffold production in which mechanical properties can be adapted layer-by-layer. Thus, scaffolds could be tailored to match the depth-dependent mechanical properties of articular cartilage. However, precise information about gradients of mechanical properties in articular cartilage is limited. In this study, we demonstrate the use of the nanoindentation technique to measure the zonal elastic modulus on human femoral condyle cartilage. We identified a depth-dependent change of local elastic modulus. From the superficial zone towards the bone the elastic modulus varied between 0.02±0.003 MPa and 7.91±0.69 MPa, respectively. Zonal and patient-to-patient differences were corroborated by visualizing the depth-dependent GAG and collagen type II content in histological sections. Measuring precise local mechanical properties by nanoindentation and using these as target values for 3D printed scaffolds can help in development of functional scaffolds with tailored properties for future tissue engineering approaches.

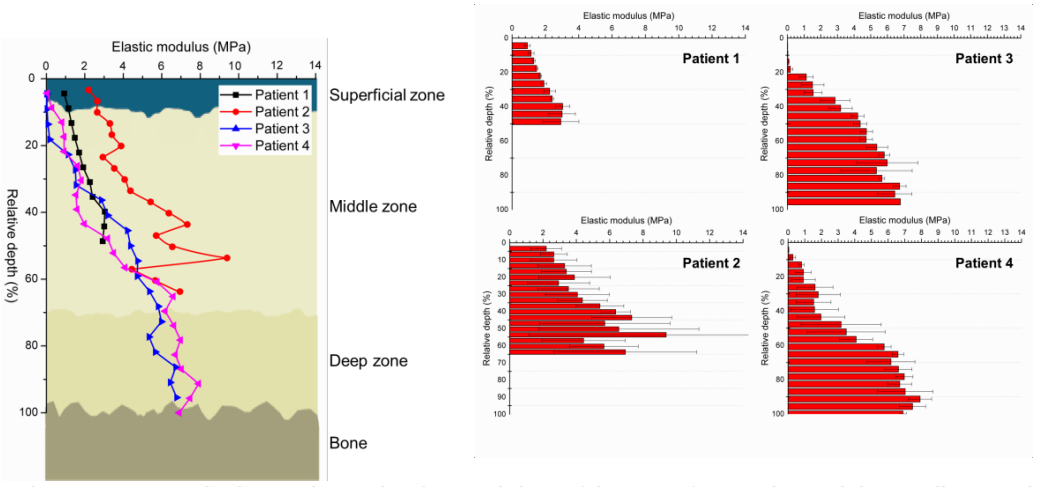

Figure 14: Depth-dependent elastic modulus of human femoral condyle cartilage. The relative depth corresponds to the distance between superficial layer (0%) and bone (100%) and was normalized for each sample; Patient 1 and 2 were not measured to 100% since the fixed number of indentations did not reach to the bone (thicker cartilage). For each specimen, measurements were repeated 3 times.

P706 Co-culture of endothelial and myogenic stem cells: strategies and applications for tissue engineering

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The tissues of the human body are composed of a multiplicity of cell types. The entirety of cells promotes tissue homeostasis, metabolism, growth and repair. Therefore, co-culture of different cell types more accurately represents the structure of real tissue and the cellular interactions within. Consequently, co-culture systems are being increasingly used in biomedical research. Nowadays, co-culture becomes a promising tool in tissue engineering research to induce and study various effects on cell differentiation, proliferation, migration, etc. Thus, our first aim was to create a novel type of co-culture based on rat endothelial progenitor cells (EPC) and rat myogenic progenitor cells (Myo). The field of nanoparticles is a rapidly developing, but there is still much remaining to be elucidated concerning their possible health effects. Little is also known about the impact of nanoparticles on stem cells. Therefore, the second part of our study is directed at the elucidation of ZnO and TiO_2 nanoparticle and carbon nanotube (CnT) effects on EPC, Mio cells and their co-culture.

Our data shows that ZnO nanoparticles had a stronger effect on EPC and Mio cells alone than on their co-culture. However, the smallest tested concentrations of ZnO stimulated the proliferation of co-culture. Moreover, the nanoparticles also affected EPC morphology, adhesion, migration, and angiogenic potential. CnTs had a remarkable effect on the adhesion of tested cell populations. Co-culture was rather resistant to TiO_2. In conclusion, the effect of nanoparticles on co-culture differs from pure ELP and Mio cell populations.

Third, we combined the developed co-culture with a PDMS-based hydrogel. Data obtained suggest an exciting opportunity to create novel 3D tissue-like models *in vitro*.

P707 Oxidative status predicts quality in human mesenchymal stem cells

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Human bone marrow-derived mesenchymal stem cells (MSC) are adult progenitor cells with great potential for application in cell based-therapies. From a cell based-therapies perspective there are two limitations to MSC use (1) these therapies require large numbers of cells and long-term expansion of MSC in-vitro promotes replicative senescence, (2) patient variability is a challenge for defining MSC quality standards for transplantation. This study aimed to determine whether low or high oxidative status (OS) of MSC correlate with changes in cell expansion and differentiation potentials.

We investigated functional aspects of mitochondria, such as cell metabolic activity indicators and expression of antioxidant enzymes. Furthermore, we tested if senescence induced changes in OS of MSC could be counteracted by methylene blue (MB), an alternative mitochondrial electron transfer known to enhance cell bioenergetics.

MSC isolated from donors of the same age showed distinctive behaviour in culture and were grouped as weak (low CFU and short-life in-vitro) and vigorous MSC (high CFU and long-life in-vitro). In comparison to weak MSC, vigorous MSC had OS characterized by lower mitochondrial membrane potential, lower mitochondrial activity and fewer reactive oxygen species production, as well as reduced mitochondrial biogenesis. Vigorous MSC had a significantly higher expansion potential compared to weak MSC, while no differences were observed during differentiation. MB treatment significantly improved expansion and differentiation potential, however only in vigorous MSC.

In conclusions, these results demonstrate the importance of mitochondrial function in MSC in-vitro, and that cells with low OS levels are better candidates for cell-based therapies.

P708 Tumor targeting therapy and magnetic hyperthermia on breast cancer cells by doxorubicin-bioconjugated carboxylic acid magnetic nanoparticles

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INTRODUCTION: Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide, accountings for 25% of all cancer cases and 15% of all cancer deaths among females [1]. Nanocarriers can be effective anticancer drug delivery systems for preventing and treating breast cancer. The aim of the present study was to produce a dual targeted drug delivery system and magnetic hyperthermia for more effective treatment of breast cancer by using carboxylic acid functionalized iron-oxide nanoparticles (CA-SPIONs - Fe3O4) loaded with doxorubicin which were able to be localized in target tissues and internalized within the desired cells by application of an external magnetic field.

METHODS:
The electrostatic loading of doxorubicin (DOX) to SPIONs was achieved through the electrostatic interaction between the carboxyl group of carboxylic acid functionalized iron-oxide nanoparticles and the amine group of DOX. After formation of the drug-nanoparticle conjugates, they were separated from the free drugs via magnetic separation and washing in the aqueous medium.
The therapeutic effect of DOX-CA-SPIONs was evaluated by MTT assay on the following cell lines: MCF-7 (Human breast cancer cells ER+/PR+), Triple negative MDA-MB-231 breast cancer cells, MCF-10A (Mammary epithelial cells). In this way we tried to verify the selectivity of magnetic hyperthermia treatment induced by SPIONs on killing breast cancer cells respect to normal mammary epithelial cells.
For hyperthermia treatment, cells were put in the incubator at 46°C for 30 minutes, corresponding to a temperature dosage of 90 cumulative equivalent minutes at 43°C, or else cells will be left in the incubator at 37°C. At 48 h after hyperthermia, cells viability will be tested. The in vitro efficacy was also tested without hyperthermia treatment.

RESULTS: The cytotoxicity of DOX loaded CA-SPIONs was compared with free drug DOX in the absence and presence of magnetic field, also the probable cytotoxicity of the targeted and non-targeted blank nanoparticles were checked. Cell viability assay showed that in MCF-7 and MDA-MB-231 cells the cell survival percentage was decreased significantly ($p<0.05$) in targeted nanoparticles group compared to free DOX and naked magnetic nanoparticles in most drug concentrations both in absence and presence of magnetic field without toxic effects on normal mammary epithelial cells (MCF-10A). These data indicate that DOX-CA-SPIONs and the presence of magnetic hyperthermia led to increasing cytotoxic effects of the nanoparticles on breast cancer cells.

DISCUSSION & CONCLUSIONS: The results demonstrated the potential of the DOX-CA SPIONs to achieve dual tumor targeting by magnetic field-guided in breast cancer cells.

P711 Effect of TGF-β1 on the plasminogen activation system, TIMP-1 and N-cadherin expression of stem cells from the apical papilla (SCAP) and the related signalling pathways

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Recently clinical revascularization/revitalization procedures have been successfully developed to repair/regenerate the pulp tissues lost due to infective necrosis of dental pulp and root canal in teeth with immature root formation. The induction of blood clot (containing growth factors such as TGF- , PDGF, bFGF etc.) formation into root canals and the ingrowth of stem cells from apical papilla (SCAPs) are suggested to be the critical factors for the clinical success of revascularization and apexogenesis. may regulate the cell proliferation, migration, matrix turnover, differentiation of the ingrowth SCAPs. Then new extracellular matrix formation and cell differentiation are important for overall pulpal repair and regeneration processes. Matrix formation is tightly regulated by plasminogen activation system [plasminogen activator inhibitor-1 [PAI-1], urokinase plasminogen activator [uPA], uPA receptor [uPAR] and tissue inhibitor metalloproteinase (TIMP) that prevent the collagen degradation. On the other hand, N-cadherin is a cell adhesion molecule popularly enhanced the differentiation of odontoblasts, cementoblasts, and osteoblasts as well as tissue mineralization. The effects by TGF- 1 can be mediated by ALK5/Smad-dependent or other signalling pathways. To know more about the role of TGF- 1 and SCAPs in the processes of revascularization, apexogenesis and future tissue engineering, we studied the effect of TGF- 1 on the plasminogen activation system, TIMP-1 and N-cadherin expression in SCAPs. Briefly, human stem cells from the apical papilla (SCAPs) were obtained and cultured from teeth with immature root apex due to orthodontic extraction by the approval of Ethics Committee, National Taiwan University Hospital. SCAPs were cultured and characterized to express various stem cell markers (Stro-1, CD146 etc.). SCAPs were exposed to TGF- 1 with/without pretreatment and co-incubation by SB431542 (ALK5/Smad2/3 inhibitor) and U0126 (MEK/ERK signalling inhibitor) for 24 hours or 5 days. Culture medium was collected for enzyme-linked immunosorbant assay of PAI-1, uPA, and soluble uPAR (suPAR). Cells layers were used for extraction of RNA or proteins. Protein expression was analysed by western blotting. The results showed that TGF- 1 stimulated PAI-1 and TIMP-1 secretion of SCAPs, but partly inhibited uPA secretion and showed no marked effect on suPAR secretion. Similarly, TGF- 1 also induced PAI-1, TIMP-1 and N-cadherin protein expression and inhibited uPA expression of SCAPs. TGF-1–induced PAI-1 secretion can be attenuated by SB431542 and U0126, TGF- 1–induced PAI- 1, TIMP-1 and N-cadherin protein expression can be attenuated by SB431542. These results indicate that TGF- 1 possibly stimulates matrix deposition and cellular differentiation via induction of PAI-1, TIMP-1, N-cadherin and the suppression of uPA in SCAPs. These events are differentially regulated by ALK5/Smad2/3 and MEK/ERK signalling pathways. Understanding the effects of TGF- 1 on SCAPs is helpful for future tissue engineering, regeneration and revascularization of pulpodentin complex as well as apexogenesis of dental roots.

P712 The effect of adipose tissue derived mesenchymal stem cells for the regeneration of radioiodine-induced salivary gland dysfunction

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Radioiodine (RI) ablation is an excellent treatment modality for the removal of residual thyroid cancer. After RI treatment, patients often complain of painful salivary gland (SG) swelling, xerostomia, taste alterations, and oral infections, and thus, this conditions subsequently diminish quality of life for the patients with thyroid cancer. Nevertheless, no satisfactory therapy has been devised to treat SG dysfunction after RI treatment. Nowadays, cell-based therapy has been reported to restore the damaged SG tissue. This study was aimed at determining whether adipose tissue derived mesenchymal stem cells (ASCs) could prevent SG dysfunction after RI treatment. Four-week-old female C57BL/6 mice were divided into three groups; a normal control group, a RI-treated group (0.01 mCi/g mouse, orally), an ASC and RI-treated group. Salivary flow rates and lag times were measured, and morphologic and histologic examinations and TUNEL (terminal deoxynucleotidyl transferase biotin-dUDP nick end labeling) assays were performed. Changes in salivary ^{99m}Tc pertechnetate uptake and excretion were followed by single-photon emission computed tomography. Salivary flow rates and lag times to salivation in the ASC and RI-treated group were better than in the RI-treated group. Histologic examinations of SGs in the ASC and RI-treated group showed more preserved parenchyma compared to the RI-treated group. Fewer apoptotic cells were observed in ASC and RI-treated group than in the RI group. In addition, the patterns of ^{99m}Tc pertechnetate excretion were better in the ASC and RI-treated group compared to the RI group. In conclusion, local delivery of ASCs could protect from SG damage and thus, offers a possible means of preventing SG dysfunction by RI.

P713 Specific moieties carrying nanotracers for gene therapy and tracking the human mesenchymal stem cells

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Stem cell therapy based on human mesenchymal stem cells (hMSCs) has shown great promise for various disease treatments. However, traditional stem cell-mediated therapy is limited due to their multipotent differentiation ability (uncontrolled spontaneous differentiation) and the difficulty in monitoring cells after implantation in vivo. Here, we report a new multi-functional stem cell nanotracer (M-NT) for directing controlled differentiation through gene delivery, as well as tracking stem cells with dual-modal imaging (Optical and CT imaging). The M-NT was prepared through a facile surface modification process of ~100 nm-sized gold nanoparticles with catechol-functionalized branched polyethylenimine (C-bPEI). The C-bPEI-functionalized M-NT exhibited greatly enhanced long-term colloidal stability in aqueous solution and a capability to complex with plasmid DNA (pDNA; i.e., pEGFP) through electrostatic interaction for gene delivery and transfection to control differentiation. M-NT/pEGFP complexes showed an enhanced transfection efficiency into hMSCs with low cytotoxicity compared with branched polyethylenimine/pDNA complexes. Accordingly, successful in vitro chondrogenic differentiation was achieved in hMSCs treated with M-NT/pSOX9 complexes. Finally, hMSCs transfected with M-NT/pEGFP complexes were transplanted into Balb/c nude mice and successfully visualized through dual-modal optical fluorescence and computed tomography (CT) imaging.

Keywords: Specific moiety; nanotracer; stem cells; pDNA; nude mice.

P714 A combined theoretical and experimental strategy to inform the choice of cell density in engineered tissue constructs to maximise therapeutic cell survival

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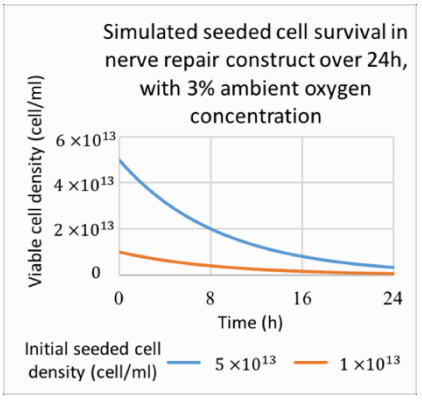
Introduction: Engineered tissue design for clinical use must involve the consideration and evaluation of the impact of many different parameters, including physical and chemical properties. Mathematical modelling is a cost effective and time efficient tool that can suggest the probable experimental outcomes of design parameter choices when implemented in combination with carefully designed experiments [1]. A theoretical framework describing the interactions between oxygen concentration and seeded cell density within engineered tissue has been constructed and parameterised using experimental data. It has been applied to the nerve repair construct context to explore the impact of different initial cell densities upon cell survival over time. The results can be used to inform the future design of such constructs.

Methods: Schwann cell-like differentiated adipose-derived stem cells were maintained in plastic compressed collagen gels with various initial seeding densities and oxygen levels. Viability was assessed using CellTiter-Glo (Promega) after 24h. A mathematical model was created to simulate the interaction between oxygen concentration and cell density within the *in vitro* gel and peripheral nerve construct scenarios. It consists of coupled differential equations with terms representing cell proliferation and death, and oxygen consumption and diffusion. Parameters within the model were assigned using values from the literature and fitting based upon the experimental results [2-4]. The model was then applied to a nerve repair construct setting.



Results: The experimental work demonstrated that initial seeded cell density has an impact upon cell survival. The parameterised theoretical model provides a fit to the initial experimental work, and has been used to simulate the impact of a range of initial seeded cell densities upon cell survival.

Discussion & Conclusions: This flexible theoretical framework can be applied to the nerve repair construct context, as well as other engineered tissue scenarios, to inform the choice of initial seeded cell density with the aim of increasing seeded cell survival.

P715 Cellular studies of electrospun PCL/biocomponent nanofibers from alternative and traditional solvents

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Bicomponent polycaprolactone/gelatin and polycaprolactone/collagen nanofibers formed by electrospinning process using alternative solvent mixture as well as perfluorinated alcohol were subjected to in-vitro cellular studies and compared. The alternative solvent system consisted of the mixture of acetic acid (AA) and formic acid (FA), while hexafluoroisopropanol (HFIP) was used as a reference, an example of traditional solvent.

The alternative solvent mixture as well as the conditions for electrospinning process were optimized as previously described [1] and let us to obtain a similar fiber morphology as those electrospun from HFIP.

The set of six types of materials consisting of PCL/coll, PCL/gelatin, both in 9:1 w/w ratio, as well as pure PCL mats as reference materials, electrospun from two types of solvents underwent cellular in-vitro studies and wettability measurement.

Tests were performed using L929 mouse fibroblast cells and human primary fibroblasts. MTT cytotoxicity test was carried out on extracts and showed that all type of materials are not cytotoxic.

In order to assess cellular response in direct contact, the cells were cultured for 3, 5 and 7 days on all types of materials and afterwards SEM images were taken as well as fluorescent dying of nuclei and cytoskeleton were performed. DNA proliferation test, XTT and resazurin based tests were carried out up to three days.

Obtained results of cellular studies proved that the biopolymer addition facilitates cell adhesion and spreading on the surface of nonwovens in comparison to pure PCL materials. Similarly, wettability measurements showed a lower water contact angle for samples containing collagen/gelatin, with a small but noticeable difference in regard of solvent used in electrospinning.

Bicomponent electrospun nonwoven materials from AA/FA mixture solvent can be an attractive alternative for using expensive and toxic perfluorinated alcohols. Exhibiting good cellular response they show potential to be used in tissue engineering as a scaffold material.

Acknowledgement

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P716 Effect of exosomes from human induced pluripotent stem cells on skin wound healing in diabetic mice

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Human induced pluripotent stem cells (hiPSCs) are regarded as a promising cell source for regenerative medicine. But their clinical use has not been popular because of teratoma formation. Recently, stem cells conditioned medium have attracted considerable attention as effective tools for some disease model in the point of non-cell therapy. Researchers suggested that its mechanism is related to exosomes including microRNA and proteins. To date, a few reports have demonstrated exosomes derived from hiPSCs (hiPS-Exo). In this study, we investigated the potency of promoting diabetic wound healing by the application of hiPS-Exo.

METHODS:

According to Yamanaka's report, we cultured hiPSCs (201B7, Riken) and collected hiPSCs conditioned medium. Exosomes derived from iPSCs culture medium (M-Exo) and hiPSCs conditioned medium (hiPS-Exo) were isolated using Magcapture (Wako). We did morphometric and flow cytometry analysis of M-Exo and iPS-Exo, and also evaluated the in vitro effects of hiPSC-Exo on both the proliferation and migration of diabetic mouse dermal fibroblasts by cell-counting and scratch assays. PBS, M-Exo, and iPS-Exo were respectively injected subcutaneously around skin defect in a diabetic mouse model, and their effects on wound healing were assessed. Wounds were harvested at 7 and 28 days and then processed, sectioned, and stained (CD31, alpha-smooth muscle actin to quantifyvascularity.

RESULTS:

Small vesicles (average diameter 120.0±24.5nm) were observed using transmission electron microscope only in hiPS-Exo group. CD9, 63 and 81 were positive in hiPS-Exo group. hiPS-Exos stimulated the migration of diabetic mouse dermal fibroblasts in vitro, but does not stimulated its proliferation. Injected hiPS-Exo to wound sites resulted in accelerated wound closure at day 7 and 10 compared with PBS-control and M-Exo-injected wounds ($P < 0.01$). Histological analysis showed that the Capillary Score (the number of vessels/mm2) was significantly higher in hiPS-Exo-treated wounds at day 7 but not at day 28 compared with the other groups.

CONCLUSIONS:

Our findings suggest that hiPS-Exo promoted diabetic wound healings through vasculogenesis at the early stage of wound healing. hiPS-Exo might become a therapeutic option for diabetic ulcer. Further study is still required for its detail mechanism in the future.

P718 Evaluation of commercially available cryopreservation media for the cryopreservation of MSCs expanded in a novel xeno-free medium

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Mesenchymal stem cells (MSCs) are being assessed therapeutically for a number of conditions based on their ability to differentiate into mesenchymal cell lineages such as bone and cartilage. However, heterogeneity of MSCs is typically reported in the literature and results in conflicting reports of efficacy of these cells. Much of the heterogeneity observed in the literature may be due to variations in culture conditions that may affect the final cell product. A number of factors can potentially reduce this heterogeneity, including the use of an antibody-selected MSC, growth in a chemically defined medium and the use of an automated system to reduce variability introduced to the culture system due to user and system variation. Autostem, an EU funded project aims to address this need by development of a fully automated system for the isolation and expansion of bone marrow derived MSCs. Many MSC products require cryopreservation prior to delivery to patient to facilitate long-term storage of cell products. In this study, we assessed a number of cryopreservation media for their ability to effectively cryopreserve MSCs which have been previously isolated and expanded in a novel xeno-free medium. These media were assessed based on their recovery rate, cell viability, effect on MSC differentiation ability, surface marker characterisation and proliferation. Cryopreservation media assessed were CryoStor (Sigma Aldrich), Prime XV (Irvine Scientific), CryoSOfree (Sigma Aldrich), Cellvation (MPBio), and Human serum albumin containing 10% DMSO. All cryopreserved groups were compared to fresh MSCs. Variation in recovery rates were observed between groups indicating loss of cell viability in some test groups. No differences were observed in surface marker phenotype or cell morphology.

P719 The effect of fibronectin during the isolation and expansion of bone marrow derived mscs isolated in a novel xeno-free medium

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Mesenchymal stem cells (MSCs) are progenitor cells that differentiate to form cartilage, bone and fat. MSCs also possess self-renewal abilities and immunomodulatory properties, and are thus potential therapeutic targets for regenerative medicine and tissue engineering. Currently large-scale expansion of MSCs depends on the use of serum during cell culture, in particular, fetal bovine serum (FBS). The use of FBS raises serious scientific, safety and ethical concerns. Therefore, previous work in our group has resulted in the development of a chemically defined, serum- and xeno- free medium for the production of MSC's. This serum-free medium does not require the use of attachment factors such as fibronectin to efficiently isolate MSCs. However, fibronectin may have an effect on the chondrogenic potential of these cells. To assess this MSCs were isolated from bone marrow and cultured without fibronectin, on fibronectin coated flasks or with fibronectin supplemented in the medium. The cultured MSCs were characterised based on growth kinetics, surface marker expression and tri-lineage differentiation potential. A partial loss of differentiation potential of MSCs was observed in cells expanded in the absence of fibronectin. This was observed particularly in chondrogenesis were elevated levels of the *Fibronectin* gene were observed early in chondrogenesis in conjunction with a delayed upregulation of *SOX9*, the master regulator of chondrogenesis. Fibronectin has previously been reported as an inhibitor of SOX9 and its downregulation is required for the chondrogenic differentiation of MSCs. This work indicates that in the absence of fibronectin, MSCs expanded in a serum-free medium upregulate *Fibronectin* and this inhibits chondrogenesis of these cells.

P720 The effect of changing oxygen tension during culture and differentiation of bone marrow derived mesenchymal stem cells isolated and expanded in a novel xeno-free medium

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The majority of early work on developing *in vitro* culture conditions of cells including mesenchymal stem cells has focused primarily on nutrients, pH and growth factors. This has resulted in a wide variety of media formulations and supplements with varying degrees of success for culture of stem cells. More recently, oxygen tension has also been recognized as an essential component of culture conditions for cells. Historically, with oxygen tension for cell culture receiving little attention, atmospheric or ambient oxygen levels were primarily used. To a large extent this continues to be the standard oxygen tension for the expansion of the majority of mammalian cells including MSCs. In contrast, the majority of stem cells *in vivo* exist in far lower oxygen tensions. Lower oxygen tension in tissues, such as bone marrow (1-5%), known to harbour stem cells raised the question as to whether or not these lower oxygen tensions were essential for the maintenance of stem cells. With that in mind a number of studies have been carried out which report that altering of oxygen tension during culture of MSCs does alter the phenotype of these cells. Numerous studies have reported an effect of oxygen tension on growth, tri-lineage differentiation potential and production of cytokines. However, these studies have generated conflicting data regarding the effect of hypoxia on MSCs. Although some of this can be attributed to experimental conditions, there are also reporting issues. These typically occur in studies which report an effect of switching MSCs from one oxygen tension to another on varies aspects of MSC phenotype. What is often unclear is the timing of this switch. This can be a switch during culture prior to performance of the assays or at the time of the assays themselves. In this study, MSCs were isolated and expanded in atmospheric 'normoxia' (21% O2) and 'hypoxia' (2% O2) in standard 10% serum-containing medium and a novel serum-free medium. These cells we then expanded for two passages prior to being passages in the oxygen level they were isolated in or switched to the other group. These cells were expanded for another passage before undergoing a detailed characterisation including surface marker expression, tri-lineage differentiation and cell stress response. This study provides a detailed evaluation of the effect of changing oxygen levels on MSCs in standard serum-containing medium and in a novel xeno-free medium.

P721 Development of cell manufacturing incubator integrating isorator technology for maintaining aseptic environment for an extended period of time

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Incubator is one of the most critical devices in the manufacturing of stem cells for regenerative medicines. Stable cell culture in a safe and highly reproducible environment (equivalent grade A) is required for broad commercialization of cell manufacturing. We have developed an incubator that can provide an aseptic environment stably for an extended period of time by applying isolator technology. The incubator, which can be decontaminated internally with vapor hydrogen peroxide, can also maintain the aseptic environment over an extended time period with a leak tight structure and positive pressure control. HEPA-filtered unidirectional airflow is employed, providing a high-degree cleanliness and a uniform temperature and humidity distribution. A water supply room is filtered and separated from the main unit, thus reducing a contamination risk during supplying water and allowing to control humidity levels at a given value. By connecting the incubator with cell processing isolator via aseptic transfer interface, cell processing/culture can be conducted in a continuous aseptic environment, preventing cross contamination. In conclusion, the newly developed incubator fulfills the requirements for commercialization of cell manufacturing. The R&D has been conducted with support from the Japan Agency for Medical Research and Development.

P722 Engineering of axially vascularized bone grafts towards the treatment of avascular bone necrosis

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INTRODUCTION: The standard of care for avascular bone necrosis (AVN) includes the use of pedicled or free autologous bone grafts, which are associated with morbidity and availability constraints.[1,2] To overcome these bottlenecks stromal vascular fraction (SVF) cells of human adipose tissue have previously been used to engineer osteogenic and vasculogenic grafts.[3] This study aims at combining such osteogenic/vasculogenic engineered grafts to a vascular bundlein order to manufacture pedicled bone graft substitutes, which were then tested in an ectopic model of AVN.

METHODS: SVF cells from human adipose tissue were seeded into hydroxyapatite cylinders and cultured for 5 days in a perfusion bioreactor system. Constructs were then inserted into hollow cylinders of devitalized bone, mimicking necrotic bone in AVN. In nude rats, a ligated arteriovenous bundle was created from the deep inferior epigastric vessels and introduced into the SVF-seeded constructs through a central drill hole. After up to 8 weeks subcutaneous implantation constructs were assessed by histology and microtomography with cell-free constructs as controls.

RESULTS: After 1 week in vivo, constructs with or without SVF cells were fully vascularized, but cell-based constructs displayed significantly higher (54% more) blood vessel density. At 8 weeks, bone tissue was formed only in cell-seeded constructs. Implanted SVF cells contributed both to bone and blood vessel formation in vivo, as documented by staining for human-specific ALU sequences. Rat-derived osteoclasts and tissue remodeling (M2) macrophages were found predominantly in SVF-seeded constructs. The surrounding necrotic bone was revitalized in both groups, as assessed by the ingrowth of blood vessels.

DISCUSSION & CONCLUSIONS: We successfully engineered pedicled bone graft substitutes by combining a vascular bundle with SVF seeding. In the ectopic AVN model, the vessels arising from the central pedicle efficiently revitalized the necrotic outer shell, while the SVF cells were essential to attract/polarize M2 macrophages and to induce bone and vessel formation in the inner core. Longer implantation times are now required to investigate the dynamics of osteoconduction into the outer layer of the construct, which would bear relevance for the treatment of AVN.

P723 Collagen-chitosan membranes crosslinked with EDC for guided bone regeneration

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The low bone availability is a fairly frequent problem in Implantology. When a dental piece is lost, either by a caries or disease, a reabsorption of the vestibular cortical occurs, that in some cases, makes it difficult or impossible to place implants. In order to resolve these difficulties, the bone augmentation technique known as Guided Bone Regeneration (GBR) has been developed. In this procedure, bone graft (decellularized bone) is placed in the cavity left by the extraction of a dental piece and a membrane (barrier material) is surgically inserted over the filled bone defect, in order to promote the formation of bone tissue in front of the connective and epithelial tissue in the healing process [1].

The membranes can be developed using various polymers of natural or synthetic origin. Currently, natural polymers, such as collagen and chitosan, have been widely used in tissue engineering because they promote cell adhesion, growth and differentiation [2,3]. The objective of this study was to develop collagen-chitosan membranes improving their mechanical and enzymatic stability by chemical crosslinking with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), to be used in guided bone regeneration. The membranes were prepared at different proportions of collagen and chitosan by solvent evaporation, crosslinked with EDC and characterized in terms of morphology, thermal stability, enzymatic degradation and cell attachment, viability and proliferation.

Membranes morphology was observed by Scanning Electron Microscopy, showing an appropriate microstructure for biological applications. Differential Scanning Calorimetric showed an increase in shrinkage temperature using EDC. Enzymatic degradation test indicated that membranes treated with EDC dissolved slowly in enzymatic solution. Cell viability and attachment tests suggested that EDC treatment do not affect the excellent biological characteristics of membranes.

Acknowledgements: To CONACyT for their financial support through the project 214128 and DGAPA UNAM through the project PAPIIT RG100114.

P724 The control of stem cell differentiation using peptide-targeted delivery of biodegradable nanoaprticles

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Nanoparticle (NP)-based gene therapy, combined with new genome editing technologies (such as CRISPR/Cas9), could potentially correct inherited diseases (such as CF and DMD), acquired genetic abnormalities (such as cancer) and infections (such as HIV). Presently, *in vivo* delivery of gene-loaded NPs has proven to be inefficient and cellular internalisation must be enhanced for its full potential to be realised.

Here, we have produced gene-loaded NPs with the aim to enhance their intracellular targeting and therapeutic potential. We have used a novel cell penetrating peptide system, termed *Glycosaminoglycan (GAG) binding Enhanced Transduction (GET),* to improve endocytosis and intracellular delivery of our NPs. GET functions by targeting cell membrane heparan sulfate and has been shown to significantly improve the delivery of NPs, nucleic acids and recombinant proteins. Initially we demonstrate the efficient fabrication of nucleic acid loaded Poly (DL-lactide-co-glycolide) acid PLGA NPs with physical attributes tailored to increase tissue penetration and transfection efficiency such as small size (60-80 nm) and high encapsulation efficiency. Due to its advantages (such as lack of shear stresses and simplicity), we use nanoprecipitation to rapidly generate NPs with 23% encapsulation efficiency of pDNA which remains stable andintact.

We have tested pDNA-PLGA NP delivery and demonstrate low but significant internalisation/transfection of luciferase reporter pDNA *in vitro* (NIH3t3 cells). By coating pDNA PLGA NPs with GET-peptides, we observe several orders-of-magnitude increase (~45000- fold in SFM conditions) in reporter transfection, dramatically improving the success of productive gene transfer.

By combining nanotechnology with GET peptides we believe new regenerative medicine applications could be developed; including integrating gene editing, augmenting growth or transcription factor expression, and cellular reprogramming/programming strategies.

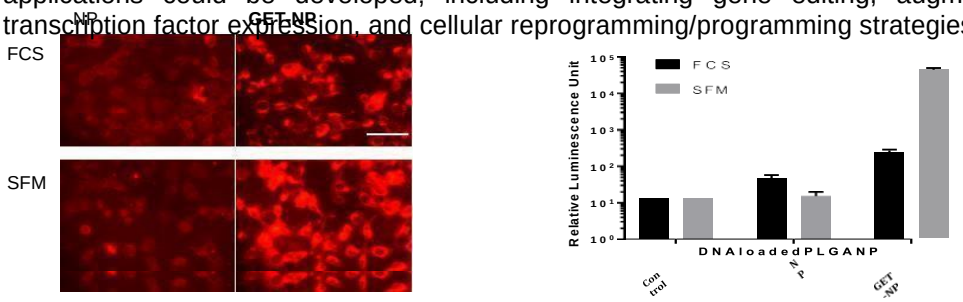

Figure 1: Transfection efficiency of NP and GET-NP. (A) Fluorescence microscopy images of NIH3t3 cells after addition of Nile red loaded PLGA NP with and without GET system in 20% FCS containing media or SFM. The GET system significantly enhances the internalization of loaded PLGA NP especially in SFM, (Scale bar 100 µm). (B) Transfection efficiency of DNA loaded PLGA NP with and without GET system. The GET system significantly enhances the transfection efficiency of DNA loaded NP in both media.

P725 Effect of TGF-ß1 on the expression of N-cadherin, plasminogen-activated inhibitor-1, TIMP-1 proteins in stem cells from human exfoliated deciduous teeth (SHED): Role of ALK5/Smad2/3, TAK1 and MEK/ERK signal transduction pathways

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Stem cells, growth factors, and scaffolds are considered to be crucial factors for tissue engineering and regeneration. Dental pulp stem cells derived from human exfoliated deciduous teeth (SHED) are discovered and recently considered to be a suitable stem cells source for regeneration of dental tissue and non-dental tissues (neuronal tissue, bone, adipocytes and for immune therapy etc.). Transforming growth factor-1 (TGF-β1) is one major growth factor that may affect many dentinogenic processes including proliferation, differentiation, tooth development, and response of dental tissues to injury. TGF-β1 is also crucial for the development, repair and regeneration of many crucial organs. Binding of TGF- 1 to receptors may stimulate Smad- dependent (ALK5/Smad2/3) and Smad-independent (transforming growth factor-activated kinase- 1 [TAK1], MEK/ERK etc.) signalling pathways to regulate cell behavior. N-cadherin is crucial for the mineralization processes by enhancing the differentiation of odontoblasts, osteoblasts, cementoblasts as well as the non-dental tissue cells such as cardiomyocytes, hepatocytes and neuronal cells. On the other hand, plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitor metalloproteinase-1 (TIMP-1) are crucial molecules to prevent collagen degradation. To know more about the future use of SHED and TGF- 1 in dental and non-dental tissue engineering and regeneration, the purposes of this study were to investigate the effects of TGF-β1 on SHED and the related signal transduction pathways. In this study, SHED were cultured from human dental pulp of exfoliated deciduous teeth and their stem cell properties were characterized (Stro-1, CD146 etc.) by flow cytometric analysis. The mRNA expression of various TGF- receptors was examined by reverse transcriptase-polymerase chain reaction (RT-PCR). SHED were exposed to different concentrations of TGF-β1. Smad2, TAK1 and ERK phosphorylation was determined by western blotting. The expression of N-cadherin, PAI-1 and TIMP-1 in SHED was analysed by western blotting or enzyme linked immunosorbant assay. In some experiments, SHED were treated with TGF- 1 with/without pretreatment and co-incubation by SB431542 (an ALK5/Smad2/3 inhibitor), U0126 (a MEK/ERK inhibitor), 5Z-7-oxozeaenol (a TAK1 inhibitor) for 24 hours or 5 days. The results showed that SHED expressed various TGF- receptors including ALK5, ALK3, ALK1, TGF- receptor II, betaglycan, endoglin as analysed by RT-PCR. Exposure of SHED to TGF- 1 stimulated the smad2, TAK1 and ERK1/2 phosphorylation. TGF-β1 stimulated N-cadherin, PAI-1, TIMP-1 proteins expression (and secretion of PAI-1 and TIMP-1) of SHED. TGF- 1-stimulated N-cadherin, PAI-1 and TIMP-1 expression can be markedly attenuated by SB431542, and partly also by 5z-7oxozeaenol and U0126. These results indicate that TGF-β1 may stimulate the matrix deposition and differentiation of SHED possibly via induction of N-cadherin, PAI-1 and TIMP-1. These effects are differentially modulated by ALK5/Smad2/3, TAK1, and MEK/ERK signal transduction pathways. TGF-β1 and SHED with/without scaffolds can be potentially utilized for future tissue engineering and regeneration of dental and non-dental tissues.

P726 Influence of processing parameters for 3D bioprinting

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Bioprinting is a new technology with great promise to revolutionize the tissue engineering field. However, the effects of the printing process on cells are largely unknown. Many groups reported a drop in cell viability after microextrusion-based bioprinting [1]. Experimental analysis and mathematical models revealed that shear stress during printing, needle diameter, geometry and printing pressure can decrease cell viability of bioprinted cells [2,3]. In this study we investigated the influence of certain processing parameters like mixing method and nozzle diameter on cell viability directly after printing. We observed a strong decrease in cell survival, when highly viscous polymer solutions, commonly used for bioinks, were mixed with much lower viscous cell suspension to create a bioink for 3D bioprinting.

Methods: P4 primary bovine chondrocytes were mixed with a polymer solution of 2% alginate and 3.5% gellan gum. Solutions were either manually stirred with a spatula (Stirred) or mixed by a two component syringe system and static mixer (Static Mixer with 18 mixing elements, MedMix Switzerland). The bioinks were extruded and printed constructs crosslinked with a solution containing 20 mM $SrCl_2$ and 130 mM NaCl for 30 min. Cell survival was assessed with LIVE/DEAD fluorescence staining 3 hrs after printing and data was analysed using Student's *t*-test (n=4) and one-way-ANOVA (n=4) with Tukey HSD posthoc testing.

Results: When cells and polymer were gently stirred, cells had a relatively low survival rate after bioprinting (76% cell viability, SD=±3%). Vigorously stirring of the bioink further compromised the cell viability (66±1%; p=0.074). Using the static mixing chamber, however, the survival rate increased to 90±2% (Fig. 1). Extruding the statically mixed bioink additionally through a 0.41 mm conical needle did not change the cell viability (88±5%, p=0.77).

Conclusions: Use of a StaticMixer significantly increased cell viability over hand mixing ($p<0.01$) and is highly recommended for preparation of cell-laden bioinks.

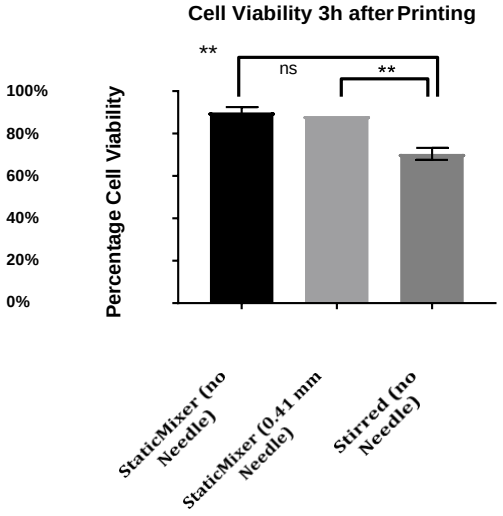

Fig. 1: Effect of mixing method on cell viability 3h after printing. Plotted is mean ± SD.

P727 *In vitro* and *in vivo* application of scaffold-free tissue reconstructed by endometrial stromal cells

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Introduction:

Uterus is an essential organ for embryo/fetus to grow until women give birth. While many women are suffering from uterine disease, they are subjected to hystoterectomy when it becomes severe, resulting in permanent infertility. On the other hand, as many women give birth to their first baby at late age, the pregnancy rate decreases. Recently, *in vitro* fertilization (IVF) has been widely carried out, but its success rate still remains low. Our group has attempted to utilize tissue engineering approaches for uterine regeneration utilizing decellularized matrices or scaffold-free tissues (SFT) [1]. In this study, we developed a novel method to reconstruct the SFT using rat endometrial stromal cells (rESCs).

Material and Methods:

We reconstructed the SFT using rESCs with a novel method and evaluated using immumohistochemical assay. Early stage of rat embryo (zygote) were harvested and then cocultured for 24 hours either on normal culture dish, rESC monolayer, or rESC SFT. The cocultured embryos were evaluated with regard to hatching and attachment. Moreover, the SFT was transplanted into Sprague Dawley (SD) rat for 3 days. The transplanted uterine sample was collected for immunohistochemical analysis to evaluate its partial uterine regeneration.

Results:

The method of fabricating SFT was successfully established. HE staining showed that the SFT was tightly formed in a three-dimensional shape while the immunostaining results represented that the stromal cell marker (Vimentin) and type I collagen (Col1) were strongly and entirely expressed in the SFT. As a result of incubating embryo on the SFT, the ratios of hatching and attachment were remarkably raised by coculturing them on the SFT, compared to the normal culture dish or rESC monolayer. The transplantation results of SFT in SD rat were also shown to regenerate uterine tissues greater and quicker than sham model. The SFT was integrated into the endometrium of native uterus involving endometrial epithelial cell layer and stromal cell layer in as little as 3 days of transplantation.

Discussions and Conclusions:

The wide applications of the SFT are expected for *in vitro* as well as *in vivo* partial uterine regeneration. The SFT exerted a superior environment *in vitro* for embryo growth including hatching and attachment. It implies a utilization of SFT as a new concept of incubator for early stage of embryo (zygote) after fertilization to keep or promote the development of embryo during *in vitro* fertilization (IVF). Moreover, this SFT was applicable *in vivo* for partial uterine regeneration, but further study will be required such as pregnancy test.

P728 Piezoelectric dermal patch for wound healing

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In the process of wound repair, endogenous electric field (EF) is generated in the damaged cell layer and known to regulate cell behaviors for wound repair. Here, we developed a skin patch with a piezoelectric effect that can create EF in ruptured epithelium for active wound healing. Piezoelectric patches were developed with alignment of bi-oriented grown zinc oxide nanorods and generated electric potential at the wound site upon mechanical deformation induced by movements of animals. In vitro and in vivo experiments demonstrated that the piezoelectric patch can promote wound healing by enhanced cellular metabolism, migration and protein expression. The piezoelectric dermal patches may be an effective modality for active wound healing.

P729 Modulation of the secretory potential of in vitro adipose tissue microenvironments

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The contribution of adipose tissue (AT) both to skin homeostasis and healing after injury is still to be revealed but more and more works have been supporting and strengthen its critical role. Several strategies have been used to recreate in vitro 3D AT microenvironments although some drawbacks regarding long term stability have been hampering significant developments regarding the representation of key biochemical signalling. We herein report the development of 3D AT-like constructs with differential secretomes by combining individual building-blocks at different stages of adipogenic differentiation. Cell sheets of human adipose-derived stem cells (hASCs) were created and differentiated into the adipogenic lineage by varying different parameters. Different degrees of differentiation were achieved as confirmed by real time RT-PCR and immunocytochemistry/image analysis for adipogenic markers (FABP4 and PPARgamma, leptin, adiponectin), and lipid accumulations quantified after Oil Red O and Nile Red staining. The obtained cell sheets were then superimposed at different combinations and levels of differentiation, and kept in culture for further 7 days. The possibility to vary the secretory potential of the constructs by changing the individual cell sheets that form the constructs was demonstrated by ELISA. Angiogenic (VEGF, PDGF-bb) and anti- (adiponectin) and pro-inflammatory markers (TNFα and leptin) were considered as representative of the functionality of AT while BMP-2 and PDGF-alpha were defined as key regulators of the hair cycle.

This work shows that by modulating the degree of differentiation of the building-blocks of 3D AT-like constructs as in native tissue, it is possible to modulate their secretome and tailor it to be representative of a specific regulatory role.

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P730 Fabrication and characterization of fluorescent silk particle for tissue engineering

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Silk particles were prepared from fluorescent silk fibroin solutions of transgenic silkworms and investigated in respect to its particle size, surface charge, stability and morphology along with its cellular uptake and release of growth factors. The particles did not show any cytotoxicity. Cellular uptake studies showed the accumulation of fluorescent silk particles in the cytosol of cells. In vitro growth factor release from the particles showed a significantly sustained release over 2 weeks. Furthermore, we demonstrate the intraoperative use of fluorescent SF in an animal model to detect a small esophageal perforation. This study suggests how fluorescent SF biomaterials can be applied in biotechnology and clinical medicine.

P731 Development of a novel 3D organotypic human HepaRG-based non-invasive optical imaging platform for pre-clinical hepatotoxicity screening

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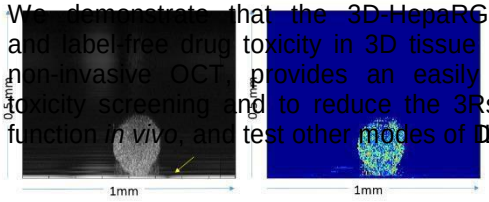
Drug-induced liver injury (DILI) is the leading cause of compound withdrawals in pre-clinical drug development, impeded by both the lack of physiologically-relevant human models and reliance on animal data. Strong incentives exist to create new human-based, *in vitro* liver models, which would offer better pre-clinical prediction of DILI. Human hepatic HepaRG cell (hepatocyte:cholangiocyte) co-cultures maintain *in vivo*-like liver-specific functions and functional polarity (intact bile canaliculi structures; enhanced cell-cell/ cell-matrix adhesions) and are considered a sustainable surrogate to primary human hepatocytes.

To develop functional organotypic 3D liver spheroids, as an approach for drug toxicity screening and as a potential alternative to animal testing, HepaRGs were cultured at different seeding densities (0-50,000) to optimize spheroidal size using ultra low-adherence plates (InSphero). H&E staining was performed (d7; d14) to ascertain viability (eg presence/absence of necrotic core) of spheroids; with optimal spheroid size/density defined as having a round spheroidal shape with no necrotic core. Optical coherence tomography (OCT) imaging system, which enables non-invasive, real-time monitoring of 3D models, was used to quantify cell viability. To further validate the 3D model, viability and cell proliferation was quantified following exposure to paracetamol at various doses.

Enhanced viability, proliferation, and metabolic competency was observed with an optimal spheroid size of 2500 HepaRG cells (also vs 2D HepaRG model). OCT was able to image 200 µm Ø spheroids non-destructively with a 5µm resolution (depth/penetration); whilst time-lapse OCT image-acquisition with optical fluctuation -maps were found to correlate directly to cell viability and calculated at each dose; giving unique insight into both drug penetration and toxicity. Finally, OCT cell viability values were integrated all over the samples and correlated to biochemical assays.

We demonstrate that the 3D-HepaRG-OCT platform has the potential to assess noninvasively and label-free drug toxicity in 3D tissue models. HepaRG-based 3D organoids combined with the non-invasive OCT, provides an easily reproducible model system for improved predictive drug toxicity screening and to reduce the 3Rs. Future work will validate the model's similarity to liver function *in vivo*, and test other modes of DILI, such as cholestasis.



1A 1B

Figure 1. OCT cross-section (x-z) of spheroids. OCT imaging was able to image the 3D cell culture through its depth. Yellow arrow points to the surface of a flat bottom 96 well plate. A) Intensity map showing dimensions and microstructure of the spheroids. B) Optical fluctuations map correlated to cell viability.

P732 Two-photon FLIM in the study of mesenchymal stem cells metabolic plasticity

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INTRODUCTION: Metabolic plasticity and the versatility of different lineages of stem cells as they satisfy their energy demands are not completely understood. In this study we investigated metabolic changes in mesenchymal stem cells (MSCs) undergoing differentiation in three directions (adipogenic, chondrogenic, osteogenic) based on the fluorescence of the metabolic co-factors NAD(P)H and FAD. Cellular metabolism was examined by monitoring the optical redox ratio (FAD/NAD(P)H), the fluorescence lifetime contributions of the free and bound forms of NAD(P)H. Two-photon fluorescence microscopy combined with FLIM was used to analyze this fluorescence in living cells. METHODS: Undifferentiated MSCs and MSCs on 7, 14, 21 days of three differentiations were imaged with a Zeiss 710 microscope coupled to a FLIM system. The intrinsic fluorescence of NAD(P)H and FAD was excited at 750 nm and 900 nm respectively by a femtosecond Ti:sapphire laser. The data were analyzed with the commercially available SPCImage software. The fluorescence lifetimes and their contributions (free and protein-bound forms of NAD(P)H: afree NADH, abound NAD(P)H) for the areas of interest were calculated by finding the global minimum of the $\chi 2$ value for three-exponential fittings. RESULTS: To estimate the general level of metabolic activity of the cells during differentiations, the fluorescence intensities of NAD(P)H and FAD were measured and represented as their redox ratio (FAD/ NAD(P)H). The optical redox ratio FAD/NAD(P)H decreased during all differentiations (0.33±0.19, 0.57±0.13 and 0.65±0.11 vs 0.77±0.13; $p < 0.000000$). This was likely to be explained by the intensive biosynthesis of lipids (adipogenic differentiation) and collagen (chondrogenic, osteogenic differentiation). To study cellular energy metabolism during adipogenic, chondrogenic, osteogenic differentiation, we analyzed the fluorescence lifetime contributions of the free and protein-bound forms of NAD(P)H. A statistically significant increase in the contribution of bound NADH was shown during adipogenic differentiation (from 29.5±1.8 % in undifferentiated MSCs to 34.6±8.2 in adipogenically undifferentiated MSCs), pointing to a shift toward oxidative phosphorylation (OxPhos). The contribution of protein-bound NAD(P)H in osteogenically and chondrogenically differentiated MSCs gradually decreased (from 40.1±3.7% to 29.3±1.8% and from 24±3.6% to 18.3±3.8% respectively), probably due to a bias toward more glycolytic metabolism. DISCUSSION & CONCLUSIONS: So we elucidate the metabolic changes in MSCs during adipogenic, chondrogenic, osteogenic differentiation, based on the fluorescence of the metabolic co-factors NAD(P)H and FAD using the methods of two-photon fluorescence microscopy combined with FLIM. Based on the data on the fluorescence lifetime contribution of protein-bound NAD(P)H, we registered a metabolic switch from glycolysis to OxPhos in adipocytes, consistent switch from glycolysis to OxPhos to glycolysis in osteoblasts and switch to more glycolytic status in chondrocytes. These data suggest the metabolic plasticity and the versatility of different lineages of MSC. ACKNOWLEDGEMENTS: This work has been financially supported by Russian Science Foundation (grants No. 14-15-00536).

P733 The role of collagenase in the isolation of adipose tissue-derived mesenchymal stem cells - aspects of risk assessment

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Collagenase is usually applied to quantitatively isolate the stromal vascular fraction (SVF) from adipose tissue. Due to the increase of indications for the use of the SVF in therapeutic applications, new aspects for the controllability of the use of collagenase in the process of tissue dissociation have to be considered.

In this study we focused on the application of collagenase in a clinical setting for the isolation of the SVF. For the reproducibility of the isolation process the storage conditions of the collagenase need to ensure the stability of the enzyme. Initially we compared the collagenase activity after storage in different buffers, concentrations, storage temperatures and storage periods.

Recent results suggest that there is no difference in decrease of enzyme activity between samples of collagenase being stored at 4 °C and -20 °C over 20 days (experiments will be extended to up to 60 days). Also differences in stability caused by storage of the enzyme at different concentrations of 1.54 PZU/ml (PZ Unit according to Wuensch) and 0.06 PZU/ml are being examined. Here preliminary results indicate that, the storage of the enzyme at the low concentration does not lead to a higher decrease in stability over 20 days when compared to the higher concentration (experiments will be extended to up to 60 days). Ongoing research also determines the influence of solving buffers like Ringer solution and PBS on the stability of collagenase during storage. Components possibly applied during the isolation of the SVF, like human albumin or fetal calf serum, and other effectors of collagenase activity, like EDTA and cysteine are being assessed for their influence on collagenase activity.

Furthermore we measured the collagenase activity at different stages of the isolation process of the SVF. The collagenase activity was determined in the native tissue, after adding collagenase solution and after 30 minutes of incubation, shaking at 37 °C. An average of 1.57 mPZU/ml of collagenase activity was determined in the native tissue. After adding the collagenase solution, an average activity of 44.8 mPZU/ml was measured in the test system. After incubation of the tissue shaking with 100 rpm at 37 °C for 30 minutes, the average activity had increased to 96.3 mPZU/ml. This led us to the question whether endogenous collagenase was released from the tissue due to the disruption during the digestion process. We therefor compared the activity in the native tissue to activities measured in tissue from the same patients after ultrasonic disintegration. The ultrasonic disruption of adipose tissue lead to an average activity of 1.2 mPZU/ml. Thus we concluded that it was not endogenous collagenase from the tissue that lead to the increase of collagenase activity in the test system during the digestion process. This research aspect needs further attention as a prerequisite for the risk assessment in the clinical setting.

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P734 Bone non union treatment by autologus cells grafts

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Background Bone non-unions remains a major concern in bone reconstructive surgery, because of limited supply of autografts, donor site morbidity, risks and complications in allografting and synthetic bone substitutes.

It was identified that cord blood, like bone marrow, is a rich source of stem cells.

The aim of presented study was to evaluate the efficacy of grafts containing alogeneic cord blood cells or autologic bone marrow cells with or without demineralized bone matrix to stimulate bone formation in nonunion and pseudarthroses.

Materials and methods: As grafts were used: alogeneic demineralized bone matrix obtained from cortical bone, autologus bone marrow nucleated cells and alogenous umbilical cord blood nucleated cells. Were studied the levels of ALP an ACP, X-ray, Computed tomography and Scintigraphy by Technetium 99m.

Results This prospective study was done on 189 patients with non-unions. The Upper limb (65 patients): clavicle – four, the arm - nineteen; the radial bone - nine; the ulna - eight; the both bones of the forearm – eight, the scaphoid bone of the wrist – fourteen, the flanges – three. The Lower limb (124 patients): the femoral bone – forty one; patella - two, the tibial bone – eighty one cases. The obtained results were evaluated by X-Ray, Scintigraphy and by CT. As control group were patients with nonunion treated by surgery using autologous cancelous bone grafts.

In sixteen cases as a grafts were used alogenous cord blood cells and the rest one hundred seventy three the autogenous Bone marrow stem cells obtained in four cases from proximal tibial bone and the rest of them from posterior superior iliac crest.

Conclusions The bone healing was obtained in 81,9% of cases, in the rest of the cases patients needed another method of treatment with autogenous cancelous bone grafts.The used cells grafts were compatible with the recipients, provides active and uniform osteogenesis in to the bone nonunion.

Key words cellular therapy, bone nonunions, nucleated cells.

P735 AUTOSTEM: a closed robotic platform for the automated expansion of mesenchymal stem cells

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In recent years, the field of regenerative medicine has been continuously gaining importance. For instance, there are between 200 and 300 clinical trials with mesenchymal stem cells (MSC) for a broad range of indications showing promising results. In order to pave the way towards availability of therapies to the general public, MSC have to be produced at a large scale. Manufacturers though are confronted with many challenges such as variations in the input cell material due to donor variability. Additionally, the final product consists of living cells with an inherent complexity. Therefore, the production process requires adherence to strict regulatoryrequirements.

However, the biopharmaceutical industry currently struggles with the lack of availability of high-capacity infrastructures. This circumstance was the starting point for the pilot project AUTOSTEM which focusses on the development of a robotic platform for production of MSC. The implementation of automated systems into biological processes opens up new possibilities with regard to higher reproducibility and increases the process robustness while minimizing the risk of human errors.

Within this project a fully automated, large-scale expansion of MSC from bone marrow will be developed with the perspective for therapeutic applications. Since safety is a major aspect, all steps of the process will be performed in closed systems that do not require any direct interaction between the human and the product. Therefore, the expansion process will be carried out under aseptic conditions within a fully enclosed robotic facility. This includes several processing steps, starting from the insertion of a disposable containing bone marrow. The subsequent expansion with large capacities will be provided by the employment of bioreactors in multi-liter scale. Accordingly, the cultivation has to be monitored and controlled with regular media exchange and automated sampling for determination of the cell count. Following the cultivation, the cell suspension will be harvested and the final cell product is formulated for cryopreservation. The latter is especially critical in regards to safety since the cell suspension has to be handled openly in order to be filled into vials for later delivery. For this reason, different areas with graduated safety levels within the platform will be defined.

One of the particular challenges within the project is that the system needs to perform different handling tasks in order to substitute the human operator. Therefore, a robot arm will be implemented for the handling and transportation of different disposables. In addition, both small (samples) and large volumes (cell suspension, medium, etc.) have to be processed automatically. This will be achieved by integration of a pipetting system for small volumes in combination with a pumping system for the management of large liquid volumes.

On top of this, the manufacturing process will be supported by software that connects the automated devices and hardware, monitors and controls the process and allows the tracking and storage of all data. This way, the automated facility will also contribute to the documentation process in the context of legal requirements.

P736 Characterization of basic mechanical properties among wet and dry unaligned electrospun collagen matrices and rabbit corneas

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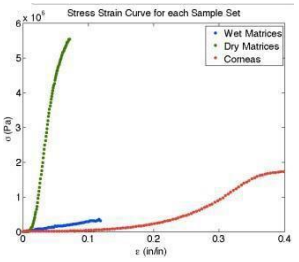
Around 285 million people around the world are visually impaired or are blind, of which 161 million are affected by corneal damage or hazing[1]. While many of these could be treated through LASIK procedures, some cornea damage requires a corneal transplant. In 2014, there were about 48,000 cornea transplants conducted in the United States alone, suggesting that the demand for corneas cannot be met by donation alone[2]. One way to resolve this issue would be to simply tissue engineer a cornea *in vitro* in order to increase the supply of corneas available for those in need. Additionally, a tissue-engineered cornea would make testing for research purposes much easier in comparison to using corneas harvested from various animals such as rabbits. Thus, it is imperative to efforts for tissue engineering an artificial cornea that the basic mechanical properties are well tuned to match those of a real cornea, as the differentiation of corneal cells has been found to be highly dependent on basic mechanical properties, such as matrix stiffness[3]. In our lab, collagen nanofibers are synthesized and collected to form extracellular matrices via electrospinning. The solution electrospun is composed of 7% collagen type I by weight, dissolved in 2 ml of glacial acetic acid. Matrices are then collected off of a copper drum and split into rectangular strips ready for mechanical testing. Rabbit corneas were dissected and removed keeping the whole cornea intact. In order to preserve the mechanical properties of the corneas, they were submerged in PBS and stretched as a whole rather than being cut into strips. Three quantities of interest were

UTS

measured, namely Young's Modulus (E), Ultimate Tensile Stress (UTS), and Strain at Failure (SF).

Strain Rate: 2 mm/min	E (MPa)	(MPa)	SF (%)
Wet Matrices	2.34 ± 1.22	0.78 ± 0.04	7.2 ± 1.7
Dry Matrices	120.7 ± 21.5	5.5 ± 2.0	14.0 ± 3.2
Cornea	46.0 ± 10.4	1.7 ± 0.2	N/A



Due to issues with slipping, there is no strain at failure reported for corneas. These results imply that there is still more work to be done to synthesize matrices with more similar mechanical properties to corneas. In particular, the Young's Modulus of wet matrices suggests the need to engineer stiffer matrices in order to better match the cornea. Future work will involve testing at different strain rates since collagen is a viscoelastic material, as well as seeding cells onto the matrices and observing their effect on mechanical properties.

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P737 Modified cell penetrating peptides for efficient gene delivery in vivo

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A major scientific goal is the development of non-viral gene carrier platforms for the delivery of exogenous DNA to cell nuclei. Many of these technologies cannot overcome challenges in low transfection efficiency, cytotoxicity and/or serum-inhibition limiting their efficacy. If these issues could be resolved then powerful technologies such as *in vivo* cell programming or gene correction could be employed therapeutically. Since their discovery ~25 years ago, cell penetrating peptides (CPPs) have been developed as non-viral delivery tools and show great promise in overcoming some of the challenges of gene transfer. At the University of Nottingham we have developed an efficient peptide-based nanotechnology for efficient DNA delivery termed glycosaminoglycan-binding enhanced transduction (GET) peptides (Dixon *et al*., 2016).

In collaboration with the Hanes lab (Johns Hopkins University, USA) PEGylated GET NPs were engineered for *in vivo* gene delivery. At 40 % and 60 % PEGylation rates complexes were able to retain colloidal stability in biological fluid and penetrate cystic fibrosis (CF) patient sputum samples. PEG GET complexes demonstrated widespread gene delivery, superior safety profiles and significantly better transfection efficiency of a reporter gene compared to PEI NPs in mouse lung models (Figure 1). Furthermore due to their superior transfection and mucus penetrating properties PEG GET technology could facilitate new approaches for *in vivo* gene therapy of lung diseases such as cystic fibrosis (CF).

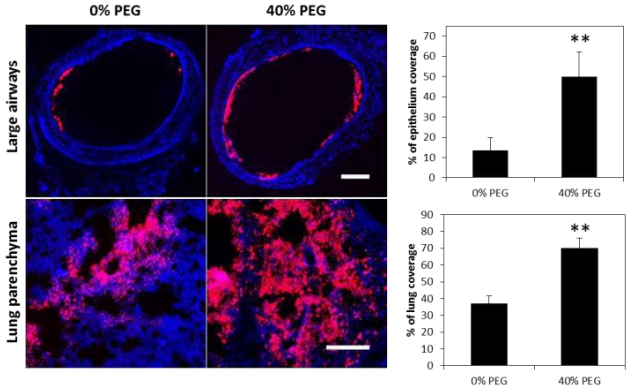

Figure 1. In vivo distribution of PEGylated GET peptide complexes in the mouse lung in vivo. Representative images of NP distribution in large airways and lung parenchyma (red). Cell nuclei are stained with DAPI (blue). Image-based quantification of coverage of NPs in large airways and distribution of NPs in the lung parenchyma. Tissues were harvested 30 min following intratracheal administration of NPs. Error bars indicate s.d., n=3.

P739 Clinically-relevant solution for hypothermic storage and transportation of human multipotent mesenchymal stromal cells

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The wide use of human multipotent mesenchymal stromal cells (hMSC) in clinical trials requires the preparation of full-scale safety and identity analysis of final cellular product or its transportation between research/medical centers, which needs additional prolonged storage of cells prior to application. Such exposure of hMSC suspensions to non-optimal extrinsic environment leads to significant decrease of viability and functional potential of cells and represents a great challenge in the field of regenerative medicine. Conventional vehicle solutions employed in cell therapy applications do not usually provide sufficient preservation of viability and specific cell characteristics during hypothermic storage. Thus, the development of new, non-toxic and efficient media, providing high viability and maintaining therapeutically associated properties of hMSC is highly relevant for successful clinical outcome.

This study was aimed on the optimization of hypothermic storage conditions for hMSC in order to improve the viability and functional characteristics of cells for further clinical applications.

hMSC were isolated from human adult bone marrow (BM) according to proper ethical guidelines. hMSC were cultured in xeno-free conditions for 2-4 passages. Hypothermic storage of hMSC was prepared at 4°C during 24, 48 and 72 hrs in several research and clinical grade media: Plasma-Lyte® 148 (Baxter Healthcare Ltd), HypoThermosol FRS (HTS-FRS, BioLife Solutions Inc), Ringer's solution for infusion (Baxter Healthcare Ltd), supplemented with 5% human serum albumin (R-alb) and developed buffered trehalose solution (BTS), prepared according to cGMP standards. Cell recovery was assessed by propidium iodide staining (immediately after storage) and Alamar blue assay (24 hrs post-storage). The stability of hMSC immunophenotype was assessed by flow cytometry. Proliferation and differentiation properties were assessed in vitro, using standard protocols.

Hypothermic storage of hMSC during 24 hrs in Plasma-Lyte® 148 or R-alb solutions resulted in more than 50% decrease of hMSC recovery and further reduction of this parameter down to 10-20% after 72 hrs of storage. In opposite, the application of HTS-FRS or BTS solutions provided significantly higher hMSC recovery rates and ability of cells for attachment and further proliferation. The recovery rates of hMSC were not significantly different between these two solutions during the whole period of storage and comprised more than 75-80% after 72 hrs of hypothermic preservation. The hypothermic storage of hMSC in HTS-FRS or BTS solutions during 72 hrs did not affect the maintenance of their specific immunophenotype and three-lineage differentiation capacity.

The obtained results show that BTS can be applied as an economic and efficient alternative to HTS-FRS for the hypothermic storage of hMSC suspensions. High efficiency together with low toxicity and simple composition opens possibilities for applying this media as universal vehicle solution, providing optimized viability of hMSC for clinical research and therapy.

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P740 Characterization of in vitro expanded human Adipose-derived stem cells: implication in regenerative medicine

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Adipose-derived stem cells (ASCs) have been in the centre of scientific and clinical attention, due to a wide range of possible implementations in tissue engineering, regenerative medicine and clinical immunology. These cells might also prove exceptionally convenient as an in vitro model for biochemical, pharmaceutical and immunological studies. The adipose tissue is an easily obtainable and an abundant source of stem cells. The data obtained in our studies, based on flow cytometry technique, show that cells cultured in vitro express high levels of ASCs markers (CD73, CD90, CD105, CD29) and low or no expression of HLA-DR, CD45, CD19, CD11, CD14. The variable level of expression was recorded for CD31 and CD34, what seems to be dependent on the medium composition. Our strategy of ASCs in vitro differentiation results in effective generation of adipocytes, chondrocytes and osteocytes (histological confirmation). The BMI (Body Mass Index) value proved to be the crucial factor influencing the ASCs capability of differentiation in various cell types (mainly adipocytes and chondrocytes). According to our observations, no significant differences in the surface markers level and differentiation potential were noted between the cells obtained from oncological patients or from plastic surgery procedures. Summarizing, our studies reveal that ASCs are a promising tool in tissue engineering. The preliminary data suggest, that adipose tissue from oncological patients may constitute a valid source of ASCs for reconstructive procedures. However, further studies are required to provide more insight into the therapeutic potential of ASCs as well as their clinical safety.

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P741 Hypothermic preservation of confluent human adipose stem cells

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Cell Sheet (CS) Engineering is based on the retrieval of hyperconfluent cultured cells as ECM-rich sheets that can be used therapeutically for the regeneration of several tissues. CS fabrication, while simple, requires cell culture facilities. Thus, ensuring adequate cell function from the fabrication site to bedside is essential to its efficiency. We tested the ability of two commercially available compounds to preserve the viability of confluent cultures of human adipose stem cells (hASC) at 4ºC. hASC were cultured to confluence in basal medium for 3 days to mimic CS fabrication conditions. Culture medium was then either replaced by a solution of Hypothermosol®(HTS) or supplemented with Rokepie®(RP) and cells were maintained at 4ºC for 3 and 7 days. Basal medium controls (BMC) at 4ºC and 37ºC were established together with non-confluent cultures mirroring all culture and storage conditions. At each time point, cell condition was assessed using 7-AAD, Annexin V and Alamar Blue. After 3 days of storage , a sharp decrease in cell viability in BMC at 4ºC was noticed, especially in non-confluent cultures. HTS and RP managed to keep cell viability values close to the BMC at 37ºC. At day 7 of storage, few viable cells were detected in BMC at 4ºC, while none were found in their non-confluent counterparts. HTS- and RP-supplemented CS presented 40% of the viability of the BMC at 37ºC, while non-confluent conditions presented much lower values. Summarizing, both HTS and RP demonstrated excellent hypothermic preservation of cells especially up to 3 days of 4ºC storage. Furthermore, confluence apparently confers protection against hypothermic insult. Surface marker characterization, differentiation potential and caspase activity after preservation are currently being assessed.

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P742 An ageing disease model from Progeria induced pluripotent stem cells identify an anti-ageing compound

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Induced pluripotent stem cells (iPSCs) represent today an invaluable tool to create disease cell models for modelling and drug screening. Several lines of iPSCs have been generated in the last 7 years that changed the paradigm for studying diseases and the discovery of new drugs to treat them.

In this work we focus our attention to vascular diseases in particular Hutchinson-Gilford Progeria syndrome (HGPS or Progeria), a devastating premature aging disease caused by a mutation in the lamin A gene. In general, patients die because of myocardial infarction or stroke at the age of 13. Therefore, in the last 5 years, researchers have used cells derived from iPSCs to model aspects of the HGPS and to screen libraries of chemicals to retard or treat the disease.

Smooth muscle cells (SMCs) are the most affected cells in HGPS patients, although the reason for such sensitivity remains poorly understood. We developed an *in vitro* mono-culture cell system to study the vulnerability of HGPS SMCs to arterial flow shear stress using a microfluidic device. HGPS SMCs derived from iPSCs presented similar characteristics observed on progerin-expressing cells and we could recapitulate *in vitro* the most important aspect of the disease, i.e., HGPS SMCs loss under flow shear stress. The results were also confirmed in SMCs collected from homozygous Lmna G609G/G609G mouse and cultured under arterial flow conditions. Microarray analysis comparing HGPS-SMCs cultured in static conditions and under slow conditions reveals that HGPS-SMCs have significant changes in extracellular matrix (ECM) secretion, specifically an enzyme. Moreover, HGPS-SMC detachment is prevented by the inhibition of this enzyme. Finally, Lmna G609G/G609G mice treated with an enzyme inhibitor for 5 weeks showed higher number of SMCs than non-treated animals. To the best of our knowledge, this is the first study documenting part of the mechanism underlining the sensitivity of HGPS SMCs to arterial flow shear stress.

Acknowledgments

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P743 Analysis of fiber diameter of electrospun collagen, gelatin, and PLLA/laminin nanofibers for the production of a bioengineered cornea

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INTRODUCTION. A leading cause of loss of vision is corneal damage. One possible solution is the tissue engineering of replacement corneas, but in order to tissue engineer, greater knowledge of corneal structure is necessary. The cornea is composed of an extracellular matrix of collagen nanofibers. These collagen nanofibers are highly aligned, with uniform fiber diameter. This structure provides support for corneal cells, and also provides an input signal to corneal cells. Changes to the structure of the matrix can affect cell phenotype. [1] We aim to optimize the phenotype of corneal keratocytes in vitro by varying the composition of the extracellular matrix.

METHODS. Three different compositions were tested: collagen, gelatin, and blended poly(L-lactic) acid/laminin, a synthetic polymer blended with laminin to increase cell attachment and viability. Nanofiber mats were created by electrospinning solutions of polymer dissolved in acetic acid (collagen, gelatin) or HFP (PLLA/laminin samples). Protocols were refined to obtain consistent fiber diameter between polymers and mat thickness viable for cell seeding.

RESULTS. A 7% w/v solution of collagen was spun to create reference mats, which were analyzed to obtain an average fiber diameter of 176.6 ± 54 nm. To maintain constant fiber diameter between polymers, multiple concentrations of gelatin and PLLA/laminin were tested until the differences were found to be statistically insignificant. An 8.5% gelatin solution gave an average of 186.1nm, and had no statistical difference from the collagen reference. Similar concentrations of PLLA/laminin samples gave variable fiber diameters in the 300-500 nm range. Samples will be prepared using lower concentrations to achieve the desired range.

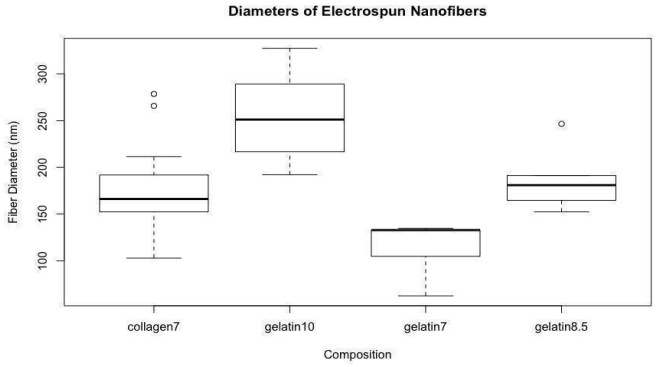

Fig. 1. Average nanofiber diameter from collagen and gelatin solutions of varying concentrations. Sample size n = 11, n = 8, n = 5, n= 6, respectively.

DISCUSSION. These studies will eliminate fiber diameter as a possible input signal. We will seed cells on these mats and analyze their response in order to determine which polymer is optimal for the eventual synthesis of a bioengineered cornea. This study will also answer fundamental questions of cellular response to nanoscale extracellular matrix features, which will inform tissue-engineering studies more broadly.

P744 Accelerating critical-sized bone defect repair using gene therapy: design of highly efficient gene-activated scaffolds for bone tissue regeneration

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INTRODUCTION: The incorporation of bioactive molecules into scaffolds for tissue engineering might enhance their therapeutic potential; however, the efficacy of such systems is dependent on a safe, effective platform for controlled and localised therapeutic delivery. Gene-activated scaffolds developed within our lab which allow for controlled, sustained release of reporter transgene *in vitro*[1,2] As osteogenic-angiogenic coupling is a crucial step in bone regeneration, the aim of this study was to mimic normal fracture repair by delivering osteogenic and angiogenic genes within a scaffold and evaluating the ability of the cell free gene-activated scaffold to accelerate repair of critical sized bone defects.

MATERIALS AND METHODS: Previously optimised chitosan nanoparticles (NPs) were used to deliver plasmid (p) DNA encoding bone morphogenetic protein-2 (pBMP-2), vascular endothelial growth factor (pVEGF) and the combination of pBMP-2+pVEGF, and protein expression was quantified using ELISA. Cellular response was measured *in vitro* by quantifying calcium deposition. An *in vivo* study was carried out to evaluate distribution, host cell infiltration and transfection efficiency/functionality with explants imaged using confocal microscopy 7 days post-implantation. Cell free gene-activated scaffolds were then implanted into 7 mm calvarial defects (n=8) in male Wistar rats and new bone formation by host cells was assessed using micro-computed tomography (µCT).

RESULTS: The chitosan NPs were capable of efficiently delivering therapeutic genes to mesenchymal stem cells (MSCs), up-regulating encoded growth factor expression; in turn significantly enhancing MSC-mediated osteogenesis when compared to untreated controls ($p<0.001$). The dual combination of pBMP-2+pVEGF induced MSCs to produce approximately 2000 µg of calcium per scaffold, significantly higher ($p<0.001$) than all other groups. Seven days post-implantation, endogenous cells had infiltrated throughout the scaffold as indicated by DAPI staining. GFP positive fluorescence indicated successful transfection of host cells *in vivo,* confirming the functionality of the gene-activated scaffold. Just 28 days post-implantation, the pBMP-2+pVEGF-activated scaffold induced a significant, and consistent, acceleration in bone tissue formation, significantly higher than the pBMP-2 gene-activated scaffold, gene-free scaffold and empty defect controls ($p<0.001$) as indicated by microCT (**Fig. 1**). Positive staining for CD31, a marker for endothelial cells, indicated new vessel formation – again most apparent in the pBMP-2+pVEGF-activated scaffold.

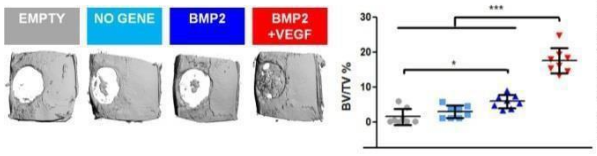

Figure 1: 7mm calvarial defects were introduced in male Wistar rats (n=8). After 28 days, microCT scanning revealed that new bone formation in the animals treated with the BMP-2+VEGF gene-activated scaffold was significantly higher than control groups ($p<0.001$) and the defects were fully bridged. Data plotted shows mean ± standard deviation. n=8 and $p<0.05$ is represented by *, $p<0.01$ by ** and $p<0.001$ by ***

Discussion: This study has highlighted the critical role of osteogenic and angiogenic coupling in new bone formation. Calcium production by MSCs seeded on pBMP-2+pVEGF gene-activated scaffolds *in vitro* is comparable to that seen following delivery of high doses of recombinant BMP-2 protein[3]. The gene-activated scaffold effectively delivered reporter genes to host cells *in vivo* without causing off target effects, indicating safety. Furthermore, the level of bone formation at the exceptionally early time-point of 28 days was comparable to that achieved following recombinant BMP-2 protein delivery on similar scaffolds in our lab after 8 weeks *in vivo*[3]. This cell-free gene-activated scaffold therefore represents a new 'off-the-shelf' product capable of accelerating bone repair in critical sized defects.

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P745 Smooth muscle tissue expansion for purposes within regenerative medicine

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Objectives:

We hypothesize that reconstructive surgical treatments could be facilitated if autologous tissues could be expanded *in vivo*. We have previously focused on autologous urothelial cell expansion for surgical treatment of severe malformations in the urogenital tract. The aim of this study was to further increase our knowledge in how to expand smooth muscle tissue for reconstructive purposes. We therefore studied minced detrusor micro-transplants in respect to proliferation, migration and reorganization, *in vitro* and *in vivo,* and, with or without minced urothelium in order to evaluate if detrusor muscle could expand in analogy with what we have found for urothelium.

Methods:

We used a porcine model to obtain minced smooth muscle and urothelium from the bladder. *In vitro* cultivation was performed with explant technique for minced detrusor and urothelium for morphological assessments, and the technique of plastic compression in collagen scaffolds for histological analyses. *In vitro* studies were analysed after 2-3 weeks. *In vivo* one-step procedure was performed by harvesting a bladder tissue sample, followed by mechanical preparation, mincing, and transplantation of autologous minced tissue in a 1:3 expansion rate on 3D Silastic tubes into the abdominal subcutaneous fat to cultivate for 4-5 weeks. Histological assessments included quantification of newly regenerated smooth muscle and urothelium. Transplants were randomly assigned to detrusor =5, detrusor and urothelium =6 and urothelium =7. Sham tubes =2, without minced tissue, were used as controls.

Results:

In vitro cultivation demonstrated cells of smooth muscle origin after 2 weeks for both explant technique, as well as in collagen scaffolds with minced tissue. Minced smooth detrusor muscle proliferated and reorganized around the tubular scaffold *in vivo*. Histology confirmed smooth muscle origin. Co-transplantation with minced urothelium *in vivo* demonstrated smooth muscle proliferation but no detectable urothelium. Shams without minced tissue showed no smooth muscle or urothelium around the tube.

Significance:

Minced smooth muscle cells from detrusor proliferated and reorganized *in vitro* and *in vivo*, with and without co-transplantation with minced urothelium. When co-transplantation with minced urothelium, smooth muscle tissue from the micro-transplants regenerated and formed continuous structures but urothelial cells disappeared in our model. The method is easy and fast to perform and further studies could lead to a method of tissue expansion *in vivo*, without the need of laborious cell expansion in a laboratory environment.

P747 Laser Microtomy for Histology and Histochemistry of Implants and Hard Tissue

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Histological analysis often is an important, yet laborious part of preclinical studies in regenerative medicine. The preparation of hard tissue samples or samples containing implants is especially challenging. Routine methods in histology like rotary or sledge microtomes are limited in the variety of samples they can cut. Samples with implants like stented vessels or undecalcified bone samples damage the blades quite fast. A lot of implants are too hard to cut and ground sections must be prepared as an alternative. Unfortunately, their quality varies, due to high section thickness and uneven laboratory skills. Other limitations include low throughput and kerf/grinding specimen loss.

Laser microtomy is a novel method of preparing thin histology sections. Plastic embedded thin sections of high quality can be generated for non-decalcified hard tissue or implanted tissue (e.g. stented vessels). Near serial sectioning at 10 µm is possible. We are showing sections of stented vessels (swine coronaries) and bones (e.g. sheep spine, rat and mouse femur and tibia) and others including polymer implants. Metal are not cut by the laser. However, the laser cuts the surrounding tissue and the interface is well preserved while the metal leaves a clear imprint. One critical benefit is that the great material loss associated with ground sectioning (generally 1-5mm) is virtually eliminated. Another advantage is a big time saving in section preparation. Thereby, laser microtomy can work economically and very efficiently producing section of equal or greater quality.

One important aspect of establishing the new method is to integrate it into the routine processes of an existing lab. Common stains like Hematoxilin and Eosin (H&E), Masson Goldner Trichrome (MG), Levai Laczko, McNeal or Verhoeffs Elastica show the expected results for each stain, comparable to sections prepared with a microtome. The results show tissue architecture and cellular details clearly.

Special detection of enzyme activity inside bone samples like Tartrate-Resistant Acid Phosphatase (TRAP) for osteoclast activity was tested. The enzymatic activity in osteoclasts could be demonstrated by TRAP staining after laser microtomy, implicating that the laser cutting did not destroy enzyme function.

For the first time, we show that immunohistochemistry staining can be performed for common antigens as Col-I, CD31 or SMA. The results are very encouraging and indicate that thin sections of undecalcified hard tissue and implanted tissue prepared by laser microtomy may be used for investigative histopathology through immunohistochemistry. This contribution shows that laser microtomy opens a new range of possibilities in histological analysis of hard tissues and implant evaluation.

P748 Cell manufacturing with an aseptic transfer interface to be standardized

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We have developed an aseptic transfer interface to be used for manufacturing regenerative medicines, utilizing a new design concept of flexible modular platform (fMP). Regenerative medicines cannot be terminally sterilized, therefore require a high level of aseptic manufacturing environment. Regenerative medicines also need to be manufactured in a smaller batch manufacturing process than conventional pharmaceutical products. The cell culture process could take a long period of several months depending on the cell type, and the manufacturing system consists of various automatic and manual devices. The fMP is a solution to ensure substantial reduction in manufacturing cost by efficiently utilizing these manufacturing devices. The fMP integrates isolator-based various automatic and manual cell-processing manufacturing devices as a manufacturing module group and connects the portable module group to the aseptic transfer interface, allowing flexible and efficient cell manufacturing. In the research and development (R&D)of fMP and aseptic transfer interface, mainly automatic operating module (cell culture and media replacement), incubator module, material introduction module, transfer pod and manual operation module are designed. By using an aseptic transfer interface, it has been demonstrated that each manufacturing module is universally and aseptically removable and the manufacturing module group is efficiently operational. Standardization of the aseptic transfer interface is progressing under the leadership of Japan. The R&D has been conducted with support from the Japanese Ministry of Economy, Trade and Industry, and the Japan Agency for Medical Research and Development.

P749 Biodegradable elastomeric scaffolds of poly(glycerol sebacate): key synthesis issues

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Poly(glycerol sebacate), PGS, is an elastomeric biodegradable polyester increasingly proposed in a variety of biomedical applications, such as cardiac, vascular, cartilage or neural tissue engineering, or for the repair of perforations in the tympanic membrane. It is prepared by polycondensation of sebacic acid and glycerol, which yields a viscous prepolymer still easy to handle, followed by its curing to crosslink it at variable density.

Herein, synthesis parameters such as the curing temperature and time, and the molar ratio between reactants were systematically modified to correlate them with the physicochemical, mechanical and biological properties of the resulting polymer networks. In each case, the efficiency of the manufacturing process was quantified through the relative mass effectively crosslinked, determined by its insolubility in tetrahydrofuran. These results evidence the need of rinsing PGS in an affine solvent following synthesis, to remove non-crosslinked chains that would otherwise easily diffuse to the surrounding medium. Swelling in water allowed calculating the crosslinking density through Flory-Rehner equation. Surface degradation in water was followed by mass loss.

Equimolar mixtures of reactants cured at 130ºC or higher, for 48 h or longer, yield hydrophobic surface-degradable rubbers with suitable features for their use as scaffolds in tissue engineering applications, without significant differences among them. However, milder conditions gave poorly crosslinked swellable networks, which hydrolyze easily in bulk mode. On the other hand, an increase of hydroxyl groups in the reactive mixture led to sticky and difficult to handle materials, whereas an excess of carboxyl groups led to a fast mass loss by hydrolysis intensified at high pH.

This prepolymer, dissolved in tetrahydrofuran at a ratio of 70% w/v, could be successfully cured in porogen templates made out of sintered sodium chloride particles having 212 - 250 microns in diameter, at a prepolymer/porogen mass rate of 1/3, with no need of vacuum and at 130ºC during 48 h. Next, samples were rinsed in water to dissolve salt particles followed by the protocol previously set up to eliminate non-crosslinked chains. The scaffolds showed appropriate porosity and pores interconnectivity and biological performance when cultured with mouse fibroblasts.

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P750 PLA/PCL electrospun membranes of different fibres diameter as culture supports and delivery of albumin thereof

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Polymeric nanofibre membranes of polylactic acid (PLA), polycaprolactone (PCL) and a 50:50 wt mixture of both were manufactured by electrospinning. The objective of the work was, on the one hand, to find out the electrospinning conditions, that allow obtaining nanofibres of different diameter, which can meet different requirements in terms of mechanical properties when anchoring the membranes to culture devices, degradation and loss of properties with time in aqueous media, biological development as culture supports, and delivery of molecules entrapped within. On the other hand, once membranes with nanofibres of satisfactorily dissimilar diameters were obtained, the electrospun solutions were analysed, as well as the loading and delivery of bovine serum albumin from the membranes, and the results were correlated with the diameters obtained. After a thorough study to acknowledge how the electrospinning conditions influenced the fibres diameters of these polyesters, membranes consisting in nanofibres of 0.8 or 1.8 μm in diameter (fibre d.) were obtained, using chloroform-methanol as solvents, a distance to collector of 13 cm, a voltage of 20-25 kV, with different conditions for the solvent ratio (solvent r.) and polymer concentration (polymer c.), as shown in the table below.

	PLA		PLA/PCL 50:50 wt		PCL	
Fibre d.	Solvent r.	Polymer c.	Solvent r.	Polymer c.	Solvent r.	Polymer c.
0.8 μm	2:1	8%wt	70:30	8%wt	70:30	10%wt
1.8 μm	2:1	15%wt	80:20	10%wt	2:1	15%wt

Table 1. Chloroform-methanol weight ratio in the solutions and polymer concentration leading to sub-micron and over-micron fibres.

The viscosity of the solutions was also determined and found to be three times higher in those leading to thicker fibres. The density of the electrospun solutions did not appear to be a relevant parameter in obtaining different diameters. However, their surface tension, measured following Tate's law, did have a relevant influence on it. Particularly, surface tensions below 160 mN/m yielded under-micron fibre diameters, whereas those around 225 mN/m yielded diameters in the vicinity of 2 microns.

Bovine serum albumin (BSA) was used as a model protein in the preliminary drug release assay. It was successfully loaded in the membranes up to 12%wt with respect to the polymer weight in the solution, and its presence did not seem to vary the morphology of the membranes significantly. The release of this protein from the membranes was followed by their immersion in PBS solution for up to 21 days. The concentration of BSA was determined by using a BCA protein assay kit and ultraviolet spectrophotometry, and with the help of a calibration curve. After 10 days, the percentage of BSA released from the electrospun membranes was similar to that from analogous films. However, the pattern release in short times differed according to the fibres diameter, being much slower from the thick fibres.

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P751 MicroRNA-mediated chondrogenic differentiation of mesenchymal stem cells

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Introduction: MicroRNAs (miRNAs) negatively regulate gene expression through base-specific interactions. Although miRNAs are important in differentiation, little is known about their roles in chondrogenic differentiation. Therefore, the aim of this study was to identify miRNAs involved in chondrogenesis of human mesenchymal stromal (stem) cells (MSCs).

Materials & Methods: A library of 2048 miRNA mimics (Dharmacon) was transfected in GMP-compliant human bone marrow MSCs (3 donors) and assessed for early chondrogenesis in a high-throughput system. Six miRNA mimics that stimulated early chondrogenesis were transfected in MSCs that were cultured in pellets for four weeks. Hereafter, the pellets were analyzed for their collagen and glycosaminoglycan (GAG) content by biochemical assays and histology. Real time PCR was used to measure expression levels of chondrogenic and hypertrophic genes. In addition, alkaline phosphatase (ALP) activity was measured and potential gene targets of the miRNAs were identified using mirsystem.

Results: 22 miRNA mimics stimulated early chondrogenic differentiation. MiRNA mimics for hsa-miR-15b-3p, 138-5p, 139-3p, 432-5p, 520a-3p, and 520h increased GAG and total collagen content and type II collagen deposition in pellet cultures. No differences were found in aggrecan gene expression, overexpression of miR-15b-3p, 139-3p, 432-5p and 520h increased type II collagen gene expression levels. Increased ALP activity and type X collagen gene expression was found for hsa-miR-139-3p and 520a-3p. The miRNAs that stimulate chondrogenesis had 12 gene targets in common, mainly genes involved in the regulation of transcription. Gene ontology analysis of targets also identified pathways involved in neurotrophin signaling, osteoblast differentiation and cell morphogenesis.

Conclusion: Overexpression of several specific miRNAs can stimulate chondrogenic differentiation of MSCs and it depends on the miRNA whether hypertrophic differentiation is stimulated as well. This is very promising for cartilage repair by progenitor cells as chondrogenesis and cartilage production can be induced without terminal hypertrophic differentiation.

P759 Characterisation of monocyte-laden hydrogel based on hyaluronic acid and gelatin

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Hydrogels as a material for tissue engineering and regenerative medicine have received increasing attention due to their versatility and capability to closely mimic an extracellular matrix (ECM). This work is focused on a hydrogel material based on a combination of hyaluronan (HA) and gelatin (GTN). It is well known that HA and gelatin GTN are promising materials for the purpose of tissue engineering. HA is a naturally occurring glycosaminoglycan and it is one of the major components of extracellular matrix and connective tissues. GTN is a natural polymer prepared by a partial hydrolysis of collagen, the chief component of the connective tissues. Both compounds can play an important role in the several applications of tissue engineering and regenerative medicine, thanks to their biodegradability and biocompatibility.

The aim of this work was a development of hydrogels based on their combination for incorporation of monocytes (THP-1). The hydrogel should provide a suitable environment for their successful cultivation and could serve as a cell laden material for immunomodulation. Phenolic groups were introduced into the structures of both polymers. Thanks to this modification, the polymers could be mutually cross-linked by a radical reaction initiated by H_2O_2 and mediated by horseradish peroxidase (HRP). The system is suitable for encapsulation of many types of cells due to the mild conditions of performed cross-linking reaction. In this study, the biocompatible hydrogel matrices served for monocyte cells incorporation. The work was focused on the preparation of hydrogels scaffold with incorporated THP-1 cells. The mechanical and rheological properties of the used hydrogel system was tested in order to standardize the material. In consequence, it was studied the influence of the presence, proliferation and differentiation of incorporated cells on the hydrogel properties during a cultivation. IL-4 was chosen to enhance a monocyte differentiation into M2 macrophages.

The activity of the incorporated cells and its influence on used biomimetic hydrogel served as in vitro stability of the developed material.

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P760 Extracellular matrix hydrogel derived from decellularised intestinal tissue for the 3D-culture of intestinal stem cells for tissue engineering applications

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Introduction:

Patients suffering from irreversible intestinal failure often require an intestinal transplant. This is unfortunately limited by poor survival (65% 1-year survival) and a scarcity of donor organs. Intestinal tissue engineering is one potential solution to this problem, whereby an intestine is harvested from a donor, decellularised, and recellularised with the patient's autologous intestinal stem cells. These cells are isolated from intestinal crypts and require 3D cell expansion in a commercially available hydrogel, Matrigel. However, since Matrigel is not approved for clinical use, this represents a major limitation in an otherwise extremely promising treatment option. This study aims to characterise and optimise an extracellular matrix hydrogel derived from decellularised intestinal tissue for use in intestinal tissue engineering applications. Due to its origin, the gel already possesses the extracellular matrix (ECM) components of the native tissue thus providing a familiar natural environment to the intestinal cells, and providing appropriate cues for cell behaviour and growth.

Methods:

Decellularisation and gelation protocols were developed specifically for newborn porcine intestinal tissue. Decellularisation was characterised using scanning electron microscopy (SEM), histology, immunohistochemistry and quantification of collagen, elastin, glycosaminoglycans and DNA content. The ECM gel was characterised with rheology, spectrophotometry for turbidity, chick chorioallantoic membrane (CAM) assay for angiogenic potential and immunogenicity, SEM, atomic force microscopy and proteomics. Cytocompatibility of intestinal stem cells in the gel was assessed with a CellTitre-Glo assay SEM, daily imaging andimmunofluorescence.

Results:

An ECM hydrogel was successfully produced from decellularised intestinal tissue. Rheology and turbidity experiments showed a gelation time of approximately 20 minutes, and proteomics showed a collagen-rich matrix with a plethora of other components including fibronectin, laminin myosin and fibrillin. Intact collagen fibres were evident from SEM results. Angiogenic potential on the CAM was statistically similar to the Matrigel control. Cytocompatibility results showed comparable cell growth and phenotype to Matrigel as confirmed withimmunofluorescence.

Conclusion:

A non-immunogenic, cytocompatible and stable hydrogel derived from decellularised intestine tissue, represents a promising tool for use in a plethora of applications within the field of intestinal tissue engineering, primarily for the 3D culture of intestinalorganoids.

P761 A UV crosslinked IPN hydrogel loaded with ADSC to promote vascularization for tissue engineered skin

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Tissue engineered skin grafts have already achieved successful translation into the clinic with reasonable efficiency. The current challenge is related to their take and survival on wound beds after implantation which depends heavily on the rapid ingrowth of blood vessels from the underlying wound bed. In this study we describe a gelatin/hyaluronic acid based UV crosslinkable interpenetrating network (IPN) hydrogel that allows effective attachment and proliferation of stem cells and that can stimulate new blood vessel formation. This biodegradable hydrogel was designed to act as a dermal substitute both to improve the take of tissue engineered skin substitute and also to serve as the dermal layer of a potential tissue engineered bilayerskin.

The synthesis of IPN hydrogel is shown in Figure 1. The IPN hydrogel provided a suitable microenvironment for adipose derived mesenchymal stem cell (ADMSC) proliferation as shown by the filopodia observed in confocal micrographs and SEM (Figure 2). Cells were readily incorporated into the hydrogels which were robust to handle and stable (degraded only 50% over 3 weeks *in situ)*. *Ex ovo* studies using chick embryos demonstrated that stem cell loaded hydrogels increase vascularization by up to 3 fold compared to their cell free counterparts. Also there was a dose related increase in the length of endothelial cell sprouts in the chick aortic ring assay when co-cultured with the stem cell loaded hydrogels (Figure 3).

It is concluded that the GelMA/HAMA IPN hydrogels can be easily assembled in the laboratory to contain patients's own mesenchymal stem cells and effectively support the growth and survival of MSCs which could then stimulate angiogenesis. Such a construct would be valuable in stimulating blood vessel formation in poorly vascularized chronic wounds, which is a growing burden for patients and health care providers worldwide.

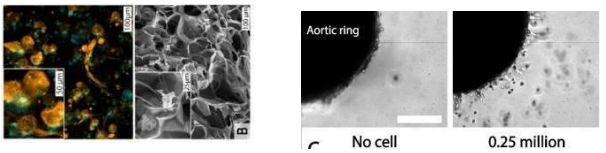

Figure 2. Confocal and SEM micrographs of ADMSC loaded hydrogels. Orange:

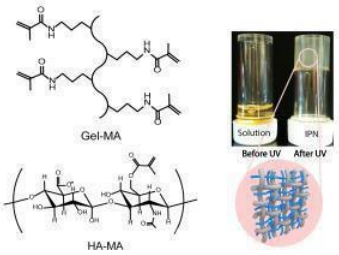

Figure 1. Synthesis and crosslinking of GelMA/HAMA IPN hydrogels.

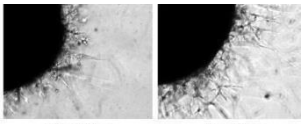

Figure 3. ADMSC loaded hydrogels inducing endothelial cell sprouts.

Acknowledgements: We would like to acknowledge the Scientific and Technical Research Council of Turkey for scholarship (BIDEB 2211/C and 2214/A) and BIOMATEN for the facilities and support.

P763 Hydrogel complex containing bioactive molecules for regenerative repair of locally ionizing radiation damaged tissue

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Polysaccharide microparticle-pluronic F127 hydrogel complex containing bio-active molecules(PM-PF), such as substance P and transforming growth factor-β1, was prepared for regenerative repair of local ionizing radiation (IR) damaged skin. The morphologies of microparticles were observed by scanning electron microscope, and quantification and release of bio-active molecules of the prepared PM-PF hydrogel complex were measured. The BALB/c/bkl mice were locally irradiated to their limbs with a single 40 Gy dose of Co-60 gamma rays to induce a skin injury. Hematoxylin and eosin staining of IR damaged skin showed acanthosis and hyperkeratosis in epidermis, and damage of hair follicle/skin appendages, adipose, panniculus carnosus in dermis. When the PM-PF hydrogel complex was injected in IR damaged skin, IR damaged skin was repaired. Thus prepared PM-PF hydrogel complex has great potential for use in regenerative repair of IR damaged skin.

P764 Evaluating the effect of matrix stiffness on corneal fibroblast phenotype in the development of a tissue-engineered cornea

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Corneal damage is one of the leading causes of vision loss worldwide. There is currently a shortage of donated corneas to combat the demand for corneal transplants. This deficiency is exacerbated by the increasing popularity of Lasik procedures which leave corneas unsuitable for transplant. Thus, the development of a tissue-engineered cornea is of great interest to resolve the donor shortage. The cornea's refractive properties are contingent on its transparency which is associated with the quiescent state of corneal fibroblasts. In corneal fibroblasts' quiescent state, the light scattering protein alpha smooth-muscle actin (α-SMA) is down-regulated while the marker proteins aldehyde dehydrogenase class 1A1(ALDH1A1) and transkelotase (TKT) are expressed.[1] In previous studies, matrix stiffness has been shown to alter the differentiation of stem cells leading to divergent cell lineage[2]. This leads us to hypothesize that corneal fibroblasts can be influenced into their quiescent state by modifying matrix stiffness. This study focuses on quantifying the effect of matrix stiffness on the regulation and expression of α-SMA and ALDH1A1 in both 2D and 3D environments. Cell studies in 2D were carried out for 7 days on collagen- coated polyacrylamide gels with stiffness of 0.2 kPa, 2 kPa, 10.6 kPa, 19.9 kPa, and 34.88 kPa. Western blotting was used to quantify protein expression of α-SMA and ALDH1A1; however, no discernable pattern was observed between stiffness and α-SMA or ALDH1A1 expression in the 2D collagen environment. In order to conduct 3D studies, a repeatable method of producing crosslinked collagen gels of varied stiffness was established. By soaking 0.5% collagen gels in solutions of 0.6%, 0.06%, and 0.006% glutaraldahyde and performing compression tests on each, we are able to produce collagen gels with varied stiffness (Figure 1). Corneal fibroblast cells will be cultured on these 3D collagen matrices and evaluated for expression of TKT, ALDH1A1 and α-SMA. These studies represent an important step toward creating viable tissue-engineered corneas.

Percent Glutaraldehyde	Stiffness (kPa)
0.5% Collagen	
0%	7.2 ± 0.28
0.006 %	29.5 ± 5.2
0.06 %	31.4 ± 8.9
0.6 %	37.6 ± 24.9

Figure 1: Stiffness results from compression testing of crosslinked collagen gels.

P765 Bioadhesive gelatin hydrogel formed by dual enzymatic cross-linking to assist in situ tissue regeneration

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Introduction: Hydrogels are receiving more attention in regenerative medicine applications due to their structural similarity to biological tissue, such as high water content and hydrodynamic properties. For clinical applications, hydrogels should meet such requirements, including facile preparation, robust mechanical properties, excellent biocompatibility and good adhesive strength to surrounding tissue. Here, we present a facile approach to prepare an injectable gelatin hydrogel with enhanced adhesiveness as cell/drug carrier for regenerative medicine, using dual enzymatic cross-linking of horseradish peroxidase (HRP) and tyrosinase (Tyr) (Fig.1).

Methods: Gelatin-hydroxyphenyl propionic acid (GH) polymer was synthesized as previously described[1]. Gelation time of hydrogels was determined using a vial tilting method by varying concentrations of HRP (0.65-2.61 U/mL) and Tyr (0.25–5 kU/mL). The mechanical properties were measured using a rheometer. Tissue adhesive strength of hydrogels was measured by modified ASTM F2255-05 method, using porcine skins as the substrate materials and commercial fibrin glue as the control bioadhesive. For biodegradability test, hydrogels were incubated in PBS solution containing collagenase. Cell viability was evaluated by 3D culture of human dermal fibroblasts (hDFBs).

Results and Conclusions: The GH hydrogels were formed rapidly and their properties (gelation time, mechanical strength) could be controlled by varying HRP and Tyr concentrations. Importantly, the highest tissue adhesive strength of 34 kPa was achieved for dual-enzymatically cross-linked hydrogels, which was superior to single HRP-cross-linked hydrogels (19 kPa) and fibrin glue (7 kPa). Additionally, all hydrogels were completely degraded when treated with collagenase. After 3 days culture, the encapsulated hDFBs were almost viable within the gel matrices, indicating excellent cyto-compatibility. From these results, we expect that dual-enzymatically cross-linked gelatin hydrogels with strong adhesiveness are potential as injectable platforms to deliver cells/drugs for tissue regeneration.

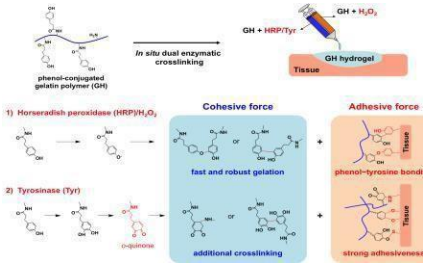

Fig.1. GH hydrogels formed by dual

Development Program) of MOTIE/KEIT (10062079, Development of tissue adhesive/healing biomaterials with adhesion strength over 150 kPa).

P766 Lysyl-oxidase-like 2 (LOXL2)- mediated biomimetic cross-linking of collagen and elastin hydrogels for improved vascularisation of tissue constructs

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A major challenge in tissue engineering is the promotion of rapid vascularization for survival of engrafted cells in order to repair and regenerate injured tissues. One of the strategies proposed consists in *in vitro* pre-vascularizing the tissue constructs, before implantation. Early blood perfusion of the engineered tissue thus relies on inosculation of the host vasculature with the network generated *in vitro*. In this context, there is growing interest for hydrogels resembling native ECM for productive cell encapsulation, even though such biomaterials suffer from poor handling properties. The aim of our study thus focused on the synthesis of enzymatically cross- linked hydrogels that would combine good handling properties (approx. 1kPa) with efficient capillary formation and cell invasion. Most of the chemical cross-linkers are not compatible with cell encapsulation as they raise toxicity issues or result in too extensive cross-linking that limits cell invasion. Here, we chose a biomimetic approach based on the use of lysyl-oxidase-like 2 (LOXL2) for preparation of type I collagen or elastin cross-linked matrices. LOXL2 is a copper- dependent secreted enzyme that belongs to the LOX family of enzymes responsible for the physiologic cross-linking of collagens and elastin.

Using recombinant human LOXL2 purified in our laboratory from the secretion medium of eukaryotic cells, we found that LOXL2 deaminates lysines of type I collagen hydrogels and of coacervated tropoelastin. This results in cross-linking and increased stiffness of these hydrogels, as measured by oscillatory rheology and atomic force microscopy (AFM), as well as increased resistance to enzyme digestion. We could thus synthesize low concentration collagen gels (2mg/mL) with elastic modulus above 1kPa.

We further investigated the impact of collagen cross-linking on angiogenesis by culturing endothelial cells under conditions that allow formation of capillaries. Pore size in collagen hydrogels was first assessed using second harmonic generation in two-photon microscopy. Whereas increasing hydrogel stiffness by modulation of pH increased stiffness but decreased pore size, LOXL2-mediated cross-linking of hydrogel increased the size of the pores. The related increase in stiffness together with pore size resulted in increased capillary formation by HUVEC.

In conclusion, we are able to synthesize elastin and collagen hydrogels biomimetically cross-linked by LOXL2. Tuning stiffness and pore size of collagen hydrogels reveals features that make them *ad hoc* biomaterials for tissue engineering applications as they are not only easier to handle but also have physical properties that promote vascularization.

P767 ECM from decellularised tissues as an additive for polysaccharidic hybrid gels

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The use of decellularised tissues represents a valid and emerging alternative over traditional synthetic scaffolds, which have limited ability to mimic the sophisticated tissue specificity1. Within the tissue engineering context, gels composed by decellularised tissues have been produced through enzymatic digestion followed by basic pH treatment2. Nevertheless, low viscosity, stability and reproducibility often limit their applicative potential. Herein, ECM, obtained from porcine blood vessels, was imbedded within alginate gels and compared to both alginate and alginate/gelatin gels aiming to process decellularised tissues in diverse physical forms and therefore broaden their application.

Porcine blood vessels were decellularised1 and gels were further obtained adapting the procedure previously described2. Gels containing ECM or gelatin (8 mg/ml) and different concentrations of alginate (2-20 mg/ml) were produced by internal gelation ($CaCO_3$ 2,8% w/v, D- (+)-gluconic acid δ-lactone 0,5% w/v). The alginate samples were obtained preserving the final polymer concentration (10,13,18, 28 mg/ml). Rheological characterization was performed by time, frequency and temperature sweep analyses3. Stability tests were conducted using cell culture medium (complete DMEM medium) from 3 hours up to 7 days. Additionally, preliminary biological characterization was assessed through DNA content after seeding EA.hy 926 for 1 day.

ECM-loaded alginate gels (AlgECM) samples were successfully obtained for all the concentration tested. All the samples could be removed from a mould while retaining the shape. The storage and loss moduli of all the tested alginate concentrations were frequency-independent, with the storage modulus higher than the loss modulus, therefore exhibiting gel behaviour. Higher final polymer concentration resulted in gels with higher complex viscosity. Overall, AlgECM samples showed higher values of both storage and loss moduli and higher stability in the medium comparing with unloaded alginate gels. Samples obtained with gelatin could not be produced at polymer concentrations lower than 18 mg/ml. The AlgECM samples remained stable in cell culture medium; samples with the lowest concentration of alginate (2 mg/ml of alginate and 8 mg/ml w/v of ECM) degraded after 7 days. A first biological characterization indicated an increased number of cells for AlgECM gels compared to alginate and alginate/gelatin samples.

A novel gel composed of alginate and native vascular decellularised ECM is here proposed. AlgECM gels able to combine the properties of its components. Alginate improved ECM gels reproducibility and allowed the tailoring of gels rheological properties through the variation of alginate concentration. The use of ECM should promote the creation of a tissue-specific material, able to enhance cell growth and proliferation. However, a wider biological characterization should be conducted to test the ECM influence.

P768 Hydrogel impregnation of bone chips allows prolonged cefazolin release Guy Putzeys[2], Christiane Duportail[1], Kathleen Croes[2], Michaël Boudewijns[2], Lieven Thorrez[1] [1]*KU Leuven, Kortrijk, Belgium,* [2]*AZ Groeninge, Kortrijk, Belgium*

Introduction

To prevent infections after orthopedic surgery, intravenous antibiotics are administered perioperatively. Cefazolin is widely used as the prophylactic antibiotic of choice. Systemic antibiotic therapy may however be less effective in longstanding surgery where bone allografts are used. Bone chips have been shown to be an effective carrier for certain types of antibiotics. Bone allografts impregnated with antibiotics may therefore provide the necessary local antibiotic levels for prophylaxis. To be efficient, a prolonged release from these bonechips is required. In contrast to vancomycin, for which prolonged release has clearly been proven effective from Osteomycin®, a commercially available impregnated bone allograft, no prolonged release bone chip preparations have been described so far for cefazolin. We developed a protocol to bind cefazolin in the porous structure of bone chips by means of a hydrogel composed of proteins naturally present in the human body.

Material and methods

Three types of bone chips were evaluated: fresh frozen, decellularized frozen and decellularized lyophilized. Bone chips were incubated with 20 mg/ml cefazolin or treated with liquid hydrogel containing either 1 mg/ml fibrin or 1 mg/ml collagen and 20 mg/ml cefazolin. The cefazolin hydrogel was distributed in the porous structure by short vacuum treatment. Bone chips with cefazolin but without hydrogel were either incubated for 20 min- 4h or also treated with vacuum. Cefazolin elution of bone chips was carried out in fetal bovine serum and analyzed by Ultra Performance Liquid Chromatography – Diode Array Detection.

Results

Soaking of bone chips without hydrogel resulted in a quick release of cefazolin, which was limited to 4 hours. When vacuum was applied elution of >1 µg/ml cefazolin was measured for up to 36 hours. Combination with collagen hydrogel resulted in a higher cefazolin concentration released at 24 hours (3.9 vs 0.3 µg/ml), but not in a prolonged release. However, combination of decellularized frozen bone chips with fibrin hydrogel resulted in an initial release of 533 µg/ml followed by a gradual decline reaching the minimal inhibitory concentration for S. aureus at 72 hours (1.7 µg/ml), while not measurable anymore after 92 hours.

Discussion

Processed bone chips with hydrogel-cefazolin showed a markedly prolonged cefazolin release. When combined with a fibrin hydrogel, high initial peak levels of cefazolin were obtained, followed by a decreasing release over the following three days. This elution profile is desirable, since high initial levels are important to maximize anti-bacterial action whereas low levels of antibiotic for a limited time may stimulate osteogenesis. It is important that antibiotic release is ending after a few days as prolonged low levels of antibiotics are not clinically helpful and may lead to antibiotic resistance. Further preclinical studies are warranted to show effectiveness of hydrogel-cefazolin impregnated bone chips.

P769 Development of photo-crosslinkable gelatin and κ-carrageenan for adipose tissue engineering

Liesbeth Tytgat[1,2], Hugo Thienpont[2], Heidi Ottevaere[2], Peter Dubruel[1], Sandra Van Vlierberghe[1,2]

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Introduction

The development of injectable hydrogel carriers for adipose tissue regeneration has recently gained increasing attention as a result of the exponential growth of lipofilling procedures performed in the clinic[1]. In addition to cosmetic considerations, adipose tissue reconstruction is also attempted for patients suffering from congenital defects, trauma or surgical resections including woman undergoing lumpectomies after breast cancer treatment. In the present work, photo-crosslinkable gelatin and κ-carrageenan hydrogel building blocks were developed for subsequent processing via two-photon polymerization (2PP) forming injectable scaffolds.

Materials and methods

The amine groups present in gelatin B and the hydroxyl functionalities of κ-carrageenan were modified using methacrylic anhydride to obtain methacrylamide-modified gelatin (GEL-MOD) and methacrylated κ-carrageenan (CAR-MOD), respectively. The degree of substitution (DS) of GEL-MOD was 97% while the DS of CAR-MOD was 15%. The materials developed were characterized in depth via several techniques including (HR-MAS) ^{1}H-NMR spectroscopy, infrared spectroscopy, atomic force microscopy, swelling and gel fraction experiments and rheology.

Results and discussion

In a first part, the occurrence of phase separation between GEL-MOD and CAR-MOD was studied via atomic force microscopy. Interestingly, it seems that no phase separation occurs between both materials, which is an important characteristic towards 3D printing of scaffolds with uniform mechanical properties. The mechanical and swelling properties of the hydrogel were influenced by various parameters including the hydrogel composition and concentration. Furthermore, the results of the gel fraction experiments indicated an efficient crosslinking during which most methacrylamide and methacrylate moieties were consumed resulting in gel fractions exceeding 90%. Preliminary results of the ongoing *in vitro* cell assays with adipose-derived stem cells to evaluate the hydrogel biocompatibility are promising. The results will be presented at the meeting.

Conclusion

Gelatin- and κ-carrageenan-based hydrogels were successfully developed and characterized. The gel fraction, swelling and mechanical properties of the developed hydrogels can be influenced by varying both the hydrogel composition and its concentration. Future perspectives include 3D printing of the gelatin- and k-carrageenan-based scaffolds via 2PP.

Acknowledgement

The authors would like to thank the Research Foundation Flanders.

(P770)

P770 ECM-mimicking biomembrane as a directional delivery system to control biological events in cell laden hydrogel

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Once a material is implanted into the body, adverse immune reaction can be triggered leading to inflammation and even implant failure. In order to promote tissue integration and maturation after implantation, two main parameters must be considered: interactions of the materials with i) the host immune system and with ii) the host vasculature. Delivery of growth factors is an indispensable part of tissue engineering to modulate immune response after implantation or to promote angiogenesis. To deliver the bioactive molecules in a controlled manner (spatial, temporal), they must be first entrapped in a release platform. To elaborate an effective release platform, specific parameters such as the degradation rate of the biomaterial or the physical and/or chemical interactions between the biomaterial and the molecules. In order to retain and protect growth factors/cytokines in the material, ECM components such as collagen, gelatin or hyaluronic acid are good candidates[1].

Here, we describe a detachable membrane based release system composed of ECM components

(Gelatin and HA derivative) that can be attached to cell-laden hydrogels to achieve directional release of bioactive molecules. This way, the release of cytokines/growth factors can be started at a desired point of tissue maturation or directly in vivo. This ECM- mimicking membrane is prepared by spin-coating process and by using HA-tyramine we can obtain an interpenetrating network of gelatin and HA double crosslinked. Gelatin is crosslinked with microbial transglutaminase and HA-tyramine is crosslinked through the dimerization of tyramine to dityramine with horseradish peroxidase mediated reaction. This double crosslinked interpenetrating network improve drastically the stability of the membrane and the mechanical properties compare to gelatin membrane only crosslinked with transglutaminase.

As a proof a concept, two models were developed. First this release platform were used to load and release VEGF in a cell laden hydrogel with endothelial cells for angiogenesis purposes. The release of VEGF resulted in extensive sprouting. Secondly, the system was utilized for immunodulation purposes in order to direct macrophage to M2 phenotype (anti-inflammatory and pro-healing). For this, IL-4 was loaded and released in a cell-laden hydrogel with THP-1 cells. The release of IL-4 resulted in THP-1 cluster formation in the hydrogel and downregulation of pro-inflammatory markers. For all these experiments, immunostainings were performed and characterized with confocal microscope, cytokines were quantified in the supernatant and gene expression was checked RT-PCR.

These results have shown that this ECM-mimicking can act as a reservoir for bioactive molecules. This modular release system can be used with 3D environment to control tissue maturation and growth after implantation and this can significantly improve the level of control over cell phenotype for tissue engineering applications.

P771 Establishing the crosstalk between macrophages and fibroblasts or endothelial cells in 3D hydrogel microenvironment for tissue engineering applications

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Resident macrophages play an important role in the maintenance of homeostasis and in wound healing following tissue damage. Macrophages can orchestrate the immune response via secretion of cytokines and growth factors and also control the behaviour of other cell types that are active in wound healing such as fibroblasts and vascular endothelial cells. In light of this, we hypothesized that the incorporation of macrophages in tissue engineering; such as in the case of cell-laden hydrogels for connective tissue replacement would have a beneficial effect on the maturation and the organisation of the tissue. In order to test this hypothesis, activated THP-1 macrophages were co-encapsulated with human fibroblasts and vascular endothelial cells in soft gelatin hydrogel and their 3D organization, viability and cytokine secretion profiles were quantified with SEM/Confocal microscopy, Alamar Blue and image analyses and ELISA tests respectively.

The encapsulated macrophages form clusters in combination with the other cell types present in 3D environment; which was not observed when only macrophages are encapsulated. The presence of the macrophages has a boosting effect on the metabolic activity within the system. In co-encapsulation condition with fibroblasts, the presence of macrophages resulted in a downregulation of TGF-beta and upregulation of Activin A. The effects can be further controlled by direct incorporation of pro- or anti-inflammatory cytokines such as IL-4. In the presence of endothelial cells; macrophages induced downregulation of PDGF; the levels of IL-6 are consistently increased in the absence of macrophages whose presence over time downregulated the expression of this pro-inflammatory cytokine.

In conclusion, the presence of macrophages in the microenvironment of an engineered tissue has substantial effects on the behaviour of the co-encapsulated cells and particularly in the cytokine microenvironment. This can be used to facilitate the maturation of the engineered tissue and having immune-competent artificial tissues that can cross-talk with the host immune system once implanted.

Acknowledgement: IMMODGEL (602694)

(P773)

P773 Influence of surface modified poly(*L*-lactic acid) films on the differentiation of human monocytes into macrophages

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Macrophages play a crucial role in the biological performance of biomaterials, as key factors in defining the optimal inflammation-healing balance towards tissue regeneration and implant integration. We investigate how different surface modifications performed on poly(L-lactic acid) (PLLA) films would influence the differentiation of human monocytes to macrophages. We tested films without modification, surface-modified by plasma treatment (pPLLA), or by combining plasma treatment with different coating materials, namely poly(L-lysine) (pPLLA-PLL), and a series of proteins from the extracellular matrix: collagen I (pPLLA-COLL I), fibronectin (pPLLA- FN), vitronectin (pPLLA-VTN), laminin (pPLLA-LMN) and albumin (pPLLA-ALB). While all the tested films are non-cytotoxic, differences in cell adhesion and morphology are observed. Monocyte-derived macrophages (MDM) present a more rounded shape in non-modified films, while a more elongated phenotype is observed containing filopodia-like and podosome-like structures in all modified films. No major differences are found for the expression of HLA- $DR^+/CD80^+$ and $CD206^+/CD163^+$, and for the ability of MDM to phagocytize. Interestingly, MDM differentiated on pPLLA present the highest expressi on of MMP9. Upon differentiation, MDM in all surface-modified f ilms produce amounts of IL-6 compared to non-modified films. After S, stimulation with the potent inflammatory agent LPS, pPLLA, pPLLA-PLL and pPLLA-FN films the reveal a significant reduction on IL-6 secretion, while opposite effect is observed with IL-10. Of note, in comparison to non-modified films, all surface-modified films induce a significant reduction of the IL-6/IL-10 ratio, a valuable progn osticator of pro- versus anti-inflammatory balance. These findings give important insights about MDM-biomaterial interactions, while strengthening the need for tailor-informed biomaterials.

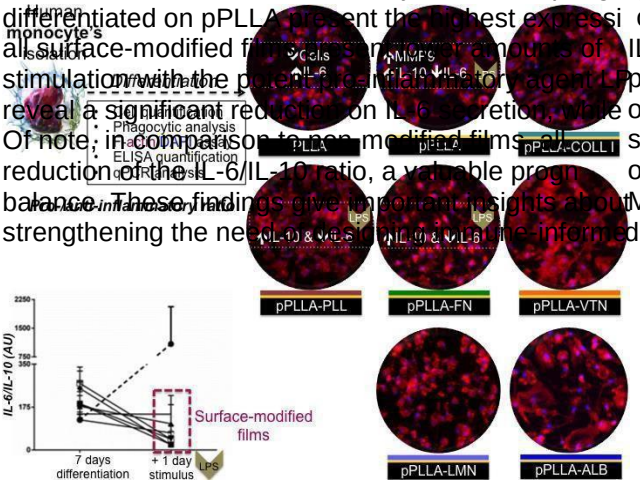

Figure 1 – Monocytes differentiated in macrophages after 7 days of culture. An additional LPS stimulus was applied for 24h.

P774 Dissecting mesenchymal stem/stromal cells secretome to modulate the inflammatory response of degenerated intervertebral disc

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Intervertebral Disc (IVD) degeneration is considered the major cause of spine disorders and disability worldwide. This process occurs naturally with age and is associated with increased vascularization and innervation around the IVD as well as an intense inflammatory reaction. The current treatments can alleviate the associated low back pain (LBP) but do not resolve the underlying cause, frequently contributing to the aggravation of the problem in adjacent structures.

Mesenchymal stem/stromal cells (MSCs)-based therapies for LBP and degenerated IVD have been increasingly explored. The regenerative potential of MSCs is widely demonstrated in a variety of tissues, as well as their immunomodulatory capacity. Besides, MSCs paracrine competence is being increasingly appointed as its main therapeutic factor, with many studies reporting promising results with the cells secretome, which could be an added advantage when considering the highly questionable MSCs survival in the harsh hypoxic and avascular environment of degenerated IVD.

So, this work aims to evaluate the immunomodulatory potential of MSCs secretome in IVD degeneration and to explore how MSCs pre-conditioning with hypoxia levels and pro-inflammatory stimuli affects MSCs immunomodulatory potential in degenerated IVD.

For that, human bone marrow-derived MSCs when confluent, were pre-conditioned for 48h with IL-1β (10 ng/mL), in either normoxia (21%O2) or hypoxia (6%O2), maintaining cell viability, metabolic activity and phenotype throughout all conditions. The different MSCs secretomes (secMSCs) were collected and used to culture bovine IVD punches in pro- inflammatory/ degenerative culture conditions (puncture+IL-1β stimulus) in hypoxia (6% O2), using an ex vivo model previously established in the group. Control groups of non-stimulated IVDs and IVDs co-cultured with MSCs were performed in parallel.

After 48h, the secMSCs-treated IVDs remained viable and the levels of pro-inflammatory markers of degenerated IVDs were down-regulated (IL-6, IL-8, TNF-α) as well as the production of inflammatory key players PGE2 and TGF-β were reduced, when compared to untreated IVDs. Cell pre-conditioning with hypoxia or pro-inflammatory cytokine IL-1β did not improve the immunomodulatory properties of sec-MSCs in degenerated IVD although, IL-1β-stimulated MSCs exhibited a pro-inflammatory profile, by up-regulation of IL-6 expression and increase of PGE2 and IL-8 production. When IVDs were co-cultured with MSCs, the decrease in inflammatory cytokines levels was also observed, although not as intensely. Regarding ECM degradation, at the same early timepoint, neither secMSCs nor MSCs seemed to be able to counteract the down- regulation of expression of collagen type II and aggrecan induced by the inflammatory/ degenerative model. Nevertheless, treatment with secMSCs down-regulated matrix-degrading enzyme MMP1 (increased when co-cultured with MSCs), while treatment with the MSCs themselves decreased expression levels of MMP3 (increased in secMSCs-treated IVDs). Overall, the results demonstrate the potential of secMSCs to control inflammation in degenerated IVD, but weather this potential could be used to treat LBP remains to be highlighted.

P775 Titanium induces expression of metallothioneins in primary human monocyte-derived macrophages and enhances their intracellular bacterial killing capacity

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Titanium is widely used as implant material for dental and orthopedic applications. Major problems of titanium implants include chronic inflammation, recurrent bacterial infections and implant failure. Macrophages are essential regulators of inflammation related to foreign body response. Metallothioneins (MTs) are known to bind metal ions (including zinc) essential for regulation of inflammation and bactericidal activity of macrophages. However response of primary human macrophages to titanium and the role of MTs in this response was not examined up to date. The expression of the MT family and the MT dependent intracellular zinc level as well as bacterial killing capacity of macrophages were determined in response to titanium.

Expression of MT-1F, MT-1G, MT-1X and MT-2A mRNAs was upregulated in M0 (control), M1 (IFNγ-stimulated) and M2 (IL-4 stimulated) primary human monocyte-derived macrophages propagated on titanium disks, and was also induced by titanium microbeads in contrast to nanobeads. The highest level of upregulation was detected in M0 for MT-2A (fold change of 12.5) by Affymetrix and MT-1G (fold change of 130) by RT-PCR. Zinc is a target of MTs and it can induce MT expression. However intracellular zinc levels were not changed in macrophages propagated on titanium on all time points analyzed (1, 3, 24h and 6 days). The intracellular killing capacity of Staphylococcus epidermidis was elevated in M1 propagated on titanium disks correlating with up regulation of MTs. Titanium-induced overexpression of MTs in macrophages has a compensatory effect on the maintenance of intracellular zinc homeostasis and supports intracellular bacterial clearance under pro-inflammatory conditions.

P776 Biological effects of hyaluronan (HA): what to consider in HA-based biomaterial development

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HA is a biocompatible and biodegradable polysaccharide with the ability to promote wound healing and establish tissue homeostasis. These are the main reasons why is it a favorite choice in biomaterial development. But most of the HA properties are mediated through a plethora of enzymes and binding partners which might be deregulated during tissue damage and inflammation. Thus, the knowledge about HA metabolism and signaling are essential for its proper utilization in tissue regeneration and repair. Same attention should be pay to the proper characterization of HA used in all stages of research and development of medical devices and biomaterials. Contamination with different substances (proteins from animal tissues or bacterial residues) or even HA fragments can skew the outcome of biological tests especially in immune response evaluation.

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P777 Surface modification of pancreatic islets using tissue-adhesive polydopamine-coated poly(lactic-co-glycolic acid) microspheres for local drug delivery

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Islet transplantation becomes a hopeful treatment for type 1 diabetes. Nevertheless, a big loss of transplanted islets due to immune reactions and thrombosis leads to the early graft failure. Therefore, drug delivery to islet microenvironment is essential to improve the transplantation outcomes. In this study, we aimed to exploit mussel-inspired linkage using polydopamine to immobilize PLGA microspheres as a drug delivery system onto the surface of pancreatic islets. PLGA microspheres with homogenous size were prepared by emulsion-evaporation method. Functionalization of microspheres with dopamine solution 1 mg/mL was conducted in bicarbonate buffer (pH=8.5) under a constant stirring for 1 h. At this condition, dopamine was auto-polymerized to form polydopamine. FTIR spectra indicated the formation of polydopamine after oxidation of dopamine. Moreover, scanning microscopic images revealed the rough surface of functionalized microspheres due to bound polydopamine nanoparticles. Polydopamine-coated microspheres were conjugated on the surface of islets in HBSS buffer (pH=8.0). Fluorescence and confocal images demonstrated well-distributed microspheres on the surface of islets. Furthermore, the viability of microsphere-conjugated islets performed by using CCK-8 assay and live/dead assay showed no significant differences compared to unmodified islets. In conclusion, owing to versatile adhesion properties of polydopamine, this system could potentially be used to deliver various drugs to prevent immune rejection as well as thrombosis after islet transplantation.

P778 Influence of the secretome of adipose-derived stem cells on M1 macrophages *in vitro*

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Introduction: Chronic wounds remain as a significant burden for patients and healthcare system. An important component in the pathogenesis of non-healing wounds is dysfunction of macrophages. In normal-healing wounds, the classically activated pro-inflammatory “M1” macrophages present at the early phases are replaced over time by alternatively activated “M2” macrophages, which stimulate angiogenesis and extracellular matrix deposition. However, most types of chronic wounds are characterized by persistence of the M1 phenotype. Strategies based on the use of mesenchymal stem cells (MSCs) have recently gained significant interest for the treatment of chronic wounds. In particular, accumulating experimental evidence suggests that MSCs favor the transition of M1 to M2 macrophages. These effects seem mostly mediated by paracrine factors secreted by the MSCs, including soluble factors and extracellular vesicles. However, the immunomodulatory role of the different components of the MSC secretome in the M1 to M2 transition remains largely unknown.

Aim: The aim of this study was to assess the effects of the different components comprising the MSCs secretome on M1 macrophages *in vitro*

Materials and methods: Cultured human adipose-derived stem cells (ASCs) were incubated in medium depleted of extracellular vesicles. The medium was harvested after 24 h and designated as the whole supernatant (WS). The WS was further divided into a soluble fraction (SF) and an extracellular vesicle (EV) fraction. The size and distribution of particles contained in the different fractions was determined by nanoparticle tracking analysis (NTA). M1 macrophages were obtained from the THP-1 human monocytic cell line after incubation in a pro-inflammatory cocktail. The M1 polarized cells were conditioned in the WS, SF or EV fraction for 48 h. The conditioned cells were labeled using antibodies against the CCR7 and CD163 receptors (as markers for M1 and M2 subtypes) and assessed by fluorescence microscopy. Additionally, qRT-PCR was used to assess the transcriptional activity of *CCR7* and *CD163* genes.

Results: While particles in the range of 40-180 nm (corresponding to exosomes and microvesicles) were found in the WS and EV fractions, no particles were detected in the SF fraction. Treatment with all three fractions attenuated the M1 phenotype, as evidenced by a decreased number of CCR7 and an increased number of CD163 positive cells. qRT-PCR analysis also revealed an anti-inflammatory effect from all three fractions, as evidenced by a significantly decreased transcription of CCR7 in the conditioned cells. However, only the WS and EV fractions significantly increased the transcriptional activity of CD163. These results suggest that the M1 to M2 transition appears to be mostly favored by components present in the EV fraction rather than in the SF counterpart.

Conclusions: Our results indicate that EVs released by MSCs may play an important role as mediators of their pro-inflammatory properties. MSC-derived EVs may therefore represent a novel cell-free treatment for non-healing wound conditions.

P779 Surface-engineering of pancreatic islets with immunosuppressant loaded PEGylated polymeric nanoparticles as a novel strategy toward improving survival time of xenografted islets

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Transplantation of pancreatic islets is currently emerging as a successful treatment for type 1 diabetes. However, the rejection of transplanted islets by the host immune system is still a big obstacle for its widespread application. To overcome this problem, FK506; a well-known immunosuppressive drug was encapsulated in dopamine-functionalized poly(lactide-co-glycolide)-poly(ethylene glycol) nanoparticles (PLGA-PEG-DOPA NPs) and immobilized onto the surface of pancreatic islets for active prevention of immune reaction. By Michael reaction between dopamine moiety and collagen matrix, islets were fully covered by nanoparticles without any negative effects on viability and functionality. The morphology and viability of surface-modified islets were well-preserved after co-culture with xenogenic splenocytes, while control islets were notably destructed. Additionally, the level of TNF-α secreted by splenocytes was lower in surface-modified group. In xenotransplantation model, the median survival time (MST) of surface-modified islets (17.33 ± 1.31 days) significantly increased in comparison to that of control group (10.00 ± 0.873 days). Thus, surface-engineering pancreatic islets with FK506 loaded nanoparticles would be a promising strategy toward improving immunoprotection in xenotransplantation.

P780 The role of interleukin-6 and nuclear factor kappa beta one in MSC-osteoclast-crosstalk in an in-vitro co-culture model for osteogenesis

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Aims: Bone biology is highly complex regulated. The crosstalk between osteoblasts and osteoclasts, the bone forming and bone resorbing cells, so far is poorly understood, and little is known on how errors in this circuit play a role in pathogenesis of different diseases. This study's objective was to investigate which parameters regulate the crosstalk between mesenchymal stem cells and monocytes during osteogenic differentiation and osteoclastformation.

Methods: Monocytes were isolated from full blood samples via gradient centrifugation and CD- 14 positive selection via magnetic cell sorting. Mesenchymal stem cells (MSCs) were isolated and expanded from bone marrow, taken from femoral heads discarded as "surgical waste" during arthroplasty. MSCs minimum requirements were proven using FACS and through differentiation.

MSCs were precultured to subconfluence in 12-well plates, then Monocytes added to the culture. Cultures were then kept in osteogenic (DAG) medium or osteoclastic (OC) medium for up to 21 days. AlphaMEM medium was used as control, as were monocultures of each cell type separately. Osteogenic differentiation was monitored through ALP activity and Alizarin Red staining; Osteoclastic differentiation was shown through formation of multinucleated cells and TRAP staining. Medium supernatant was analyzed for differentcytokines.

Results: We could show that the presence of CD-14+ monocytes and, even more so, the presence of unseperated monocytes, enhanced and accelerated the osteogenic differentiation of MSCs, up to a point where MSCs would mineralize even in control medium. Nuclear factor kappa beta one (NFkb1) was upregulated and could be seen relocalized into the nucelus in the MSCs.

Via ELISA, we could identify a considerable increase of IL-6 secreted from the MSCs in the presence of monocytes, which effectively inhibited osteoclast formation in the coculture. Monoculture experiments confirmed that IL-6 inhibited osteoclast formation in a dose dependent manner. Neither IL-1b, a potent inductor of IL-6 secretion, was present in the supernatants, nor was RANKL.

Impact: Our results suggest that certain triggers from monocytes - not IL-1b or RANKL - can act via NFkb1 to enhance IL-6 secretion form MSCs during osteogenic differentiation. We found that MSCs will inhibit the formation of mature osteoclasts, while being strongly pushed towards osteogenic lineage in our co-culture model of bone cell crosstalk.

Both molecules have been shown to be of considerable importance in bone biology, with their exact roles being highly complex and not fully understood. We hope to employ this model in future research to address whether or not cells from diseased tissues (such as metastasis) or donors (such as osteoporosis patients) show disabilities in this pathway.

P781 Has a pure low molecular weight hyaluronan got the ability to activate immune response in mouse macrophages?

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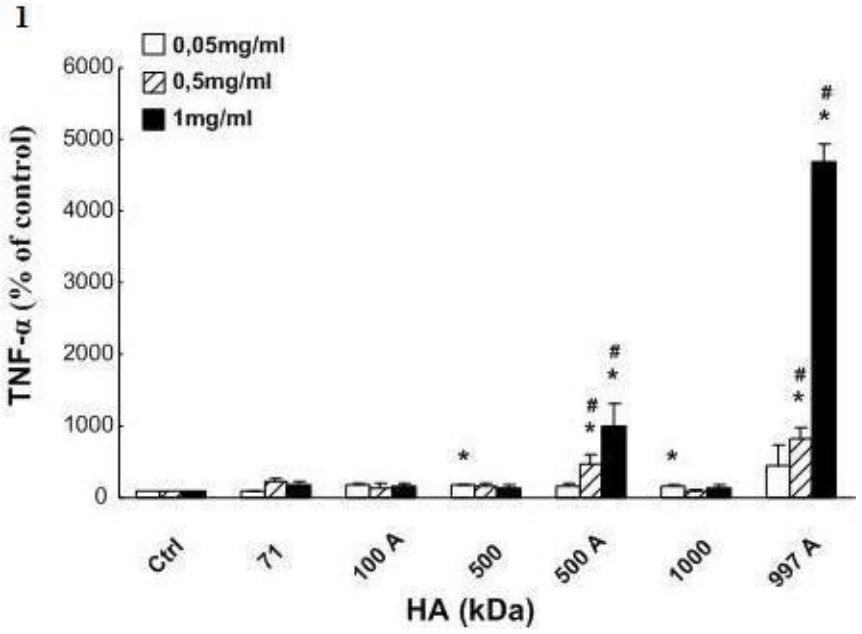
Biological properties of hyaluronan (HA) are often discussed in relation to its molecular weight. Typical example are the contradictory proinflammatory effects of low molecular weight HA and homeostatic properties of high molecular weight HA. Recently, with the development of new and more sensitive analytical methods we are able to revise this point of view. We tested the effect of different molecular weight of HA (range from 11kDa to 1000kDa) on activation of mouse macrophages cell lines as well as primary mouse macrophages isolated from bone marrow. Main methods used were ROS and NO production analysis, inflammatory cytokine production by ELISA and western blot analysis. The results indicate that the most important player in HA- induced inflammatory response is the purity of HA. In Figure 1 the HA of animal origin (100 A, 500 A, and 997 A) is able to significantly stimulate the proinflammatory TNF- alpha production in BMDM macrophages. This increase correlates nicely with the increased amount of contaminants in these preparations. Thus, the while the effects of molecular weight are can still be discussed, the necessity of high purity of HA used in biomaterial developments is indisputable.



P782 Cultivation of immune cells in HA/GTN-based hydrogels

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Immune system has evolved to defend the body against all foreign bodies and it cannot distinguish between a therapeutic device, detrimental foreign body or infection. The inflammatory response that ensue any implantation of medical devices in many cases can lead to chronic inflammation or in extreme cases rejection and destruction of the implanted system. For biomaterials in general, one solution is to develop immunomodulatory coatings that will attenuate the immune response.

Hydrogels based on natural biomolecules like hyaluronan and gelatin (partially hydrolysed collagen) can serve as an extracellular matrix-mimicking non-immunogenic coating to the implants and at the same time as a microenvironment suitable for cell cultivation. Macrophages have a critical role in the initial response to the foreign materials in the body. Following the initial injury, there are two types of macrophages present in the wound: M1 type macrophage is present during early stages of wound healing and it induces inflammatory process. Later, M1 switches to M2 type macrophage that induces healing process and supports cell proliferation. Tissue macrophages are supplemented by incoming monocytes.

We focused on the interaction of immune cells with biomaterials based on tyraminated hyaluronic acid (HA-TA) and hydroxyphenyl derivative of gelatine (GTN-HPA). The interaction of monocytic cell line and primary monocytes seeded on wells which were coated with hydrogels of various compositions was studied. Further, the cells were encapsulated and cultured within hydrogel in order to assess the influence of long-term contact of cells with the material. IL-4 was introduced to culture medium in order to support monocyte differentiation into M2 type macrophage and the real cell response was assessed.

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P783 Immunoregulatory and anti-microbial Cystapep 1 peptidomimetic - novel strategies of drug design to promote wound healing

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Wound infections are one of the biggest challenges for pharmaceutical industry and modern medicine. They can impede the healing process, generate chronic wounds formation and systematic inflammations. It creates an enormous sociological, economical and medical issue. Therefore we observe an increasing demand for new chemotherapeutics against opportunistic skin pathogens. Cystapep 1 (A-20) is a human cystatin C derivative structurally based upon its N terminal fragment. This compound shows strong antibacterial properties against S. aureus and S. pyogenes which are associated with skin - related infections. A-20 effect on human primary keratinocytes proliferation, cytotoxity and allergic potential have been analysed. The XTT and LDH assays were used to determine the effect of A-20 on proliferation and cytotoxicity of normal human keratinocytes. Allergic potential of A-20 peptide was assessed using ELISPOT technique (IL-4, IL-5 and IL-13 releasing by human PBMCs) and Basophil Activation Test (CD63, CD203c and CCR3 antigens expression on basophils). The obtained data showed that, A-20 is not cytotoxic to human keratinocytes and does not inhibit their proliferation in concentrations up to 50 µg/mL. Simultaneously, A-20 proved to stimulate proliferation of keratinocytes in lower concentrations. Moreover, it does not exert activation effect on PBMCs or basophils in vitro. Additionally, A-20 inhibits IL-4 and IL-13 production by concavalin stimulated PBMCs in vitro. The acquired data suggest potential implementation of A-20 in treatment of wound infections therefore enhance wound healing.

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P784 Therapeutic effect of allogeneic Bone Marrow-derived MSCs for aortic aneurysm

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BACKGROUND: The pathogenesis of aortic aneurysm (AA) is chronic inflammation caused by atherosclerosis. We have previously reported that intravenous injection of autologous mesenchymal stem cells (MSCs) reduced the morbidity rate of aortic aneurysm by immunomodulation, anti-inflammatory and tissue repair properties of MSCs. Meanwhile, the MSC provides an immunotolerance to the recipient, and clinical studies have demonstrated that allogeneic MSC transplantation prevents graft-versus-host disease. Allogeneic MSCs would be an alternative cell source for cell therapy instead of autologous MSCs. The purpose of this study was to investigate whether allogeneic MSCs have similar therapeutic effects as autologous MSC in a mouse model of aortic aneurysm (AA).

METHODS: *In vitro*: Bone marrow-derived MSCs were isolated from different strains of mice, C57BL6J (as autologous MSCs; C-MSC) and BALB/C (as allogeneic MSCs; B-MSC). Characterization of C-MSC and B-MSC was analyzed by flow cytometery. One-way mixed lymphocyte culture (MLC) was used to assess T lymphocyte reactivity against allogeneic cell populations. *In vivo*: The AA induced mice (continuous angiotensin II administrated aged apolipoprotein E KO mice, background; C57BL6J) were intravenously injected 1×10^6 C-MSC or B-MSC with 0.2 ml saline (n=10 C-MSC group, n=7 B-MSC group) or 0.2 ml saline only (n=10, saline group). After two weeks of injection, we evaluated the existence rate of AA, AA diameters, EVG stain for elastin fiber, and matrix metalloproteinase (MMP)-2 and -9activity.

RESULTS: *In vitro*: Flow cytometric analysis of cultured C-MSC and B-MSC demonstrated that both MSCs were similarly positive for Sca-1, CD44, CD73 and CD106, but negative for CD11b, CD34, CD80, CD86, MHC class II and c-kit. The C-MSC and B-MSC groups suppressed T-lymphocyte activation compared with allogeneic splenic lymphocyte co-cultured. *In vivo*: The existence rate of AA was decreased. The C-MSC group was 40% and the B-MSC group was 43%, respectively, whereas 100% in the saline group. The AA diameters were significantly reduced in the C-MSC group (1.4 vs 2.29 mm, $p<0.01$) and the B-MSC group (1.36 vs 2.29 mm, $p<0.01$) compared with the saline group. In contrast with the saline group, the elastin fiber was observed to prevent of degradation in the C-MSC and B-MSC groups. The enzymatic activities of MMP-2 and MMP-9 were significantly decreased in the C-MSC group and the same as the B-MSC group compared with the saline group.

CONCLUSION: These results suggest that allogeneic MSCs might be an alternative cell source for treatment of AA.

P785 TNF-ALPHA as the regulator of imflammation-induced activity of adipose tissue derived mesenchimal stem cells

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Mesenchymal stromal cells (MSC) participate in tissue regeneration and tumor growth, via secretion of bioactive molecules and their behavior strongly depends on cellular microenvironment. Regeneration takes place in presence of leukocytes, which are abundant in course of tissue remodeling. Cytokines and other factors secreted by leukocytes create inflammatory environment influencing MSC activity as well as that of immune cells themselves. Here we used TNF-α treatment at both physiological (5ng/ml) and supraphysiological (100 ng/ml) concentrations to model inflammatory microenvironment and studied ROS involvement into migratory, proliferative and secretory responses of adipose tissue-derived MSC(ASC).

We have shown that in MSC, TNF-α induce MMP9 expression and triggers some key intracellular signaling pathways: Akt, small GTPase Rac1, p38 MAP-kinase. Some of these pathways lead to ROS generation so we examined ROS generation in ASC treated with TNF-α using either DCF-DA or HE-staining. Furthermore, we observed using MTT assay that TNF-α enhances ASC proliferation as well as promotes entering into G2 phase as was tested by flow cytometry. This TNF-α effect was reduced by treatment with synthetic antioxidants (ebselen, tiron). TNF-α-treatment induces expression and secretion of several inflammatory and angiogenic factors in ASC: IL-1β, IL-8, MCP-1 and VEGFA. In our real-time PCR experiments, MCP-1 mRNA expression triggered by TNF-α was dramatically inhibited while VEGF expression was increased by antioxidants. TNF-α also moderately increase of ASC chemotaxis in transwell assay.

We conclude that TNF-α induce migration and expression of MMP9 in MSC; these effects could possibly mediate the influence of inflammatory environment on MSC activities related to regenerative processes or tumor growth.

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P787 Assessment of injectable myocardial matrix derived from decellularized bovine cardiac tissue for mesenchymal stem cell culture

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Cardiovascular diseases cause the decrease in both number and regenerative capacity of cardiomyocytes over time. Cardiac tissue engineering aims to repair, regenerate or replace damaged tissue. Using extracellular matrix (ECM) as a scaffold may have significant impact on promoting cell adhesion, growth and proliferation. Also it can be degraded into nontoxic products and can be recognized by the body with mild immune response. Injectable ECM offers a minimally invasive delivery. Here, we evaluated the properties of injectable myocardial matrix as a scaffold for rat bone marrow mesenchymal stem cell (MSC) culture. In the study bovine heart tissue was decellularized to obtain an acellular scaffold using a protocol including physical and chemical treatments, followed by lyophilization and sterilization. Acellularity was confirmed by spectroscopic and histological analyses. After then, decellularized ECM was enzymatically digested and following incubation at 37ºC, the injectable myocardial matrix was obtained. The gelation time was determined by turbidimetry, protein content was evaluated by the Lowry assay, and the glycosaminoglycan content of the ECM was determined by using the 1,9- dimethylmethylene blue assay. MSCs were seeded onto the obtained injectable myocardial matrix. Viability and proliferation of attached cells were determined by the MTT assay. DNA content analyses revealed a significant decrease in the DNA level in decellularized bovine cardiac tissue. This result was consistent with histochemistry analyses. Turbidimetry analyses demonstrated that gelation started after approximately 15-20 minutes of incubation at 37ºC. Lowry assay showed injectable myocardial matrix protected its protein content at the rate of ~50% compared to the native tissue. Injectable myocardial matrix preserved its GAG content at around 80%, which is crucial for cell attachment and viability. MTT assay showed that MSCs proliferated by time. Findings demonstrated that the obtained injectable myocardial matrix supports the attachment and growth of MSCs, and may have potential as an injectable scaffold for use in cardiac repair.

P788 A cost-effective method of (atelo)collagen type 1/3 (COL1/3) isolation from human placenta and its *in vitro* characterization in 2D and 3D cell culture applications

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Collagen-based biomaterials are a gold standard scaffold material in a broad range of tissue engineering (TE) and clinical applications. These collagens are still mainly of non-human origin and are reported to provoke immune responses in up to 5% of all patients. In this study, we established a cost-effective method of atelocollagen type 1/3 (COL1/3) isolation from human placenta based on pepsin digestion followed by subsequent salt precipitations and diafiltration steps.

COL1/3 was biochemically characterized by determining residual DNA content, proving the absence of impurities by SDS-PAGE analysis combined with total amino acid quantification and identifying the isolated collagen types by western blot. *In vitro* cytocompatibility of COL1/3 was demonstrated in 2D using primary rat hepatocytes and in 3D by a sprouting assay of HUVEC cells.

Mean values of COL1/3 yields were 550 ± 71 mg/100 g blood free wet weight placenta tissue; n=7). Residual DNA content (0.36 ± 0.11 µg/mg dry weight; n=7) was not significantly different to xenogenic collagen fleeces registered for clinical use. Collagen type I and III were present on Western blots and the absence of other proteins was confirmed by SDS PAGE. The amino acid content of COL1/3 was shown to follow the general characteristics of the collagen family. *In vitro* in 2D cultures, primary hepatocytes adhered at a higher density to COL1/3 and maintained the hepatocytic phenotype over the 4 days of culture compared to the gelatin control and in 3D spheroid-based angiogenesis assays, COL1/3 significantly increases the number of sprouts upon addition of VEGF compared to rat tail collagen.

We established an effective method of isolating a highly pure atelocollagens type 1 and 3 fraction from human placenta which can be compared to those obtained from commercial vendors. COL1/3 clearly demonstrated its usefulness in *in vitro* 2D and 3D cell culture applications thereby making it a potent cost-effective biomaterial for fully allogenic TE with an increased overall safety profile for patients in clinical settings.

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P789 Injectable hydrogel- PolyHIPE composite biomaterials for cartilage regeneration

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Injectable materials, and hydrogels in particular, have become promising for tissue repair. However, weak mechanical properties can limit their application. In this study, injectable composite materials have been fabricated consisting of a hydrogel containing, polyethylene glycol diacrylate (PEGDA) and methacrylated hyaluronic acid (Me-HA) plus porous particles obtained by polymerisation in a high internal phase emulsion template (polyHIPE particles). The resulting composite materials may overcome the drawbacks of hydrogels alone, especially in terms of poor mechanical properties, and the presence of highly porous particles in this composite system is expected to enhance cell proliferation and migration. Rheological, equilibrium swelling and degradation experiments have been performed to investigate the applicability of the system for cartilage tissue engineering. Rheological studies showed that storage modulus increased with increasing polyHIPE particle mass fractions and could be tuned by varying the ratios of materials used, particle size and UV intensity. Furthermore, to provide degradability, dithiothreitol (DTT) was incorporated into the crosslinked polymer network. Increasing the amount of DTT resulted in rapid degradation of PEGDA-based hydrogels. Mesenchymal stem cells (MSCs) encapsulation and differentiation to chondrocytes have also been examined using these composite hydrogels.

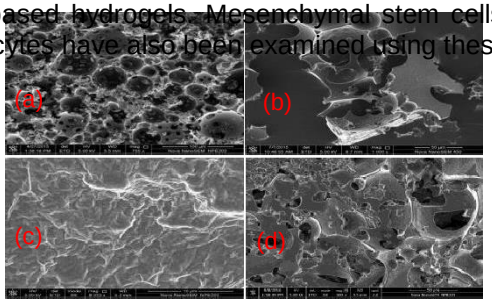

Fig.1. SEM images for (a) polyHIPE, (b) polyHIPE particle, (c) PEGDA/Me-HA hydrogel (d) PEGDA/Me-HA/polyHIPE composite hydrogel.

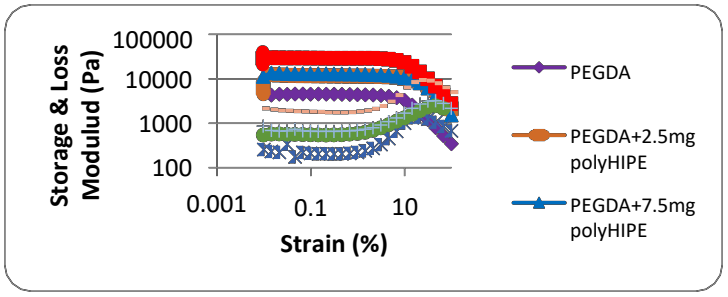

Fig.2. PolyHIPE concentration impact on storage modulus and loss modulus of PEGDA-based hydrogels.

P790 In vitro and in vivo comparison of nanoparticular hydroxyapatite pastes of different particle content and size

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Autologous bone grafting is considered the current gold standard for the treatment of low weight bearing bone defects. A promising alternative is nanoscale hydroxyapatite (HA), showing good biocompatibility and the potential to support bone formation.

In this study, various bone substitute pastes of different HA content and particle size were evaluated in vitro using classical 2-dimensional (2D) cell cultures versus 3-dimensional (3D) cell culture test systems. The effect of the pastes on several human primary cell sources as well as cell lines was assessed. In addition to the in vitro analysis, the pastes were characterised using X-ray powder diffraction (XRPD), thermogravimetric analysis as well as transmission electron microscopy (TEM).

Subsequently, two pastes were selected and compared with the commercially available HA paste Ostim®, autologous bone and empty defects, at two time points (6 and 12 months) in an ovine scapula drillhole model using micro-CT, histology and histomorphometryevaluation.

2D cultures showed a significant negative impact of nanoscale HA on cell activity. This effect was found reduced in 3D cell cultures. TEM images showed numerous nanoparticles located within the cell cytoplasm. As most promising candidates, pastes with low HA concentration of 38% (nHA-LC) and with a high concentration of 48% (nHA-HC) were selected.

In vivo, the nHA-LC paste supported bone formation with a high defect bridging-rate. Compared to nHA-LC, Ostim® (35 % HA content) showed less and smaller particle agglomerates but also a reduced defect bridging-rate due to its fast degradation. The nHA-HC paste formed oversized particle agglomerates which supported the defect bridging but showed a low bone formation in the defect site due to limited space.

Interestingly, the treatment of the defect site with autologous bone tissue did not improve bone formation or defect bridging compared to the empty control. We concluded that the particle-specific agglomeration behaviour highly influences the in vivo material resorption and bone formation.

P791 Fabrication of visible marker on decellularized tissue for ultrasonography via reactive sputtering

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Decellularized tissue is one of biomaterials derived from living bodies. The decellularized tissue is generally prepared via chemical or physical treatments. Since the decellularized tissue not only contains no viruses and cells, which are the causes of immune rejection, but also retain the native collagen fiber organization, it is useful as tissue replacement materials and tissue-engineered materials for regenerative medicine. However, the decellularized tissue is the living body-derived material, so that it is indistinguishable from tissues in a living body using non-invasive diagnostics such as ultrasonography, magnetic resonance imaging (MRI), and computed tomography (CT) when it is placed in a body. Therefore, a distinguishable marker for the non-invasive diagnostics is required. In this study, titanium oxide which shows excellent cytocompatibility was partially deposited on the decellularized tissue via reactive sputtering.

The decellularized tissue was prepared by high hydrostatic pressure treatment. For the partial deposition, a honeycomb-structured mask was put on the tissue before sputtering. Reactive sputtering has two sputtering mode, which are “metal mode” and “compound mode”. In this study, reactive sputtering was carried out in metal mode and the oxygen gas was used as a reactive gas. The estimated thickness of the film is 100 nm from the calibration curve of film thickness and sputtering time. After sputtering, the tissue was put on a simulated organ which is made of polyurethane, then it was observed by ultrasonography. As a control, a non-sputtered decellularized tissue and a silicon wafer were prepared and they were also observed by ultrasonography.

The ultrasonographic image of the partially-sputtered tissue was shown in Fig.1. As a result, sharply defined dots which attributed to the partially-sputtered titanium oxide were observed (arrows). On the other hand, the non-sputtered tissue could not be observed (Fig.2). The ultrasonographic image of silicon wafer was fogged due to halation (Fig.3). From these results, it was demonstrated that the partial sputter-deposition of titanium oxide will be useful for observation of the decellularized tissue via non-invasive diagnostics *in vivo*.

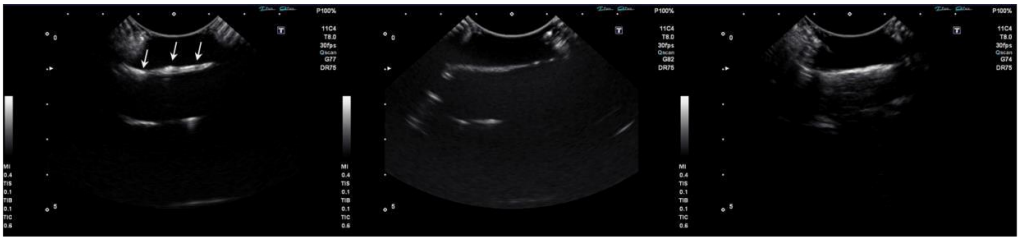

Fig.1. Ultrasonographic image of the partially-sputtered tissue.

Fig.2. Ultrasonographic image of the non-sputtered tissue.

Fig.3. Ultrasonographic image of the silicon wafer.

P792 A Novel Micro-Fibrillar Hybrid Injectable Biomaterial for Vocal Fold Tissue Engineering

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Injectable biomaterials with tissue-mimetic chemical composition and microstructure play an essential role in vocal fold (VF) tissue engineering. In general, injectable biomaterials should be biocompatible and biodegradable, and provide a tissue-mimetic microenvironment. The VF-specific biomaterials should also be mechanically stable under dynamic mechanical stimulations involved in phonation. Biomaterials containing collagen I (Col-I) and chitosan have been studied for various soft tissues. However, their potential use for VF engineering has been overlooked. Long-term exposure to Col-I containing biomaterials was previously shown to increase scar formation. Collagen III (Col-III) is known to modulate scar formation, and has been reported to significantly decrease in scarred tissue. In the present study, we proposed a novel injectable tissue-mimetic biomaterial composed of both collagen types I and III in glycol-chitosan, considering their co-presence in vocal fold tissue and the Col-III:Col-I ratio of 1:1 reported in VF literature [1]. The proposed biomaterial has a micro-fibrillar porous structure. The presence of collagen fibrils yields extracellular matrix-mimicking microenvironment to investigate the interactions between cells (fibroblasts in the present study) and the macromolecular components and morphometric parameters of the scaffold. Also, this can be used as a potential scaffold to fabricate engineered implants inside tissue culture bioreactors. Col-I/glycol-chitosan and bulk glycol-chitosan hydrogels were used as positive and negative controls, respectively. The hydrogels were characterized using atomic force microscopy, scanning electron microscopy, and micro-computed tomography for their morphological properties such as fibril thickness, pore size and total porosity. The thickness of the embedded heterotypic fibrils in the developed biomaterial was found to be consistent with the results of our recent study on in vitro fibrillogenesis of heterotypic collagen fibrils [1]. The swelling capacity, biochemical and mechanical stabilities as well as cell-scaffold interactions, viability, proliferation, cell adhesion and cell morphology were studied. The proposed hydrogel was shown to support cell adhesion and provide longer half-life compared with hyaluronic acid-based counterparts commercially used for VF engineering. This may thus decrease the need for regular reinjections currently performed for voice recovery. The proposed injectable biomaterial therefore combine the enhanced biochemical and biomechanical stability of a recent bulk glycol-chitosan hydrogel [2] with the cell adhesion characteristic of both collagen types I and III, and the role of Col-III in preventing scar formation. The proposed hydrogel could be a promising candid

P793 A minimal invasive injectable hybrid hydrogel that provides linear support for oriented nerve growth

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Injectable hydrogels are capable of mimicking the molecular and mechanical cues of the extracellular matrix (ECM) found in native tissues, but currently lack control over their structural architecture after injection in the body. This control is essential to guide and direct cells for regenerating complex tissues with oriented ECM, such as the spinal cord. Therefore, we engineered a minimal-invasive biomaterial that can form an anisotropic matrix after injection in the patient's body. The designed system is a hybrid hydrogel, containing short electrospun fibers. The fibers are fabricated from poly (lactide-co-glycolide) (PLGA), rendered magnetic by incorporating superparamagnetic iron oxide nanoparticles (SPIONs), and mixed within a neuro- compatible soft fibrin gel. After injection, short magnetic fibers can orient in the direction of an applied low external magnetic field, while the fibrin gel crosslinks to interlock the aligned fibers. This method enables the formation of a stable anisotropic architecture that is preserved after removal of the magnetic field. The orientation rate of the short fibers increases with increasing SPION concentration, enhanced magnetic field strength, and shorter fiber length. For example, short fibers with a diameter of approximately 700 nm and 50 µm length, containing 10 wt% SPIONs, oriented in low 100 mT magnetic field within 50 s. When a low concentration of short fibers (0.015 v%) was aligned and fixed within a fibrin gel, the obtained anisometric structure was already able to induce cell alignment. L929 mouse-derived displayed one dimensional cell growth along the fiber orientation, with the cells acquiring an elongated morphology and cytoskeleton structure. In contrast, fibrin gels without fibers, thus lacking unidirectional guidance cues, led to cell growth in all three dimensions, isotropic morphologies, and F-actin filaments stretched in all direction. To test the potential of the newly developed material to direct nerve growth, dissociated primary neurons and full dorsal root ganglions (DRGs), derived from chick embryos, were inserted inside the hydrogels. Hydrogels without short fibers or randomly oriented fibers resulted in radially extending neurites, while the anisotropic hydrogels triggered the neurites to grow along the fiber direction after they come in contact with the cell-adhesive fibers. In addition, we observed that the presence of short oriented fibers within the 3D fibrin gel enhanced the rate of neurite extension.

In conclusion, the simplicity and versatility of our approach enables the formation of unidirectionally oriented structures *in situ* with controlled features that stimulate fibroblasts and nerve cells to grow in a linear manner. This newly designed hydrogel overcomes some of the limitations of the existing injectable regenerative materials, and fills a major need in the field. It can be applied as a therapeutic material and a tool to investigate the effect of different design parameters of an anisotropic matrix on physiological and pathological processes *in vivo*.

P794 Manufacturing porous microspheres which successfully support bone formation and stimulate an increased vascularisation response

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Introduction:

Injectable cell-seeded scaffolds are an interesting route towards in situ repair of bone. The combination of scaffolds with cells is aimed to reduce healing time by supporting the formation of natural bone tissue. For this approach to be successful the injectable bone fillers need to enable rapid self-vascularisation because their delivery method prevents the inclusion of pre-formed vasculature. As a three-dimensional (3D) cell support matrix for cells, porous microspheres have many advantages over their non-porous counterparts; they can provide enhanced nutrient diffusion and greatly increased surface area [1]. We investigated porous microsphere manufacturing to produce scaffolds capable of supporting MSC-like cells and measure their vascularisation response using a chick Chorioallantoic membrane assay (CAM).

Methods:

Microspheres were formulated from a photocurable acrylate-based high internal phase emulsion (HIPE). Porous microspheres via a T-junction microfluidic device at 80% porosity. Microspheres were coated with poly-acrylic acid via plasma deposition. hES-MP cells were cultured on microspheres for up to 60 days and the cell ingrowth was investigated through use of a microtome and H&E staining. CAM assays were conducted over 7 days for testing the angiogenetic potential of pre-seeded and non-seeded microspheres.

Results:

Cell viability was observed on PolyHIPE spheres coated with poly-acrylic acid. Within the cell culture, multiple small microsphere aggregates were formed after a few days, which then increased in size as single microspheres and other small aggregates combined with each other. By the 30-day time point, cells were present within the internal pores and the morphology of these cells appeared to resemble that of osteocytes. Increased vessel number was observed in the CAM around scaffolds pre-seeded with cells but not around scaffolds without cells.

Discussion:

This investigation found that tailored porous microspheres were able to support hES-MP cells over 30 days and that cells pre-seeded in the scaffold produced a better angiogenic response than when either was used in isolation. hES-MP cells were shown to grow on the scaffold and agglomeration of the microspheres and cells was observed. Calcium was detected within the samples and osteocyte-like cells were observed within the porosity of the microspheres. Despite not stimulating an angiogenic response scaffolds without cells were found to recruit native cells from the CAM. Porous microspheres have the potential to be used as a bone graft material offering superior vascularisation response.

P795 A new culture platform for cost-efficient adherent cell expansion

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Cell therapy production platforms benefit from closed, automated bioreactor approaches to improve handling and process control, while reducing costs. To compete with conventional monolayer culture it is crucial that such an approach is able to obtain sufficient cells and that it yields the same final product. We demonstrate culture of two cell types (bone marrow-derived mesenchymal stem cells and adipose-derived stromal cells, MSC resp ASC) in a closed bioreactor system to high cell numbers for therapeutic applications.

For MSC expansion, a one vial of cryopreserved MSCs (P1) were seeded in a bioreactor (Scinus Cell Expansion system) and cultured in parallel with controls. For bioreactor and spinner conditions, dissolvable microcarriers were used and environmental control was used to maintain pO_2 (20%), pH (7.3) and temperature (37 ^{o}C). MSCs were harvested from all conditions and analysed using: visual inspection, flow cytometry, cytokine stimulation assays, inhibition of T-cell proliferation, karyotyping, and medium analysis for metabolites. For ASC, 20 million cells were cultured in the Scinus for two weeks using the same process control settings.

Starting from 1 million cells, up to 1.9 billion MSCs were cultured (range 1.4-1.9 billion) in under three weeks (range 15-19 days, figure 1). All MSCs displayed correct morphology (figure 2), marker expression, differentiation and safety profiles, as well as inhibition of T-cell proliferation. For ASCs up to 500 million cells were obtained (range 260-515 million). Medium expenditure was reduced up to 50% compared to monolayer procedures. Total cell numbers were efficiently harvested (>85%, fire 2 bottom right) by one operator in one hour through dissolution of the microcarriers, resulting in a single cell suspension.

The ability to reliably obtain high cell yields, while maintaining identity, potency and safety, is crucial for success of new cell culture platforms. We present a closed-system approach that can yield up to two billion cells with retention of identity and potency. Cells are quickly and efficiently harvested and operator time is reduced significantly, providing a user-friendly and cost effective alternative to monolayer culture for adherent cells such as MSCs and ASCs.

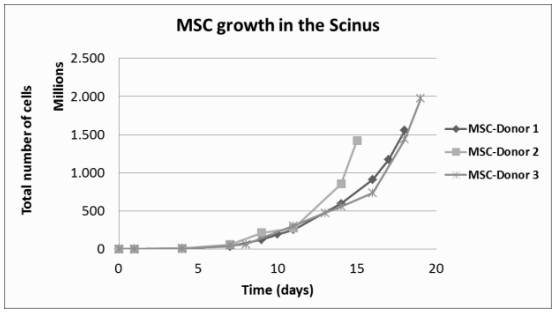

Figure 1: MSC expansion in the Scinus

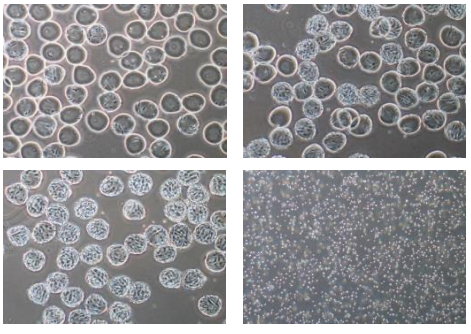

Figure 2: Visual inspection day 4, 11, 14 and harvest

P796 Introducing the SkinCreator – make bioengineered skin available for everyone – a Skintegrity Zurich project

Stephan C. Fox[1], Thomas Biedermann[2], Marianne Schmid Daners[1], Ernst Reichmann[2], Mirko Meboldt[1]

[1]pd|z Product Development Group Zurich - Department of Mechanical and Process Engineering - ETH Zurich, Zurich, Switzerland, [2]tbru Tissue Biology Research Unit - Universities Children's Hospital Zurich - University Zurich, Zurich, Switzerland

Research in Tissue Engineering is pushing the boundaries further and further creating highly promising personalized regenerative medicine therapies. In the late 1970s first autologous epidermal keratinocyte sheets were transplanted onto patients in the USA, making it the first part of an organ created in a laboratory from the patients' cells to our knowledge.

A very important milestone in the process of clinical implementation was reached 2016 in Zurich with the successful completion of a clinical phase I study using dermo-epidermal bioengineered skin equivalents, called denovoSkin, a product of the Tissue Biology Research Unit at the Children's Hospital Zurich. A clinical phase II will start soon, including Burn and Reconstructive surgery centres in Zurich, the Netherlands and United Kingdom.

Based on these advances, the Tissue Biology Research Unit and the Product Development Group Zurich of ETH Zurich initiated the SkinCreator project. It is part of the Skintegrity initiative, a flagship project of the Hochschulmedizin Zürich.

The mission of the SkinCreator project is to make bioengineered skin available for everyone. What are the future challenges for research and how can we solve them? Making human autologous bioengineered skin a worldwide available and applicable clinical gold standard for the treatment of skin defects requires a safe, robust and cost-effective production. The nature of the therapy as an autologous treatment is based on a biopsy from the patient. Because of this, a mass production in advance is not possible. This and other traditional ways of cost reduction through high volumes, called economies of scale are not effective or applicable to lower the cost for a personalized therapy. We analysed the process from the extraction of the skin biopsy to the surgical transplantation of the skin equivalent and created an interdisciplinary knowledge base throughout medicine, biology and engineering.

We suspect the major challenges for an all-encompassing application of the denovoSkin to be the high complexity of the laboratory process and the resulting expertise required from the technicians as well as the high-tech infrastructure needed. These could benefit from the transition from manual to automated production of skin substitutes making them more secure, more reliable and more affordable.

To cope with these challenges, the research of the SkinCreator project focuses on developing a robust and easy-to-apply process by incorporating specially tailored devices of automation. This will allow to minimize the interactions of the technician and the required expertise, paving the way for a future adoption worldwide in the clinical application.

P797 Morphology-based real-time evaluation of culture condition

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By the advances of stem cell culture technology, various culture protocols have been reported for more cost effective and stable cell culture. There are numerous protocols by the combination of culture media, plates, coating materials, and handling skills. However, it is difficult to find the most appropriate protocol since there are too many parameters to be evaluated. Therefore, commonly, once examined and succeeded protocol is used for long time and it is considered to be risky to examine new protocols. Such conservative protocol preservation is sometimes costly, and losing the chance to establish better protocol with new materials. Moreover, when a facility require new and optimized protocol for establishing cost effective original manufacturing process, or search for suitable protocol for new cells that have not yet been investigated in the past, we still need a laborious and costly examination process. In many cases, only the proliferation rate or endpoint assay is examined in such protocol exploration process.

Cellular morphology has long been known as an important indication to evaluate the cellular culture status. However, the rule of cellular morphology and its fitness to the culture condition has not been quantitatively defined. Commonly, the cellular morphology is evaluated ambiguously based on feeling and experiences.

We have been proposing morphology-based evaluation concept by quantifying cellular morphology as cellular fingerprint, and have verified the effectiveness of its application to both the cellular quality evaluation and its culture condition. By converting the statistics of cellular morphological parameters as morphological fingerprint, we have found that delicate cellular response to the effect of culture condition can be recorded as quantitative data, and can be compared with bioinformatics analysis. In this report we show that such morphology-based analysis is effective to profile and compare the optimum culture conditions rapidly, feasibly, and cost effectively by using only the non-stained time-course microscopic images. We here show the practical examples of phenotypic evaluation method of various types of culture conditions for mesenchymal stem cell and neural stem cell culture.

P798 Application and understanding of morphological data for non-invasive quality control of stem cell manufacturing

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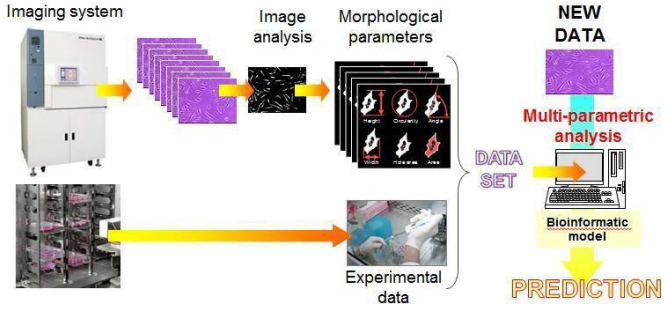
By the advances in cell culture and regenerative medicine technologies, cell therapies and tissue engineering have become not only new, but also realistic concept to treat difficult diseases. Many industrial technologies have been developed as commercial products to support the researches in this field to grow as a new industrial movement. However, there are still more engineering technologies required to speed up the industrialization for more cost effective, more stable, and more controllable manipulation of cellular products for treatment. Practically, technology for cell manufacturing is now highly required than ever.

One of the critical and seductive issues in the cell manufacturing, especially the stem cell manufacturing, is the monitoring and control of the state of cells during the total culture period. Presently, manual microscopic observation is the only practical, cost effective, and realistic way to monitor cellular status. Ironically, the conventional invasive staining or measurement technologies that have led us understand cells are not compatible for manufacturing cells for therapy. Therefore, imaging is now regarded as an important technology to understand the status of cultured cells in real-time manner. Supported by the successful legends in cell culture, the cellular morphology quantified by imaging technology has been reported to be effective in evaluation of cells.

We have been reporting evaluation and prediction of cellular quality (undifferentiated status, differentiation rate, remaining doubling time, media effect, and etc.) in mesenchymal stem cells, neural stem cells, and pluripotent stem cells. By our reports, we have verified that “morphological information” extracted from cellular images have significant information. However, we have also found that the concept of usage and application of morphological information is critically important for enabling accurate classification or precise predictions. However there are still no golden standard to interpret or convert such morphological information for cellular evaluation works. We here present and discuss the important aspects of morphological data for effective cellular quality control for stem cell manufacturing.



P799 Imaging regeneration after stem cell application for skeletal muscle reconstruction using diffusion tensor imaging (DTI) and magnetisation transfer (MT) measurements

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INTRODUCTION:

Urinary incontinence compromises a patient's quality of life and inflicts tremendous health care costs. Recent advances using cell therapy such as satellite stem cells (SSCs) show promising results towards correcting the underlying etiology[2] but evaluating the success of such treatments *in vivo* is difficult. In this study, we show that Magnetic Resonance Imaging (MRI) properties enable a monitoring of adult stem cell myogenic differentiation in a mouse model.

METHODS:

We have isolated, characterized and expanded human SSCs followed by injection into a tibialis anterior muscle crush mouse model. We followed up the *in situ* differentiation via MRI (4.7 T scanner) for 21 days focusing on Magnetization Transfer Ratio (MTR) and Diffusion-Tensor Imaging (DTI) properties of the *de novo* tissue and confirmed the results by histology, immunohistochemistry, western blot and real time PCR.

RESULTS:

MT measurements showed an initial MTR decrease before increasing steadily and approximating the MTR values of reference skeletal muscle tissue. DTI revealed that *de novo* generated muscle fibers are orientated in the same direction as the surrounding fibers which were not affected by the initial muscle crush injury. Cell differentiation and myofibers formation could be confirmed by increased muscle specific markers. The mean fiber length, tract volume, fiber number as well as diffusion parameters focal anisotropy and apparent diffusion coefficient approximate the values prior to injury acquisition during the process of regeneration.

DISCUSSION & CONCLUSIONS:

Human SSCs form muscle tissue *in situ* and MT-MRI allows to directly assess muscle fiber formation as a measure of myogenic differentiation. DTI there while highlights the direction of the newly formed fibers. These results will be transferable to the clinical setting as a non-invasive biomarker for the assessment of muscle tissue regeneration in patients.

P801 Personalized cement augmentation of the proximal femur using a discrete cement diffusion model

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Osteoporosis is a major health concern in virtually all developed countries with up to 9 million new osteoporotic fractures expected annually worldwide. Augmentation of osteoporotic femur using PMMA bone cement has been shown in biomechanical studies as a preventive treatment to reduce the risk of fracture. Therefore, the main goal of this work is the development of a discrete diffusion model that allows us a patient-specific planning of the technique. A correct planning and execution involve the development of an accurate method for predicting the diffusion of the cement through the porous medium of osteoporotic cancellous bone.

The proposed discrete model is based on the random-walk theory. It takes into account the cement viscosity and volume injected, pressure of injection and direction of injection. Furthermore, it is supposed that it exists some fixed particles that represent the bone trabeculae, so that cement particles will not be able to fill it. Cement is injected following the random-walk theory. Once the cement injection is simulated, a voxel mesh is generated that represents the augmented bone (bone plus cement). Additionally, the computational model has been validated with experiments. We carried out injections in several open-cell structures (Sawbones, Malmö, Sweden) with different volume fraction (porosity). Three different cement types were injected that corresponded to three different cement viscosities. The open-cell structures were cut in blocks of approximately 65x65x40 mm^3. Each block was tightly enclosed in a Plexiglas shell of 5 mm thickness acting as a cortical shell. Cement was injected through a drilled hole of 3 mm on the different block faces. Cement injection was recorded and cement flux was followed using image techniques. Once the cement was solidified, we performed compressive mechanical tests in order to quantify the improvement of the specimen mechanical properties.

The cement injection pattern was successfully predicted in all the simulated cases. All the augmented specimens increased their mechanical properties. As the cement injection increases, the mechanical properties also improved. In fact, it was observed that specimens with lower volume fractions showed a considerable increase in the mechanical properties (61.16%).

Therefore, our proposed discrete cement diffusion model will allow us to plan and improve cement augmentation in a patient-specific model. Femoroplasty significantly increased fracture load when osteoporotic femora were loaded and cement filling may have an important role in the extent to which femoroplasty affects mechanical strength of the proximal femur. This methodology will be integrated in a previously developed parametric model of the proximal femur to move our treatments towards a more personalized medicine.

P802 Integrating computational modelling with biofabrication to design and fabricate successful bone tissue engineering strategies

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Introduction

The success of a bone tissue engineering (TE) construct strongly depends on the development of a new blood vessel network (angiogenesis) from the surrounding host tissue. In this study we used an existing computational model of bone regeneration [1] for a more in depth investigation of the host environment in critical size defects as well as design and biofabricate optimized patterned cell constructs.

Results and discussion

The *in silico* model correctly simulated the formation of a non-union in critical size defects and was used to study the underlying mechanisms. In the central region of the fracture callus, we found that all cells die due to severe hypoxia, leading to bone healing arrest. This finding prompted us to explore the influence of the vasculature from the host environment on the fracture healing outcome. Interestingly, the *in silico* model predicts a considerably improved healing outcome in the case of full muscle contribution and an intermediate result if the fracture callus is only partially supplied with blood vessels from the host environment. Motivated by these results, which indicate that a more homogeneous blood supply from the host environment leads to an improved healing, we investigated whether a well-designed TE construct, with an optimized spatial pattern of cell densities in a material matrix, will improve fracture healing in large segmental defects. For example, we aimed to test whether a reduced cell density in the central region would limit the severe hypoxia and lead to healing in the case of large segmental defects. We predicted the healing outcome for nine patterns, each defined as linear cell density gradients, and ten total average cell densities. Interestingly, the results show that particular cell patterns are able to enhance the bone regeneration compared to uniform constructs. In addition, the discretization of the pattern, which is required for the bioprinting process, has a substantial effect on bone regeneration and should therefore be accounted for during the design process. Following the model-informed design, we successfully bioprinted patterned constructs and demonstrated that the gradient pattern and cell viability were maintained for three days of culturing.

Conclusion

The results of this work clearly demonstrate that the effectiveness of a TE therapy is strongly influenced by the host environment since it can serve as a source of vascularization, as well as the cell density pattern and total average cell density of the TE construct. Moreover, the proposed integrated approach of modelling and biofabrication may increase the success rate of implanted TE constructs for critical size bone defects.

Acknowledgements

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P803 Engineering of an osteo-inductive decellularized extracellular matrix using an immortalized human cell line

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Critical bone defects together with stringent clinical scenarios (e.g. avascular necrosis, osteoporosis) necessitate the development of innovative bone repair strategies. Existing treatments are associated with significant drawbacks, namely severe donor-site morbidity and infections. To address the unmet need for bone regeneration, tissue engineering has proposed cell-free extracellular matrix (ECM)-decorated scaffolds in which ECM offers a microenvironment able to drive tissue repair [Sadr, 2012].

The goal of this study is the generation of ECM-decorated scaffolds as *off-the-shelf* constructs enriched in bone morphogenic protein 2 (BMP-2), an osteogenic factor already validated in clinical use [Goutschi, 2007].

To this end, we first aimed at developing a hMSC line capable to overexpress BMP-2 (M-BMP2). We designed a lentivirus carrying the BMP-2 sequence and transduced the M-SOD line [Bourgine, 2014] generating a highly heterogeneous cell population. In order to perform the study with stable and well-characterized cells, we selected one clone from the transduced population displaying high BMP-2 secretion level (> 1µg/mL in 48 hours), death-induction responsiveness and osteogenic differentiation. Those characteristics were assessed in classical 2-dimensional culture system. Subsequently, M-BMP2 line was dynamically seeded on porous hydroxyapatite scaffolds in 3-dimension perfusion bioreactor. A 4-weeks culture protocol under osteogenic conditions promoted proliferation and bone matrix deposition [Bourgine, 2014]. The phenotypic characterization of the constructs showed an osteoblastic commitment of their cellular compartment, confirmed by fluorocytometry (CD10, CD49E and Osteocalcin) and RT- PCR (ALP, Runx2, Bone Sialo Protein and Osteocalcin). ECM decorated scaffolds were obtained after ablation of living cells by overnight triggering of the apoptotic death device. Studies are ongoing to assess the osteoinductivity of those enginieered cell-free constructs upon ectopic implantation in nude mice.

This project describes a pioneering approach fulfilling key criteria, such as *off-the-shelf*, *standardization* and *osteoinduction.* Those advantages place this approach as a highly relevant strategy, as cell-based but cell-free bone substitute material. In particular, the high degree of standardization, combined with the customization through targeted protein enrichment, represents a key advantage over human and/or animal derived native ECM associated with important quality variations and risk of disease transmission.

P804 Detergent-based decellularization strategy well-preserves macro- and microstructure of pulmonary heart valves

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Heart valve disease is an increasingly prevalent and clinically serious condition resulting in substantial morbidity and death worldwide. Biological tissue has great potential to function as bioprostheses in patients for heart valve replacement. To remove the antigenicity of the tissue, bioscaffolds must be decellularized while preserving the complex mixture of structural and functional proteins that constitute the extracellular matrix. An intact microstructure of the material is mandatory since reseeding of scaffolds can prevent calcification. Unfortunately, an optimal decellularization protocol of heart valve leaflets resulting in adequate preservation of the extracellular architecture is still lacking. Here, we compared three different decellularization strategies for their efficiency to maintain acellular scaffolds with a preserved heart valve ultrastructure. Porcine pulmonary heart valves were treated either with 1) Trypsin-EDTA (TRP), 2) a protocol using detergents in combination with nucleases (DET+ENZ) or 3) Accutase™ solution followed by nuclease treatment (ACC+ENZ). The treated heart valves then underwent histological, DNA and SEM analysis. Fresh (native) porcine pulmonary valve tissue samples served as controls. Histological and DNA analysis of leaflets decellularized with ACC+ENZ revealed complete removal of all cellular components, whereas cellular removal was incomplete in the TRP group. However, TRP and ACC+ENZ-treated valves were enlarged and showed a disrupted architecture and degraded ultrastructure. SEM analysis supported this result, as the remaining extracellular matrix appeared loose and ruptured. In contrast, fully acellular heart valves with intact architecture, layer composition and surface topography were achieved with DET+ENZ treatment. Our observations show that in comparison with TRP and ACC+ENZ procedures DET+ENZ treatment preserved macroscopic structures and microscopic matrix components *in vitro*, resulting in an excellent scaffold for further application in tissue engineering. Additional studies are needed to investigate whether *in vitro* seeding of leaflets with autologous progenitor cells before implantation leads to functional tissue engineered heart valves *invivo*.

P805 Comparison between zebrafish- and human-derived decellularized extracellular matrix (ECM) for the cell culture

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Extracellular matrix (ECM) is a cellular microenvironment which provides structural framework and viability. Especially, for tissue engineering, its development has become an issue and tissue engineers are trying to obtain ECMs to control cell behaviour. Decellularization of fibroblast monolayers are one method which is best way to obtain the ECMs. Also, ECMs derived from cells, they are not toxic or hazardous to cells compare to many other synthetic biomaterials because cells are only removed leaving behind ECM. However, previous researches showed that many of decellularized ECMs are done with human or rodent cells. Moreover, many of these studies were done with human-derived ECM to rodent cells or in oppositeway.

Recently, zebrafish are used in many biological and medical fields from past to current days. Furthermore, physiological relevance between human and zebrafish is almost similar. From this approach, many human related research have been increased and still zebrafish have been shown similar results to humans.

In this study, we used decellularized zebrafish-derived ECMs for cell application. For the establishment of a zebrafish-derived ECM model to establish the human or related research field. We used zebrafish embryonic fibroblasts (ZF-4) for the zebrafish fibroblast cell. For comparison of fibroblast-derived ECMs, we also established a human model using human lung fibroblast (WI- 38). Fibroblast monolayers were decellularized and images were taken with optical microscope for before and after ECM decellularization. Morphology of these two ECMs have different structures according to the cell culture formation. Scanning electron microscope (SEM) images were also used to compare these two different types ofECMs.

The effects of ECM's on cells were used with human hepatic carcinoma cells (HepG2). For compatibility, we first check the differences in cell proliferation by WST-1 assay. Next the secretion of Albumin, which is the key sign for hepatic functionality, was measured with enzyme- linked immunosorbent assay (ELISA). For last, differences in expression of cytochrome P450 (CYP 1A1, CYP 3A4), indicators for hepatocyte's detoxification, were analysed after treatment with acetaminophen by quantitative real-time polymer chain reaction (qPCR).

In conclusion, zebrafish-derived ECM shows similar cell behaviours as much as human-derived ECM. Cell survivability and functionality were better compare to non-ECM treated cell culture. With these results, zebrafish-derived ECM are compatible for human cells and it could provide useful ECM materials for advanced cell and tissue culture.

P807 Successful decellularization of thick-walled tissue - a multifactorial approach

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OBJECTIVE: Decellularization of xenogenic materials to enhance biocompatibility is a new strategy and presents a promising alternative to fixation treatments. The benefits of decellularized tissue include better long-term stability as well as improved endothelialization. However, it is of utmost importance, to establish a process which grants complete decellularization (DC). We evaluated different DC factors to optimize the penetration depth and to reduce the DNA content.

METHODS: Porcine aortic valves were decellularized according to different procedures. DC was performed for 24h (G1), 48h (G2) and 72h (G3) with a solution containing 0.5% SD and 0.5% SDS. Intermittent washing steps and the inclusion of sonication characterize the procedure, which was derived from previous research results. Additional treatment with DNase and α-Galactosidase was performed to further improve the DC effectivity.

The penetration depth was measured by evaluation of DAPI and HE staining. Picrosirius red staining was performed to evaluate the effect of the procedure on the collagen fiber network. The structure of the connective tissue was further analyzed by Pentachrome staining. Additionally, REM analysis was performed to assess the effect on the surface structure and the fiber network. The remaining DNA content was evaluated by quantitative DNA assays. Moreover, a quantitative GAG assay was performed to analyze the effect of DC on the GAG content of the tissue.

RESULTS: DAPI and HE staining revealed a large amount of remaining nuclei in all groups. However, consecutive DNase treatment had a significant effect. While remaining DNA was detectable in G1, G2 and G3 were fully decellularized. Quantitative DNA assays showed a significant reduction of DNA content to less than 5%. Nonetheless, complete removal of DNA content was not successful in all groups. The influence of the DC treatment on GAG was significant as well. GAG content was reduced to 50% after 24h (G1) but remained constant for G2 and G3. Picrosirius red staining revealed an intact and stable collagen network without any visible defects. Pentachrome staining substantiated these effects. However, G3 revealed a slight loosening of the fiber network. Nonetheless, the fiber network remains intact, which could be confirmed with REM analysis.

CONCLUSIONS: DC of thin tissue, such as valve leaflets or pericardium is a feasible and stable process. As soon as tissues with higher thickness have to be treated however, established processes reach their limits. Inherently, DC is always a tightrope walk between remaining DNA and damages inflicted to the tissue. Thus, creating a stable and safe process which grants successful DC is a multifactorial approach. In this study, we highlight the significant effect of DNase and α-Galactosidase on DC efficiency. Furthermore, we developed a procedure that grants successful DC of porcine aortic valves including the aortic walls while keeping the collagen structure intact.

P808 Auxetic materials in tissue engineering

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AIM: Natural biological tissues are known to display auxetic characteristics, the material becomes thicker, rather than thinner, when stretched; corresponding to negative values of Poisson's ratio. These have been demonstrated in skin, artery, tendon, embryonic tissues, and, possibly cancellous bone. Natural biomaterials can also display gradient and/or anisotropic structure and properties. Mechanical stimulation of the scaffold promotes cell proliferation and a recent study indicated enhanced proliferation may occur in an auxetic scaffold. Auxetic porous materials facilitate mass transport and, therefore, there is potential for optimal flow or delivery of nutrients, metabolic wastes and therapeutic agents. For these reasons, the development of porous auxetic materials promises to deliver improved next-generation tissue engineering scaffolds. However to date there are limited studies investigating cell behaviour on such materials, thus this study aimed to investigate the ability of auxetic materials to support mesenchymal stem cell growth and differentiation.
METHODS: To produce auxetic foam samples polyurethane foams (Custom Foams) with 80 pores per inch ('ppi') before conversion were converted into auxetic form using an established thermo-mechanical processing route. The auxetic fabric was a warp knit fabric using polyester monofilament and Dorlastan V500 yarn. The foam and fabric were cut into 5mm x 5mm x 2.5mm and 10mm x 5mm x 1mm cuboids, respectively, and sterilised in 100% ethanol, washed in PBS and cell culture media in a laminar flow hood. Each cuboid of foam and fabric was coated with 50µg/ml of fibronectin, seeded with 6.4 x103 rMSC cells/mm3, and cultured under standard conditions for up to 3 weeks in low-adhesion plates. Following cell culture for upto 3 weeks, samples were histologically processed and 4µm sections were stained for morphological analysis. Duplicate samples were snap frozen, freeze dried, and coated with 24nm of gold, for scanning electron microscopy (SEM) examination. Sixty micron sections stained with H & E and analysed by SEM were used to observe the pore structure and localization of cells in the auxetic foam. Hoechst 3342 staining and Alamar blue were used to examine cell viability.
RESULTS: Mesenchymal stem cells adhered to fibronectin coated auxetic materials and displayed cellular clusters within the auxetic foams and fabric samples. This was also confirmed by SEM which showed the presence of extracellular matrix forming bridges with the foam pores (Fig. 1).
IMPACT: Mesenchymal stem cells were successfully grown within the auxetic foam and knit fabric samples, confirming both these materials support cellular adhesion, migration and extracellular matrix depostion and thus have potential use in tissue engineering applications.
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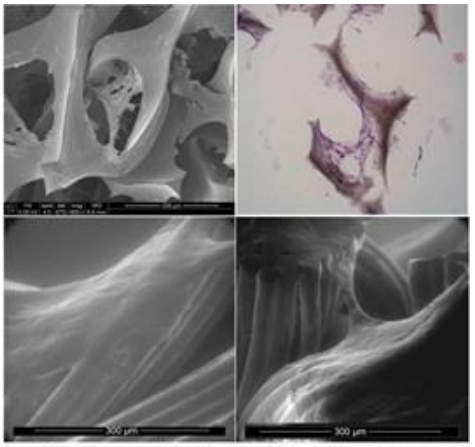

Figure 1: Scanning electron microscopy (SEM) and Haematoxylin and Eosin staining of 60µm sections demonstrating the morphology of P80 foam with clusters of rMSCs within the pores (A+B). Environmental scanning electron microscopy (ESEM) of live rMSC cells cultivated on auxetic fabric(C+D).

P809 Calcium phosphate scaffolds with high specific surface area towards improved cell response in vitro

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Specific surface area (SSA) is an important property of any material, including biomaterials. Biomaterials have to provide intimidate environment for successful biological processes in a body. It is a well-known fact that higher SSA ensures better solubility and reactivity compared to the same material with lower SSA.

Current study focuses on surface properties and cell response *in vitro* of calcium phosphate scaffolds. Calcium phosphate scaffolds available at the market have relatively low SSA (0.5-2 m^2/g) [1-3] while bone mineral has SSA of 87-100 m^2/g [4]. Therefore, in many cases autologous bone grafts are still preferred over synthetic grafts. The aim of the study was to develop calcium phosphate scaffolds with higher SSA to approach properties of bone mineral and study their cell response *in vitro*. *In vitro* cell behaviour was tested on two types of calcium phosphate scaffolds: ones with SSA 78±5 m^2/g and others with lower SSA 28±2 m^2/g. Characterization of obtained materials was done with XRD, FT-IR, SEM and BET N_2 adsorption. For *in vitro* tests MG63-GFP cells were used.

In vitro cytotoxicity results showed that higher cell viability was for scaffolds with higher SSA.

P810 Omega-7 accelerates healing of grafted skin burn wounds

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Introduction: Treatment of patients with severe large burn is complicated because of limited availability of donor skin. Thus, the robust healing of donor sites accommodating repeated skin harvests is of particular interest. Omega-7 (ω-7) is a fatty acid abundant in sea buckthorn seed oil. We have previously reported beneficial effects of sea buckthorn oil in ovine model of burn wound healing. The aim of this study was to test the efficacy of ω-7 on both third degree grafted skin burn and donor skin wounds.

Methods: Two full-thickness flame burn sites of 25 cm^2 were made on the both sides of dorsum of sheep (n=8). 24 hrs after burn, the eschar was excised down to the fascia and 30/1000 inches split-thickness skin grafts were harvested, meshed, and fitted to the wounds. 5% ω-7 (isolated from sea buckthorn pulp oil, Gangar Polivit Co. Ltd, Ulaanbaatar, Mongolia) was randomly and topically applied to one of two autografted sites. The remaining site received vehicle. After the initial treatment, the grafted sites were covered with tie-over dressings to be removed at 7^{th} day. Thereafter, the wounds were treated daily with ω-7 or vehicle for 7 days. The donor sites were also randomly allocated to 5% ω-7 or vehicle and daily treated with 5% ω-7 or vehicle starting at day 1 for 14 days. The epithelization rates (histologically) and blood flow (laser doppler) of all wound sites were assessed at 7 and 14 days after the surgery. The, total wound size and the area of open mesh interstices of autografts were determined with planimetry Image analysis as well. The donor site complete epithelization time was also determined.

Results: 1) Grafted wound sites: The size of unephithelized raw surface area was significantly smaller in treated sites compared to control sites at 7^{th} and 14^{th} day (0.40±0.06 vs. 0.46±0.05 and 0.05±0.04 vs. 0.13±0.07, p=0.01 and p=0.0003, respectively). The blood flow in treated sites was significantly greater (137±12 vs. 100±3.1 perfusion units, p=0.005) than in untreated sites 14 days after the grafting.

2) Donor sites: The complete epithelization time was significantly shorter in treated sites (8.5±2.8 vs. 12.6±1.6 days, p=0.02) than in untreated sites. Histologically, at the 7^{th} day, the percentage of wound closure in untreated sites was 88±14.3% vs. 100% in treated sites.

Conclusions: Topical application of ω-7 accelerates healing of both grafted and donor sites wounds by increasing blood flow. ω-7 should be considered as a cost-efficient and effective supplement therapy to boost healing of skin wounds. Further mechanistic studies are warranted increasing the study sample size.

Applicability of Research to Practice: The results are highly translational to the clinical practice.

P811 Evaluation of a novel detergent-based method for decellularization of peripheral nerve allografts

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Introduction: Within the field of peripheral nerve regeneration, there is a need for efficient alternatives to nerve autografts in cases of severe nerve damage. A promising option is the use of decellularized peripheral nerve allografts (DPNAs). These DPNAs provide a natural nerve architecture without the risk of immune response and thus create a favorable micro-environment for peripheral nerve regeneration. The aim of this study was to develop an improved DPNA and compare this to other, previously described DPNAs. **Methods:** Sciatic nerves of Wistar rats were harvested and randomly divided into four groups. Group A contained native nerves; group B consisted of nerves decellularized by Triton X-100 and sodium deoxycholate as described by Sondell et al.; nerves in group C were decellularized with sulfobetaine (SB)-10 and SB-16, based on the protocol of Hudson et al. and finally, nerves in group D were decellularized with Triton X-100 and enzymes as recently described by Roosens et al. Histological, biochemical and tensile analyzes were carried out to evaluate the impact of the different detergent treatments on tissue structure and extracellular matrix (ECM) composition. **Results:** While nerves of group B seemed to have undergone severe structural damage with loss of glycosaminoglycans, the nerves of group C had better preservation of the ECM, but were not completely cell-free. On the other hand, nerves of group D showed a good balance between conservation of the nerve structure and removal of cellular components. Interestingly, HE and DAPI staining confirmed the absence of nuclear remnants in the nerves decellularized in group D. In addition, important ECM components such as laminin and collagen I were well preserved, maintaining a homogeneous tissue distribution. **Discussion:** Finally, Roosens' combined decellularization method showed superior results in the decellularization of rat sciatic nerves as compared to Sondell's and Hudson's methods. However, in order to determine the potential clinical

usefulness of these decellularized nerves, it is still necessary to perform future in vivo studies.

ACKNOWLEDGEMENTS: This study was supported by the Spanish Plan Estatal de

P812 Exploring macrophage interactions with zwitterionic hydrogels

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Silicone (PDMS) has been widely used in the clinic and it is generally accepted as a ‘‘biocompatible’’ material. However, medical complications related to implanted silicone devices are increasingly reported. Female patients with silicone breast prostheses are susceptible to fibrotic encapsulation (Fig.1a) with an incidence of 50-70%, as a result of the foreign-body- response (FBR) triggered by the immune system. Thus, it has become increasingly important to develop new strategies to combat the FBR.

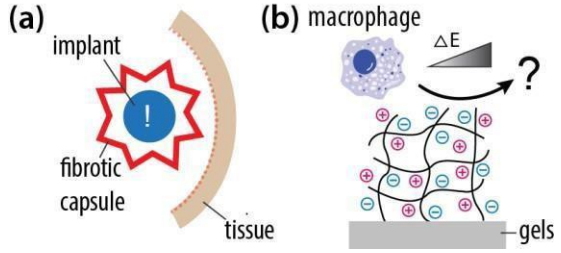

Figure 1. ? (a) Schematic showing the medical problem related to silicone implants; (b) sketch of the zwitterionic hydrogel strategy used in this study.

Inspired by the nonfouling properties of phosphorylcholine (PC) lipids in cell membrane, we designed photocrosslinkable zwitterionic hydrogels based on methacrylated PC (MPC), as potential anti-FBR materials. We seek to investigate: 1) how macrophages, the key players in FBR, interact with zwitterionic hydrogels; and 2) whether decision-making of macrophages depends on the physical properties of substrates.

MPC gels and PDMS (control) were prepared with tunable mechanical properties as validated by in situ rheometry. Human THP-1 monocytes were plated on the samples while being differentiated into macrophages by PMA for 3 days. A large number of macrophages attached onto PDMS, whereas on MPC gels a very low degree of cell attachment could be observed. Importantly, cell viability analysis of the supernatants show that most of the non-adherent cells were still alive (>90%), indicating the good cytocompatibility of MPC gels. Confocal imaging show that cells on PDMS had significantly larger sizes and with more podosome protrusions than those on MPC gels. By tuning the substrate elasticity, we found a decreasing extent of cell attachment on softer substrates, implying the contribution of biophysical factors in macrophage attachment.

In summary, our findings suggest new design principles for future anti-FBR biomaterials in tissue engineering and regenerative medicine applications.

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P813 A novel approach for the synthesis and fabrication o f naturally derived biocompatible β-TCP scaffolds through mechanical stimulation

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Naturally derived bioceramics are widely used as implants for bone and dental defects. In the present study, eggshell derived β-tricalcium phosphate (β-TCP) scaffold is fabricated using polyurethane foam, as a template to simulate a potential bone graft analogue. CaO derived from egg shells, a biological waste material, is mixed in a specific molar ratio with phosphoric acid to synthesize biphasic calcium phosphate (BCP) bioceramic material. The same molar ratio of CaO and phosphoric acid was employed to derive β-TCP by mechanically stimulating CaO raw material aided with ball milling process. The importance of mechanical stimulation in stabilizing the phase of β-TCP, a better bone resorbing material is detailed in the current report. The physico- chemical characterization of the fabricated BCP and β-TCP scaffolds was carried out using XRD, FT-IR and SEM for the analysis of phase behaviour and surface morphological properties. Similar scaffolds were fabricated with β-TCP powder obtained from a wet chemical precipitation route were compared with the developed BCP and β-TCP scaffolds of natural origin. Biocompatibility of all the groups of scaffolds is tested by performing a FDA/PI live-dead staining on adipose derived h-MSCs seeded scaffolds for a period of 21 days. Alamar blue assay was performed for the same time period in order to analyse the metabolic activity and cell proliferation ability of seeded cells on the developed scaffolds. The SEM analysis of cell seeded scaffolds in this time period was carried out for analysing the effect of the topographical change of scaffolds and the proliferating stem cells. Eggshell derived BCP and β-TCP showed enhanced microstructure and biocompatibility as compared to chemically derived β-TCP. The mechanical stimulation through ball-milling played an important role in stabilizing the phase of β-TCP rather than obtaining a mixed phase bioceramic. As β-TCP has its own advantages over BCP, the developed route of synthesizing β-TCP and developing scaffolds with improved cell adhesion and proliferation as compared to BCP pave way to develop promising bio-templates for bone tissue regeneration.

Keywords: β-TCP, Biphasic calcium phosphate, Eggshell, Mechanical stimulation

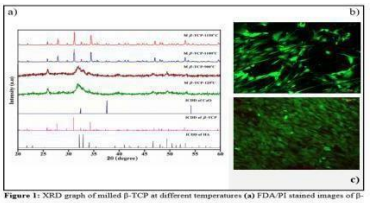

Figure 1: XRD graph of milled β-TCP at different temperatures **(a)** FDA/PI stained images of β-TCP scaffolds after 7days **(b)** and 21 days **(c)** obtained from a Fluorescence microscope.

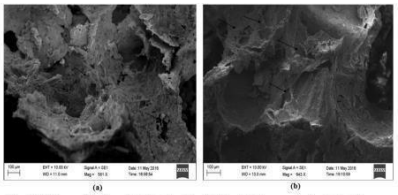

Figure 2: SEM images of β-TCP scaffolds after 7days (a) and 21 days (b) black arrows showing attached cells

P814 Keratins from Human Hair and Wool: isolation, characterisation and gel formation studies

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Keratins are natural proteins and can be extracted from wool and human hair by reduction methods [1-4]. Keratins have been found having potentials for the treatment of chronic wound as functional wound dressing materials. They can be fabricated into porous foams, sponges, mats, films, sheets, gels, microfibers, and bulk materials. In this study, keratins were isolated from human hair and wool using two methods, namely Na_2S method and Shindai method according to published procedures [3,4]. The results showed that Na_2S method achieved a higher overall yield (Table 1). The isolated keratins were characterised by NMR, FTIR, Raman, size exclusion chromatography (SEC) and Scanning Electron Microscopy (SEM). The thiol content of keratins were determined using Ellman's reagent (5,5'-DITHIO-BIS(2- NITROBENZOIC ACID, DTNB). The molecular weights of keratins were determined by SEC using Agilent 1100 HPLC instrument equipped with BioSep Sec 2000 column and UV detector at 30°C. BSA and Lysozyme were used as the standards. The flow-rate was 1.3ml/min and the eluent was phosphate buffer of pH 7.44. Keratins showed two peaks in SEC curves with Mp as 60 KDa and 13.5KDa. The isolated keratins were used to prepare hydrogels in the present of PEG diacrylate (entries 5 and 6 in Table 1) to tailor the hydrophilic/ hydrophobic and mechanical properties of the gels. Swelling studies were performed on these gels.

Table 1: Isolation and gelation of keratins from human hair and wool

No.	Source	Isolation method	Yield (%)	Thiol Content (µM/g)	Keratin: PEG diacrylate (Weight ratio)	Gelation time*
1	Hair	Na_2S	51	13	-	-
2	Hair	ShinDai	41	35	-	-
3	Wool	Na_2S	40	25	-	-
4	Wool	ShinDai	30	15	-	-
5	Hair	ShinDai	41	35	50:50	overnight
6	Wool	ShinDai	18	15	50:50	overnight

P815 The human amniotic membrane's metabolic landscape

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The human amniotic membrane (hAM) has been used in regenerative medicine for many decades in a devitalized form. More recently, however, it became evident that the hAM is a rich source of stem cells. To exploit the regenerative capacity to a full extent, the hAM is now also considered to be applied in its vital form, containing vital cells with functional cell organelles.We investigated mitochondrial respiration, ATP concentrations, lactate concentrations, levels of reactive oxygen species (ROS) and succinate dehydrogenase in two sub-regions of vital hAM, placental and reflected amnion.

Mitochondrial respiration was monitored by high-resolution respirometry. ATP concentrations were assessed and lactate concentrations were quantified with a blood gas analyser radiometer. Levels of ROS and succinate dehydrogenase concentrations were measured with electron paramagnetic resonance spectrometry.

Placental and reflected amnion differ distinctly in mitochondrial activity, as the placental sub- region of fresh hAM shows significantly higher mitochondrial respiration. Furthermore, we found higher levels of ATP and succinate dehydrogenase in placental amnion. Inhibition of ATP synthase led to elevated lactate levels in placental amnion, indicating increased glycolysis. Interestingly, this switch was not observed in the reflected amnion. Despite higher respiratory activity, we found lower levels of intracellular ROS in placental amnion, however, higher levels of extracellular ROS.

Investigations of viable hAM demonstrated distinct metabolic differences of placental and reflected amnion. In particular, increased glycolysis and higher contents of succinate dehydrogenase in placental amnion could be an indication that these cells have a higher ability to adopt their energy metabolism according to the energy “supply and demand”. This could be an advantage, as the site of injury usually represents a harsh microenvironment with high degree of inflammatory factors, immune cells and hypoxia. Taking advantage of this knowledge could allow a more customized application of the hAM.

P816 Mimicking the structure of fracture hematomas using snake venom enzymes for the creation of an ex vivo natural designer scaffold to enhance bone repair

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Despite of the regenerative capacity of bone, the treatment of large segmental bone defects and fractures showing delayed healing or non-union remains a major clinical problem. When fractures occur the blood coagulation cascade is activated, leading to the formation of a structurally mature clot. This hematoma is crucial to the initiation of bone healing as it serves as a reservoir for growth factors as well as a space for cell infiltration, and a guiding structure for ingrowing blood vessels. In our previous studies we found discernible structural differences in blood clots formed in healing (1 mm) vs. non-healing (3 mm) cortical rat defects. The 1 mm clot had a more porous structure and thicker fibrin fibers, and repaired bone more rapidly compared to 3 mm clots. Recently, snake venoms have been explored medically for uses other than anti-venom, since the pro-coagulating factors are structurally and functionally similar to those normally found in mammals. Therefore, the aim of this study was to explore the ability of the pro-coagulant *ecarin*, isolated from saw-scaled viper venom (*Echis carinatus*), to alter the structural properties of a blood clot in an attempt to mimic the hematoma of self-healing fractures in order to improve bone healing.

Whole blood was collected from mice and one human volunteer. Purified *ecarin* was added at various concentrations from 0.05 U/mL to 2.5 U/mL to citrated blood, which was allowed to coagulate fully before 4% paraformaldehyde was added and the clots were fixed. Cell and fibrin fiber morphology was analyzed by scanning electron microscopy.

The results showed distinct clot morphologies dependent upon the concentration of *ecarin* and the species used. Un-citrated mouse whole blood showed normal disc-shaped erythrocytes, which were entangled in a loose fibrin mesh. Erythrocytes of human whole blood also had normal morphology, however, the fibrin mesh was packed more densely. At the lowest concentration (0.05 U/mL), half of the erythrocytes showed signs of crenation in the mouse blood clot. In contrast, only a few crenated cells were seen when *ecarin* was added to the human blood. At 0.6 U/mL, all cells appeared crenated when mouse blood was used, whereas only less than half of the human blood cells appeared crenated. At 2.5 U/mL, the erythrocytes appeared distended in mouse blood, while in human blood the cells appeared more crenated. Fibrin fibers were thinnest (~50 nm) at 0.05 U/mL, thicker (80-120 nm) at 0.6 U/mL and thickest (150-200 nm) at 2.5 U/mL.

This study demonstrates that the morphology of blood clots can be effectively manipulated using *ecarin*. In addition, fibrin fiber thickness is concentration dependent and resembles normally coagulated blood clots at the lowest concentration. Interestingly, this study showed that the morphology of blood clots was also species dependent. Although preliminary, these results suggest that snake venom coagulating factors could act as natural biological additives to produce *ex vivo* blood clots that could be used as a novel therapy to enhance bone repair. However, how this would affect the repair of bone is not clear and requires additional studies to confirm these findings.

P818 Screening of Anabolic and Anti-inflammatory Effect of Biological Small Molecules for Treatment of Osteoarthritis

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Osteoarthritis (OA) is the most prevalent degenerative joint disorder that affects millions of patients worldwide. Due to the poor self-healing capacity of articular cartilage, there is currently no effective and standardised treatment available, neither for repair nor for prevention of onset or progression of this disease. The pharmacologic therapy for OA shows efficacy in pain relief but is frequently associated with adverse side effects that lead to the transition from pharmacological to biological therapy.

In this study we tested 40 small molecules with biological structure which are extracted from herbal Chinese medicine. Using a high-throughput screening method, the chondrogenic effects of a selection of 40 TCM (traditional Chinese medicine) compounds were assessed on human osteoarthritic chondrocytes in pellet cultures. Specifically, the DNA content and glycosaminoglycan (GAG) synthesis of the cells in response to different doses of TCM compounds are evaluated by using Hoechst dye and DMMB assay.

Our results showed that some specific compounds significantly increased the matrix production of chondrocytes. For instance, after 14 days of treatment of the pellets with different concentrations (25, 10, 1 µM) of the compounds, 4-Hydroxybenzoic acid showed significantly higher GAG/DNA compared to the negative control group. In the next step the anti-inflammatory effects of the compounds were investigated using an inflammatory model. For this respect, chondrocytes from osteoarthritic (OA) donors were chondro-differentiated in pellets for 2 weeks. OA pellets were exposed for 72 hours to IL-1ß/TNF-α and then cultured up to 14 days in serum free medium with different concentrations (25, 10, 1 µM) of the compounds. The GAG/DNA ratio was markedly reduced after induction of inflammation by IL-1β/TNF-α. Interestingly, the GAG synthesis was re-established after 14 days of treatment with 4-Hydroxybenzoic acid, whereas no significant recovery was observed in the negative control group.

In conclusion, 4-Hydroxybenzoic acid increased cartilage matrix production of human OA chondrocytes towards a healthy phenotype and also had anti-inflammatory effects in our inflammatory model. In further experiments, a hyaluronan based release system for the delivery of the bioactive compound will be optimised and the bioactivity of released compound in terms of cartilage repair will be tested.

Successful completion of the project will provide a new minimally invasive therapy for early OA with the potential to be applied in pre-clinical studies. Ultimately, reducing inflammation and restoring the joint function will relief disabling pain in a large number of patients worldwide and will significantly lower health care costs.

P819 Surface functionalization of chemically treated Ti6Al4V alloy with mentha piperita essential oil

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Mentha piperita essential oil is a complex mixture of different natural biomolecules with proven antibacterial activity against Gram-positive and Gram-negative bacteria, fungi and viruses [1] and it has been widely used in folk medicine for various applications. Despite its increasing use in phytotherapy, application of peppermint essential oil in medicine is still quite scarce and almost no research work considers its application in combination with biomaterials. On the other hand, the possibility to employ the antibacterial properties of this essential oil together with its low resistance development risk and its low toxicity is a challenging approach for the development of smart biomaterials for prosthetic applications. The aim of the work is to combine the antibacterial properties of peppermint essential oil with those of bioactive titanium alloy for applications in which good osteointegrability and antimicrobial effects are required. The peppermint essential oil was used for the surface modification of Ti6Al4V made bioactive with a patented chemical treatment [2,3]. Different concentrations of peppermint essential oil in Ethanol (20-100%) were considered for the treatment. The obtained samples were characterized by means of XPS and FTIR analyses, wettability tests, z potential measurements and antibacterial tests. XPS analyses evidenced a significant increase in the carbon content for the surface modified samples as well as a peculiar modification of the high resolution spectra of the oxygen region. FTIR analyses highlight the presence of three peaks (2950, 2920 and 2870 cm^{-1}), attributable to chelate compounds: primary alcohols, Aliphatic -CH_3 and -CH_2 stretching, O-H and C-O stretching characteristic of the organic compounds in essential oils, on the modified Ti6Al4V alloy. The wettability tests show a reduction of the wettability after the modification with the peppermint essential that could be related to the antibacterial effect. Z potential highlight a shift of the isoelectric point to a less acidic value and the appearance of a plateau in the basic region after the surface functionalization process. The antibacterial tests shows a reduction of the adherent bacteria on the surfaces.

In conclusion, the research work shows the great potential of the use of peppermint essential oil for surface functionalization of biomaterials with enhanced antibacterial properties. Furthermore, the use of the *Mentha piperita* oil of Pancalieri is consistent with the European policy of exploitation of the local regional natural resources (Piedmont) .

P820 Bioactive and antibacterial Ti6Al4V for bone contact applications

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Silver is a well-known antibacterial agent with broad spectrum activity and low resistance development [1]. An increasing interest is registered for silver nanoparticles because of their large surface area and multiple mode of action (damage of the cell membrane, induction of ROS and release of silver ions) [2]. Despite of a wide research on surface enrichment of titanium surfaces with silver, the identification of an affective technology is a still an open issue. The aim of the present research is the surface modification of Ti6Al4V alloy in order to induce bioactive and antibacterial behaviour. The proposed surface modification process is based on a patented chemical treatment [3,4] that confers to the surface oxide layer a complex topography (micro and nano-scale roughness), and enrichment of hydroxyls groups, suitable for bone contact applications because of its bioactive behaviour (induced precipitation of hydroxyapatite). The research is focused on the introduction of a silver precursor into the process at three different steps (T1-T3) of the patented chemical treatment (CT) with additives (AD) in order to induce the precipitation of silver nanoparticles within the surface oxide layer and to avoid their agglomeration (CT+AD+Ag_T1, CT+AD+Ag_T2 and CT+AD+Ag_T3). FESEM, TEM and XPS analyses, ion release tests and antibacterial tests were performed in order to characterize the surfaces. FESEM, EDS and XPS analyses show the presence of well dispersed metallic silver nanoparticles with dimensions depending on the experimental procedure applied. TEM analyses performed on the cross section of the samples highlight different distribution of the nanoparticles in the thickness of the oxide layer. With the release test it was also possible observe a sustained and reproducible release up to 14 days for each kind of sample (Figure1). Antibacterial properties were evaluated by Kirby Bauer test and by bacterial adhesion test with *Staphylococcus aureus* ATCC 29213. Kirby Bauer test show an inhibition zone about 3mm around the samples due to the silver release from the surfaces. The adhesion test highlights a reduction of both adherent and not adherent bacteria compared with the control. Considering that infections are still a serious complication in orthopaedic and dental fields, and that bacterial resistance to antibiotics is becoming a critical problem in their treatment, the here proposed surfaces are promising for implants that require osteointegration and antibacterial activity, such as prosthetic cups, steams and dental screws.

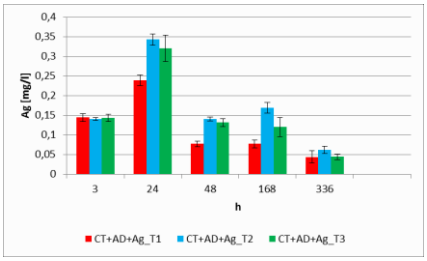

Figure 1:Silver release

[1] L.G. Ovington, Ostomy Wound Manage. 50 1S–10S, 2014; [2] M. Rai et al., Biotechnol. Adv. 27, 76–83, 2009; [3] S. Spriano et al., European Patent 2214732 ;[4]S.Ferraris et Al., J Mater Sci: Mater Med 22, 533–545, 2011

P821 Biosurfactant-based coatings inhibit fungal and bacterial biofilm on medical-grade silicone

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Introduction and aim: Microbial biosurfactants have recently emerged as a potential new generation of anti-adhesive and anti-biofilm agents for medical device coatings with enhanced biocompatibility. Aim of the work was to evaluate the ability of AC7 and R89 biosurfactants (AC7BS, R89BS) medical-grade silicone disks coatings to inhibit biofilm formation of *Candida albicans*, *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Methods: AC7BS and R89BS were extracted from *Bacillus subtilis* and *Pseudomonas aeruginosa* cell-free supernatants according to [1] and the composition of the crude extracts evaluated by ESI-MS analysis. The selected biomaterial was functionalized following two different strategies: direct BS physical absorption (silicone-BS disks) and plasma treatment followed by BS absorption (silicone/P-BS disks). Anti-biofilm activity against bacterial and fungal biofilm- producer strains was investigated by the crystal violet staining and MTT assays to evaluate, respectively, biofilm biomass and cells metabolic activity at different time-points (1.5h, 24h,48h).

Results: Chemical characterization revealed that AC7BS is a mixture of surfactin and fengycin and R89BS is a mixture of mono- and di-rhamnolipids. Both coating strategies promoted a significant inhibition of biofilm formation for all the tested strains and, in general, treatments with R89BS resulted to be more effective. In particular, on silicone-R89BS disks, biomass and metabolic activity of fungal biofilms were respectively reduced up to 73% and up to 64% at the last time-point (48h). A significant inhibition was also observed on *S. aureus* biofilms, with similar values on biofilm biomass (up to 78% at 48h) and on metabolic activity (up to 72% at 48h). Interestingly, *S. epidermidis* biofilm formation was mostly reduced by R89BS in terms of metabolic activity (up to 53% at 48h) than of biofilm biomass (up to 7% at 48h). On silicone-AC7BS disks, fungal and bacterial biofilms were respectively inhibited up to 62% and 74% at 48h. On silicone/P-AC7BS disks, biomass and metabolic activity of fungal biofilm were averagely reduced of 52% at 1.5h and of 45% at 24h and on silicone/P-R89BS disks of 68% at 1.5h and 70% at 24h. *S. aureus* biofilm formation at 24h was inhibited of 51% on silicone/P-AC7BS disks and of 75% on silicone/P-R89BS disks. Regarding *S. epidermidis*, no relevant biofilm reduction at 24h was detected on silicone/P-AC7BS disks but an inhibition of 56% was observed on silicone/P-R89BS disks. Finally, AC7BS did not inhibit fungal and bacterial planktonic cells, indicating anti-adhesive but no antimicrobial activity. Similarly, R89BS had no activity against *C. albicans* and *S. epidermidis* planktonic cells, whereas for *S. aureus* an antibacterial action was observed. Assays for the evaluation of the activity of AC7BS and R89BS on pre-formed fungal and bacterial biofilms on silicone are in progress.

Conclusions: AC7BS and R89BS are able to significantly inhibit fungal and bacterial biofilm formation on silicone and plasma treatment prior to BS adsorption seems to be a promising method for BS functionalization of medical-grade silicone.

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P822 Three-dimensional human skin wound infection equivalent as a tool for bioanalysis of antimicrobial polymeric biomaterials for wound dressings

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An *in vitro* wound infection model based on human cells is highly demanded representing a biomimetic system of the *in vivo* state of chronic wounds for the testing of antimicrobial polymeric biomaterials (AMBs). The main purpose of this 3D wound infection model is to serve as an *in vitro* tool recapitulating enough biological response for the bioevaluation of antimicrobial and wound healing properties of novel AMBs. This work was aimed at the development of an *in vitro* skin model able to maintain cell viability over time. The 3D skin equivalent was obtained having both a dermal and an epidermal compartment, by embedding human primary fibroblasts in rat tail tendon collagen type I hydrogel (mimicking skin extracellular matrix) and then seeding human primary keratinocytes on it to generate the epidermal layer. Different fibroblast seeding densities (0.04-0.16x10^6 cells/mL) and matrix concentrations (1.5-3.0 mg/mL) were evaluated to determine the cell morphological and matrix remodelling differences. This model was then inoculated with clinically challenging bacteria e.g. Staphylococcus aureus and group A Streptococcus at wound site, to generate a 3D wound infection model. The model was characterized by immunohistochemical analysis (Anti Cytokeratin 5/6, Cytokeratin 10 and Filaggrin antibodies), SEM and fluorescence-microscopy analysis using z-stack imaging (Promokine Live/Dead and Phalloidin/DAPI). To assess the viability of the system quantitatively, different cell viability and cytotoxicity assays e.g. CellTiter-Blue®, CytoTox-ONE™, RealTime-Glo™ MT, CellTiter-Glo® (Promega), XTT (Promokine) were evaluated to finally optimize the best suited assay to the respective cell types and eventually to the system. Immunohistological results demonstrated the development of dermis and remodelled intercellular connective soft tissue. The Z-stacked imaging revealed the filopodia like morphology and a uniform distribution of fibroblasts at different planes inside the matrix. However, no morphological differences were found among different collagen I matrix concentrations. The “irregular” fibril formation by rat tail tendon collagen type I constitutes an *in vivo* like matrix, however in the future we will change it with a biofunctionalized synthetic hydrogel to avoid the use of xenogeneic materials such as collagen from rat origin, that is additionally characterised by batch-to-batch variations. CellTiter-Glo® was found to be the optimal cell viability assay among those analysed. Respect to CellTiter-Glo®, CellTiter-Blue® reagent affected cell morphology suggesting an interference with cell biological activity, resulting in less reliable data. On the other hand, RealTime-Glo™ did not provide a linear signal with even as low as 2500 cell number, which made this assay unsuitable for the system. Although a model based on human primary cells is clinically relevant, it is more sensitive than a model using cell lines. Therefore, after selecting the appropriate assay, cell viability in the 3D model was measured over time and the results showed that cells kept viable inside the matrix (min. for 20 days) that is a pre- requirement of the *in vitro* model. Next step will be the evaluation of gene expression and cytokine levels by keratinocytes to identify model skin response to bacteria that will also help exploring host-pathogen interaction and thus the antimicrobial strategies.

P823 Development of biomass-based carbon composites as an anti-microbial wound dressing material

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Development of advanced wound dressings that possess anti-microbial properties at wound surface and absorbed exudate to facilitate wound healing process is a challenge study. Most of foam dressings are made from polyurethane (PU). Because hydrophilic PU foams contain a number of porous small pores that have the ability to pull exudate away from the wound bed. In this study, we fabricated anti-microbial PU foams containing silver nanoparticles and biomass-based activated carbon. Then, the mechanical property, moisture absorption speed, absorptivity, morphology and cell viability of the composite foams were characterized. Anti-microbial activity of PU foams containing silver (Ag) nanoparticles was measured by calculating the number of bacteria as a function of silver nanoparticles over time. For Ag-PU foams, the anti-microbial activities for two types of bacteria (*Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352)) reached 99.9% by adding 0.025% of silver nanoparticles after 18h. PU foams containing silver and activated carbon showed significantly effective wound healing compared to controls. It will provide crucial clue as a functional wound dressingmaterial.

P824 Medical-grade silicone coated by AC7 biosurfactant inhibits fungal biofilm formation preserving biocompatibility

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Introduction and Aim: Coating of medical-grade materials with biosurfactants (BS) produced by bacteria is a promising strategy to limit pathogenic biofilm growth on the surface of implantable medical devices. This study aimed at assessing fungal antibiofilm activity and biocompatibility of medical-grade silicone coated by a BS lipopeptide.

Methods: AC7BS was extracted from cultures of *Bacillus subtilis* AC7 according to Rivardo et al. 2009. Sterilized medical-grade silicone elastomeric disks were BS coated by physical absorption (dipping in 2 mg/ml AC7BS for 24 h and drying). Sterilized uncoated disks were used as controls. Biofilm of *Candida albicans* IHEM 2894 on treated and control disks was formed as described by Ceresa et al. 2016.

The anti-biofilm activity was evaluated at 1.5, 24, 48 and 72h. Biofilm biomass, fungal viability and percentage of biofilm-covered surface were quantified by crystal violet staining, MTT assay and scanning electron microscopy (SEM), respectively. Cytotoxicity was evaluated by LDH assay (ISO 10993) using human normal lung fibroblasts (MRC5), and testing AC7BS concentrations equal to 2.0, 1.0, 0.5, 0.4, 0.3, 0.2, 0.1 mg/mL.

Inhibition of biofilm biomass, fungal viability and biofilm-covered surface was studied in respect to controls. Statistical significance was considered for $p<0.05$

Results: AC7BS coated silicone was able to significantly counteract fungal biofilm in terms of biomass, cells viability and biofilm-covered surface at all tested time-points (Fig.1). No cytotoxic effect on eukaryotic cells was observed at AC7BS concentrations up to 0.5 mg/mL.

Conclusions: AC7BS was able to significantly inhibit fungal biofilm formation on medical grade silicone. The long-term effect on fungal biofilm and the low cytotoxicity make this BS a promising compound for realizing implantable silicone medical devices with effective antibiofilm properties.

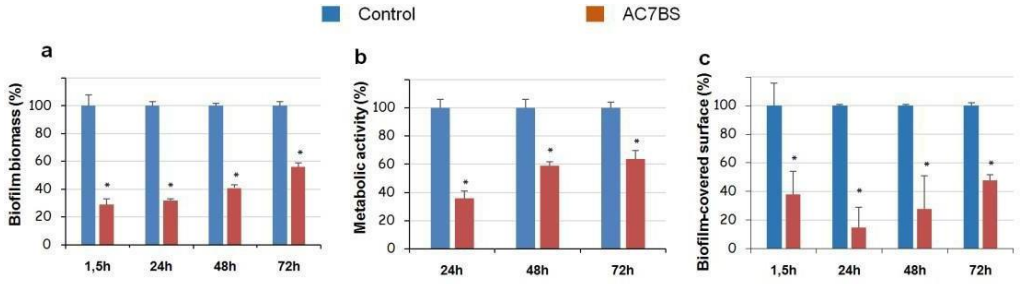

Figure 1: Antibiofilm activity of AC7BS coated silicone disks. Inhibition of biofilm biomass, fungal viability and biofilm-covered surface is reported as percentage of controls (uncoated disks). Time-points refer to biofilm maturation age. * $p<0.05$.

This research is supported by Compagnia di San Paolo.

P825 Novel natural polymers with antibacterial properties

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Preventing the rise and spread of infections has become a fundamental requirement for the successful applications of medical implants. The introduction of a device inside the human body is inherently associated with the risk of infection, defined as a foreign body related infection[1]. Generally, if a mature biofilm is formed, the only solution available is to remove the implant which increases the patient's distress and raises health costs due to the need of a second surgery. Hence, researchers have been focusing on the development of new therapeutics to prevent bacterial attachment and proliferation. One of the main strategies is to develop inherently active antibacterial materials using polymers possessing bactericidal effect or introducing antibacterial groups in their structure through chemical modification.

Polyhydroxyalkanoates (PHAs) are biological polyesters obtained through bacterial fermentation. They have been widely studied for biomedical applications due to their biodegradability and biocompatibility[2].

The main aim of this project is the production of PHAs with antibacterial properties and their modification and functionalization for their use in bone and cartilage tissue engineering applications. Two strategies are being investigated to fulfill this objective. The first is to produce PHAs with thiol groups (S-PHAs) in the side chains using bacterial fermentation. Recently, PHAs containing thioester linkages in the side chains have been shown to possess antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) both *in vitro* and *in vivo*[3]. In this work, S-PHAs were produced via bacterial fermentation using *Pseudomonas putida* KT2442 using a co-feeding experiment with fatty acids (i.e. decanoic acid) as the "good" substrates to support bacterial growth and carbon sources containing thiol groups for polymer production (i.e. 6-acetylthiohexanoic acid). The polymer showed antibacterial activity against *Staphylococcus aureus* ATCC 6538 (ISO 22196). Blending of the material with mcl and scl-PHAs is currently under investigation to produce 2D and 3D antibacterial structures to be used for bone and cartilage tissue engineering scaffolds. The use of other thio-group containing carbon substrates is also being studied to obtain a range of novel S-PHAs with inherent antibacterial properties.

The second strategy is to chemically functionalize PHAs through radical grafting copolymerization to introduce antibacterial moieties in the polymer side chains[4]. Mcl and scl- PHAs were produced via bacterial fermentation by *Pseudamonas mendocina* CH15 and *Bacillus subtilis* OK2 respectively using glucose as the carbon source. The antibacterial activity of the functionalized polymers is currently under investigation.

This work is part of the HyMedPoly project which has received funding from the European Union's Horizon 2020 research programme under the Marie-Sklodowska-Curie Grant Agreement N° 643050

P826 Dual-action fluorophosphonate titanium coatings to reduce bacterial adherence and encourage osseointegration

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In England and Wales in 2015 approximately 187,879 total hip and knee replacements were performed of which 8% (15,027) were performed due to failed implants. The main cause of failure was aseptic loosening, accounting for 37% of failed hip replacements and 40% of failed knee replacements. Previous research has found that functionalising titanium with lysophosphatidic acid (LPA) induces an increase in human osteoblast maturation on the implant surface through co-operation with active metabolites of vitamin D3. This feature, the small size of the LPA molecule and its affinity to readily bind to titanium and hydroxylapatite makes it an especially desirable molecule for bone biomaterials. Nevertheless biomaterials that also demonstrate anti- microbial properties are highly desirable.

A fluorinated analogue of LPA, (3S)1-fluoro-3-hydroxy-4-(oleoyloxy)butyl-1-phosphonate (FHBP), was employed to coat titanium discs using a one-step facile coating process. The coating was characterised using X-ray photoelectron spectroscopy, surface roughness measurements and contact angle measurements. MG-63 osteoblast-like cells were cultured on the surface of the coated titanium discs and assays for alkaline phosphatase activity were performed. To test the antimicrobial efficacy of the FHBP-coated titanium, a clinical isolate of *Staphylococcus aureus*, obtained from an infected revision surgery, was cultured on the surface of titanium discs functionalised with 0, 0.1. 0.5, 1, 2 and 5μM LPA. Bacterial adhesion was quantified at 1, 2, 6, 12 and 24 hours by live/dead counts and biofilm mass quantified by crystal violet staining after 24, 48, 72 and 96 hours culture.

Low concentrations of FHBP (500nM to 2μM) were found to significantly increase alkaline phosphatase activity (ANOVA, $p<0.005$) and significantly inhibit bacterial adhesion (Figure 1, ANOVA, $p<0.001$). These concentrations also reduced biofilm mass on the surface of the titanium.

These coatings have the potential to enhance implant osseointegration whilst simultaneously reducing bacterial attachment. This technology may reduce both septic and aseptic failure of cementless joint prostheses, ultimately prolonging implant longevity and patient quality of life.

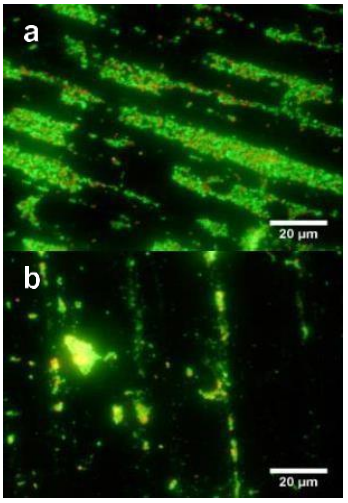

Figure 1. Live/dead staining of *S. aureus* on (a) plain titanium and (b) 1μM LPA-functionalised titanium after 24 hours.

P827 Metal and polymeric components with antibacterial surfaces fabricated via ultra-short pulsed laser ablation and thermal processing technologies

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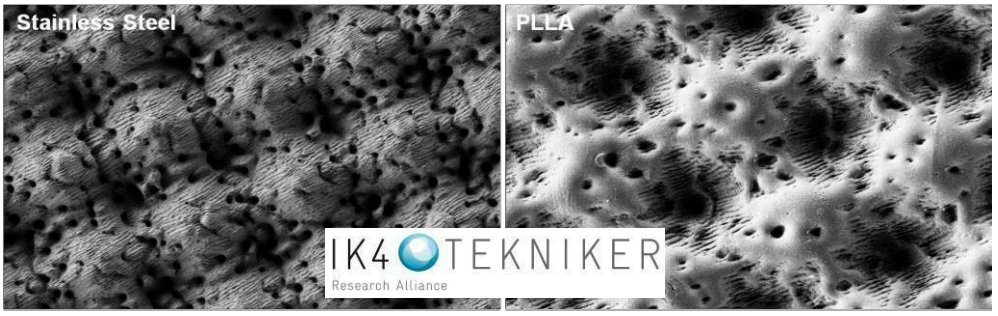
Self-sterilizing surfaces are becoming more popular as a strategy to prevent bacterial colonisation and biofilm formation on industrial components. Current antibacterial technologies involved the application of passive or active surface modification methods and the use of antibacterial local carriers or coatings. Active surface modification methods and coatings with antibacterial agents, although some of them are already in the market, still show limitations related to the induction of bacteria resistance, no effect on biofilm formation, low versatility and applicability, and a questionable long-term toxicity. On the contrary, passive methods, without releasing antibacterial agents, allow overcoming some of these limitations. Among these, surface modification with nano- and microstructures has emerged as a potential tool for fabrication of functionalised surfaces with antibacterial properties. The focus of this work is to obtain bio-inspired topographies on metal and polymeric components toward antibacterial surfaces, by the combination of direct laser topographical modification and thermal processing technologies to produce 3D parts with antibacterial surfaces.

We have tested and validated a surface pattern design that contains hierarchical structures made by means of a picosecond laser system coupled to a galvanometric scanner [Patent in progress]. This technology allows high structuring speed (up to 2000 mm/s) covering a long surface area (up to 35x35 mm^2 for long focal length optics) without moving the scanner. Longer areas can be covered by axis interpolation with the galvanometric scanner. The hierarchical structures developed contain pyramidal features (at the microscale) covered by nanoripples (*laser*-induced periodic surface structure: LIPSS) (see figure below). Nanoripples are characterized by a period of ≈ 700 nm. It is important to remark that this period depends on the laser wavelength which can be tuned within 355 nm to 1064 nm range.



Results corresponding to the antibacterial activity of these structures produced by laser in Titanium showed that the reduction on bacterial proliferation reached high values for S. Aureus and S. Epidermidis (R >70 %) [Assays made by an independent laboratory: Laboratorio Microbiología de Alimentos y Aguas: Universidad de Navarra]. The durability of the surface structures was evaluated under abrasion tests, observing that the texturing and the hydrophobic behaviour of the surface (linked to the antibacterial activity) were maintained practically unaltered. These microstructured metallic parts were used as moulds for fabrication of microstructured polymeric components by thermal processing methods. This approach was adopted in order to overtake current limitations of antimicrobial surface solutions in terms of industrial scalability, durability and versatility. In this scenario, the main challenge to face is to guarantee the transfer capability of micro and nanofeatures from the metal mould to the plastic part, reaching the final antibacterial functionality on the target materials.

P828 Surface functionalization strategies to inhibit biofilm formation on silicone materials for implantable devices

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Introduction

Silicone is frequently encountered in many biomedical applications (e.g. syringes and catheters) due to its moulding properties, chemical inertness and high oxygen permeability. However, the high level of hydrophobicity of silicone has been shown to allow bacterial colonization with subsequent biofilm formation on the surfaces of the medical devices increasing the risk of infection and limiting the devices life time. In this work, silicone surface functionalization techniques with a biological antifouling agent, AC7 biosurfactant (AC7BS), were employed in order to prevent biofilm formation.

Materials and Methods

AC7BS was extracted from cultures of Bacillus subtilis AC7. To ensure AC7BS sufficient coating, sterilized silicone disks were plasma-activated using argon as plasma gas (5 min, 15 sccm, 100W). Then, AC7BS functionalization on silicone was performed by two distinct approaches: i) AC7BS physical absorption (dipping in 2 mg/ml AC7BS solution) (Fig. 1A); ii) AC7BS covalent grafting using an aminosilane (APTES) to introduce amino groups to silicone prior to immobilization of AC7BS via carbodiimide chemistry (EDC/NHS)(Fig.1B). Morphological and physicochemical characterisation was performed on both AC7BS modified surfaces.

Results

The developed surface functionalization strategies were found to significantly increase hydrophilicity of silicone. AC7BS after physical absorption or covalent grafting procedure was confirmed by XPS and ATR-FTIR analysis. Silanisation on silicone activated surfaces was quantified by a colorimetric method and the subsequent AC7BS covalent grafting was evaluated by liquid chromatography–mass spectrometry (HPLC-MS) and corresponded to 22% of the total AC7BS amount.

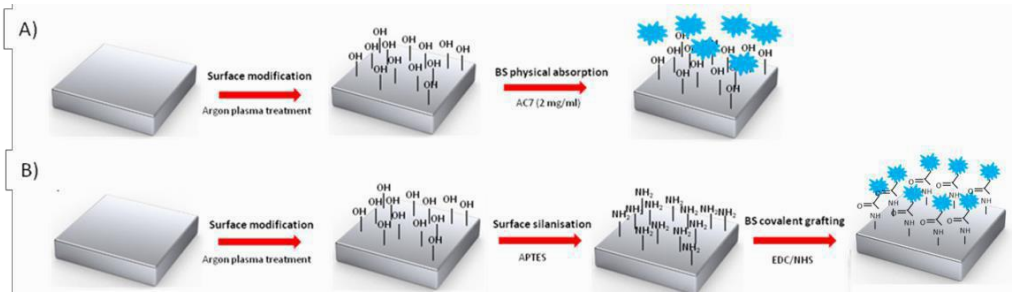

Fig.1 Schematic representation of AC7BS silicone functionalisation strategies: A) AC7BS absorption and B) AC7BS covalent grafting on activated silicone surfaces.

Conclusion

These results suggest the potential use of both functionalization approaches on silicone as anti-adhesive and antimicrobial coatings for silicone medical devices. Future works will be aimed to confirm the inhibition of fungal and bacterial biofilm growth on silicone.

P806 Tracheal reconstruction with free vascularized myofascial flap: preclinical investigation in porcine model

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Background:

The management for tracheal defect has been challenging for many years. Up to now, tracheal reconstruction methods utilizing allogeneic trachea from donor, autologous substitutes like esophagus or aortic grafts, materials from tissue engineering technique have been reported. However, none of these methods has been proved to be constantly effective. This study aimed to investigate the feasibility of the tracheal reconstruction with a vascularized muscle fascia using superior epigastric artery perforator (SEAP) flap in preclinical porcine model with tracheal defect. This study establishes the theoretical foundation for the feasibility of tracheal reconstruction using vascularized myofascial flap.

Materials and Methods:

Four female Yorkshire pigs weighing 25 to 30 kg were obtained. After general anesthesia was performed, a vertical midline incision in the anterior neck was made. The trachea was exposed. The common carotid artery and the internal jugular vein were exposed and prepared for the anastomosis. The window was made in 1 2nd and 3rd tracheal cartilage. The SEAP flap harvest was performed. The microvascular anastomosis was performed to the common carotid artery and the internal jugular vein. Then the tracheal window was covered and sutured with the muscle fascia of the SEAP flap. The skin paddle of the SEAP flap was exteriorized and sutured to the cervical midline incision. The bronchoscopic examination was done in 1 week, 4 weeks, and 10 weeks after operation.

Results:

We performed tracheal reconstruction using myofascial tissue with SEAP free flap in 4 Yorkshire pigs. The first pig with tracheal defect measuring 1.5 cm x 1 cm expired one day after the operation. Autopsy revealed the collapsed airway with intraluminal bulging of muscle tissue. The other 3 pigs' tracheal defects were made as 1 cm x 1 cm to prevent airway collapse. Among those, one pig expired 3 days after the operation due to the venous congestion and consequent flap failure. Two pigs are healthy and revealed patent airway on the bronchoscopic examination until the last follow up conducted in postoperative 10 weeks. The defect area covered with myofascial free flap was healed without serious granulation.

Conclusion:

The reconstruction of tracheal defect with free vascularized myofascial flap is feasible and highly compatible with surrounding airway mucosa. The possibility for the replacement of circumferential tracheal defects using this method combined with scaffold will be sought in the following study.

P830 Chondrogenesis-inductive nanofibrous substrate biofunctionalized with autologous growth factors from platelet lysates for stem cell based therapies

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Cartilage tissue engineering holds a remarkable potential in orthopaedics, as an alternative approach to currently available therapies. Mesenchymal stem cells (MSCs) have been used to generate engineered cartilage tissue, being the Transforming Growth Factor 3 (TGF- 3) and the Insulin Growth Factor I (IGF-I) potent regulators of chondrogenesis. Therefore, it is hypothesized that the availability of these two growth factors (GFs) at the surface of a biomaterial substrate would lead to the stable chondrogenic differentiation of MSCs. The present work aims to develop a biofunctional electrospun nanofibrous mesh (NFM) with chondrogenic inductive potential, through the immobilization of autologous TGF- 3 and IGF-I from platelet lysates (PLs).

Taking advantage of the specific and efficient interactions between an antibody and its antigen, antibodies against TGF-β3 and IGF-I were immobilized at the NFMs surface at the concentration of 4 μg/mL each. These antibodies were also immobilized over the same nanofibrous substrate at the 1:10 proportion, as used in standard chondrogenic differentiation medium. Higher binding efficiencies were observed for GFs immobilized from a pool of PL, by NFMs functionalized with mixed antibodies (99.3±0.4% for TGF-β3 and 77.5±2.4% for IGF-I). Biochemical performance of human bone marrow-derived MSCs cultured during 28 days on the biofunctional nanofibrous systems (Single or Mixed, with TGF-β3 and/or IGF-I captured from PL or from recombinant-origin (rGF)) under basal or chondrogenic differentiation media was assessed by quantification of cells viability and proliferation, and total protein synthesis, as well as glycosaminoglycans (GAGs) production. Biological data confirms the biological activity of bound TGF- 3 and IGF-I, since these biofunctional nanofibrous systems are more effective when compared to the control condition (Figure 1). The relative expression of chondrogenic transcripts confirms the genotype of hBMSCs cultured on the biofunctional nanofibrous systems. The typical round morphology of chondrocytes, as well as the Alcian Blue staining and the immunolocalization of Collagen Type II confirmed the formation of a cartilaginous ECM.

These biological results indicate that the functionalized nanofibrous substrates are able to promote chondrogenesis.

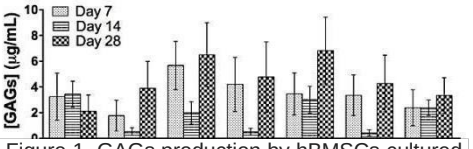

Figure 1. GAGs production by hBMSCs cultured on biofunctional nanofibrous systems (Single or Mixed, with TGF-β3 and/or IGF-I captured from PL or rGF) under chondrogenic or basal media.

The proposed biofunctional nanofibrous system can act as an effective cartilage tissue engineered scaffold, operating as a synthetic and bioactive ECM-like support of hBMSCs growth and differentiation.

Acknowledgments: We acknowledge the financial support from FCT/MCTES and FSE/POCH/PD/169/2013,for the PhD grant of MCN (PD/BD/113797/2015), and the projects SPARTAN (PTDC/CTM-BIO/4388/2014) and FRONthera (NORTE-01-0145-FEDER-0000232).

P832 Attenuation of human MSC hypertrophy in 3D culture via treatment with a retinoic acid receptor inverse agonist

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Introduction: Human Mesenchymal stem cells (hMSCs) are a promising source for articular cartilage repair [1]. Unfortunately, under *in vitro* conditions, chondrogenically differentiated hMSCs have the tendency to undergo hypertrophy that mirrors the fate of transient 'chondrocytes' in the growth plate [2; 3]. Retinoic acid (RA) signalling plays a key role in growth plate hypertrophy. Whilst RA agonists block chondrogenesis and foster hypertrophy during later stages [4,5], RAR inverse agonists (IA) enhance chondrogenesis when applied early in culture [6]. Therefore, we hypothesized that treatment of chondrogenically differentiated hMSCs with RAR IA, will attenuate hypertrophy in the during the hypertrophic stage of chondrogenesis. To test this hypothesis, we analysed early (initial chondrogenic differentiation) and late treatment (hypertrophy stage) of hMSCs with an RAR IA.
Methods: Pellets of passage 2 hMSCs were formed in V-bottom well plates by centrifugation and pre-differentiated in a chemically defined medium containing 10ng/mL TGFß (CM+) for 14 days. Thereafter, pellets were cultured for an additional 14 days under 6 conditions: CM+, CM- (w/out TGFß), and hypertrophic medium (CM- with 25 ng/ml BMP 4, w/out dexamethasone)[7]. Each of these first three conditions was additionally supplemented with the RA receptor (RAR) inverse agonist BMS493 (BMS) at 2µM after 14 days of chondrogenic pre-differentiation. One additional BMP4 group was supplemented with BMS from the beginning of chondrogenic differentiation until day 14 (CM+BMS/BMP4). On days 1, 14 and 28, pellets were assessed for gene expression (Col 2, Col 10, Col 1 and MMP13). Cartilage matrix and phenotype was assessed histologically using dimethyl methylene blue (DMMB), alkaline phosphatase staining (ALP) and collagen II and X immunohistochemistry.

Results: CM+/BMP4 hMSCs showed a hypertrophic phenotype with increased cell volume, collagen X content and ALP activity compared to CM+/CM+. Hypertrophy was reduced by addition of BMS at day 14 (CM+/BMP4BMS) and further reduced by addition from the beginning (CM+BMS/BMP4). BMS treatment resulted in smaller cells under hypertrophic conditions, higher collagen II content in chondrogenic groups (CM+/CM+BMS) and reduction in collagen X production and ALP activity in every condition. Gene expression data for hypertrophic markers, collagen X and MMP13, were upregulated under the influence of BMP4 but a distinct downregulation in MMP13 expression was down regulated upon addition of BMS during the late stage differentiation (CM+/BMP4BMS) and further reduced upon addition during early stage chondrogenesis (CM+BMS/BMP4). Furthermore, Collagen X expression was reduced by early BMS treatment.

Discussion: Chondrogenically differentiated hMSCs show hypertrophic conversion but the inclusion of the RAR IA, BMS, attenuated these hypertrophic changes as demonstrated by histology, immunohistochemistry and PCR. These findings suggest an additional approach to attenuate hypertrophy in chondrogenically differentiated hMSCs. Current studies are exploring the timing and dose of BMS to most efficaciously prevent hypertrophy. Furthermore, we are investigating the pathways through which BMS493 exerts its effects and will translate these findings in vivo.

Significance: The RAR inverse agonist BMS493 can attenuate hypertrophic conversion of hMSCs, and may be useful in producing stable engineered tissue for cartilage regeneration.

P834 A novel multi-layered and mono-fascicled nerve substitute. An in vitro and in vivo characterization study

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Introduction: Peripheral nerves (PN) are complex organs which have the function of communicating the central nervous system with peripheral target organs. PN are frequently affected by traumas, neuropathy and neoplasm. Short nerve defects can be surgically repaired, but critical gaps require the use of nerve guides or nerve grafts. Unfortunately, there are several drawbacks and limitations associated to these techniques. For this reason, the aim of this study is to generate a novel multi-layered and mono-fascicled nerve substitute containing adipose mesenchymal stem cells (ASC).

Methods: In this study, ASC were immersed on thin nanostructured fibrin-agarose hydrogels (NFAH), which were then used to fabricate a multi-layered, mono-fascicled nerve substitute (MLMF-NS). Substitutes were histologically and ultrastructurally analysed. For in vivo analysis, 1 cm of the left sciatic nerve was removed from a group of Wistar rats and the nerve was repaired by using autograft technique (controls) or MLMF-NS (n=5 in each group). After 12 weeks, animals were subjected to electromyography, functional tests, histology and volumetric analyses of distal muscles as a marker of atrophy.

Results: Ex vivo analyses showed a proliferative behaviour of the ASC, which were able to produce extracellular matrix molecules. In vivo analyses showed that nanostructured bio-artificial nerve guides could successfully bridge a critical nerve gap in the rat model. Electromyography confirmed the progressive re-innervation of the distal muscle in both groups. Time-course functional analyses showed a progressive recovery of the motor and sensory function in both groups, especially in autograft controls. Histological analyses confirmed the presence of the nerve substitute 12 weeks after implantation. Additionally, histological and the immunohistochemical analyses showed newly-formed axonal sprouts crossing the gap through the implanted substitute, especially in the autograft group. In autograft, the grafted nerves were completely remodelled and supported peripheral nerve regeneration at the epineurial, perineurial and endoneurial levels.

Discussion and conclusions: We have generated a novel biodegradable and mechanically stable multi-layered, mono-fascicled nerve substitute based on ASC and NFAH. These substitutes allowed to successfully bridge a critical nerve gap in rats, supporting a promising peripheral nerve regeneration profile from the functional and histological standpoints. These results were comparable to our previous studies in which ASC and FAH were successfully used to promote and increase peripheral nerve regeneration. However, these results were not completely comparable to the autograft group and therefore, additional improvement of this strategy is still needed in order to have an efficient and safe alternative to bridge critical nerve gaps.

Acknowledgements: This study was supported by the Spanish Plan Estatal de Investigación Científica, Desarrollo e Innovación Tecnológica, Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grant FIS PI14/1343 (co-financed by ERDF-FEDER, European Union).

P835 Generation and ex vivo characterization of crosslinked fibrin-agarose hydrogels for neural tissue engineering

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Introduction: Fibrin-agarose hydrogels (FAH) were widely used in different tissue engineered tissue models such as cornea, skin, cartilage, oral mucosa and peripheral nerve. These models showed promising results, and in the case of the cornea and skin, they were successfully transferred to the clinical use. Unfortunately, hydrogels are characterized by poor biomechanical properties, which in the case of the FAH were significantly increased by using nanostructuration techniques. Although this is an efficient method, it is still necessary to increase the FAH biomechanical properties for its use in the generation of neural devices, such as nerve guides. The aim of this study is to investigate the effects of glutaraldehyde crosslinking on the biomechanical and structural properties of FAH and nanostructured FAH (NFAH).

Methods: In this study, FAH and NFAH were generated by using human plasma, type VII-agarose and nanostructuration technique as previously described [1]. Generated hydrogels were crosslinked with 0.25 and 0.5% glutaraldehyde, subjected to rheological characterization and scanning electron microscopy structural analyses. Then, human fibroblasts were seeded on top of crosslinked and non-crosslinked FAH and the cell viability and *ex vivo* biocompatibility was assessed by live/dead, WST-1 and DNA quantification after 48h[2].

Results: Our results showed that glutaraldehyde cross-linking induced structural changes and significantly improved the rheological properties of FAH and NFAH. In addition, *ex vivo* biocompatibility analyses demonstrated that cells were viable in all conditions, although viability was more favourable when 0.25% glutaraldehyde was used.

Discussion: On the basis of our results, we hypothesize that our novel cross-linked fibrin-agarose-type VII hydrogels hold potential for use in neural tissue engineering applications, especially for the generation of biodegradable nerve guides. However, peripheral nerve regeneration *in vivo* studies will be needed to elucidate the regenerative potential of these biomaterials.

Acknowledgements: This study was supported by the Spanish Plan Estatal de Investigación Científica, Desarrollo e Innovación Tecnológica, Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grant FIS PI14/1343 (co-financed by ERDF-FEDER, European Union) and by RTC-2016-5207-1 (RETOS 2016 projects), Ministry of Economy and Competitiveness, Spain.

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P837 Mathematical model of a nerve guidance conduit combining physical and chemical gradients

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Introduction: Peripheral nerve injuries affect ~1M people in Europe and the US per year, of whom 600,000 have surgery but only 50% regain function. Although current clinical repair options have limitations (e.g. limited availability of donor tissue, donor site morbidity), tissue engineering provides a promising alternative with the opportunity to control tissue properties to guide neurite growth. However, there is no overarching consensus on how to spatially arrange biomaterials, cells and chemical factors within a repair conduit to achieve a particular functional outcome. Mathematical modelling provides a very powerful approach for evaluating conduit designs and directing experimental work, thus minimizing the need for laborious and expensive experiments[1]. The aim of this work is to inform the design of optimized nerve guidance conduits, specifically the spatial arrangement of chemical and material factors, using a combination of experimental data and mathematical modelling[1]. Methods: Fick's second law of diffusion was used to describe chemical (e.g. trophic factor) distributions, with initial/boundary conditions to mimic the experimental set-up; material factors are treated as fixed. Neurites produced at the proximal stump elongate in response to their local chemical and physical gradient environment. Simulations were run using experimentally measured parameters from the literature[2-3], and combinations of chemical and material distributions were explored to quantify the impact on neurite growth.

Results: Chemical concentration profiles evolve spatially and temporally and are predicted by the model, whereas physical profiles are fixed (Fig. 1). Model simulations show that neurites elongate in response to both the chemical and physical environment and by combining alternating chemical and physical cues, neurite elongation rates are increased

(Fig.2).Discussion and Conclusions: This *in silico* framework informs potential new designs for nerve tissue engineering. In the future, the most successful designs will undergo preliminary *in vitro/ in vivo* testing showing the benefits of an interdisciplinary approach[1]. Acknowledgements: This work was supported by the Department of Mechanical Engineering, UCL.

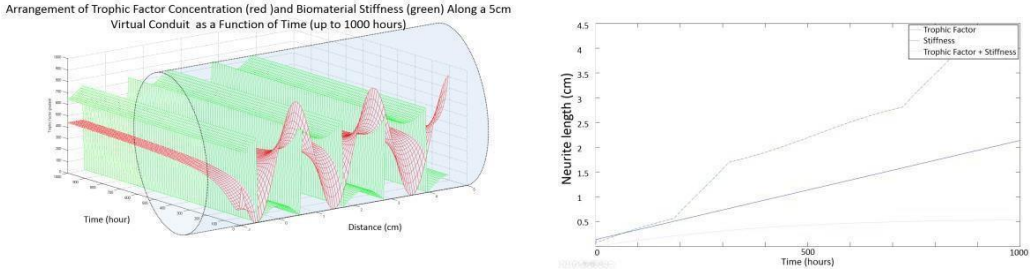

Fig.1) Example of a model of a nerve guidance conduit combining physical and chemical properties on time.

Fig. 2) Neurite growth as a function on time under different gradients (physical only/chemical only/combined)

P840 Development of aligned endothelial cells in collagen gels to promote nerve regeneration

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Background: Vascularization is important for nerve tissue engineering in terms of providing blood supply and nutrients for long-term survival of cells within construct. In addition, blood vessels in regenerating nerves have been shown to serve as tracks for Schwann cells to migrate along and thus form Bands of Büngner which promote axonal regeneration. In this study the aim was to develop a tissue-engineered construct, a tethered type-1 collagen gel with self-aligned endothelial cells that could help promote peripheral nerve repair.

Method: Type I rat tail collagen gels containing HUVECs (Human Umbilical Vein Endothelial Cells, 4×10^6 cells/ml) were cast in rectangular tethering moulds to facilitate cellular self-alignment. Aligned HUVEC gels were maintined for various times to determine optimal conditions for endothelial tube structures to form. To determine the effectiveness of the constructs as a substrate for the support and guidance of Schwann cell alignment and neuronal growth, a co- culture was established by seeding Schwann cells and neurons onto the surface of the cellular collagen sheets.

Results: Endothelial cells formed tubes after 4 days of culture, whereas at day 2 the majority of cells had elongated individually but not formed tubes. After 8 days of culture, tube-like structures were not significantly improved in terms of average length and alignment. Thus, 4-day culture period was established as the optimum culture time to achieve tubes. Schwann cells and long straight neurites were detected growing along the cell alignment axis.

Conclusions: These results demonstrated that the formation of tube-like structures using endothelial cells in tethered collagen gels can be achieved in 4 days of culture. Furthermore, the aligned endothelial cells can support Schwann cell alignment and neuronal growth *in vitro*.

P841 *In vitro, ex vivo* and *in vivo* analysis of novel 3D printed PGSm nerve guidance conduits for peripheral nerve repair

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INTRODUCTION: Three approaches exist clinically, which aid peripheral nerve regeneration; suturing, autograft surgery or the use of nerve guide conduits (NGCs). Conduits are an attractive alternative to autografts. Both synthetic and natural material-based NGCs are commercially available. Products are often simple designs with material properties far stiffer than the native tissue. This leads to current NCGs being ineffective for anything other than very short nerve gaps. We have developed a novel methacrylated version of polyglycerol sebacate (PGSm); which has mechanical properties similar to that of nerve, is biocompatible, biodegradable and can be structured by stereolithography.

METHODS: PGS was synthesised through a polycondensation reaction of glycerol and sebacic acid. Methacrylate moieties were added to create, with the addition of a photoinitiator, a photocurable material. The material was characterized by GPC, FTIR, NMR, DSC, rheology, nanoindentation, tensile mechanical analysis, water contact angle measurements and suture tests. Several forms of PGSm were produced with tuneable levels of methacrylation to control over the material properties. Long term degradation studies and *in vitro* enzymatic degradation studies were performed on PGSm NGCs. Samples were evaluated *in vitro* using NG108-15 neuronal and primary Schwann cells. *Ex vivo* studies were performed by seeding rat DRGs (dorsal root ganglion) onto hemitube PGSm NGCs. PGSm was structured by stereolithography into NGCs (5mm long / 1.3mm internal diameter) and used to bridge a 3mm mouse common fibular nerve injury, with nerve graft as control.

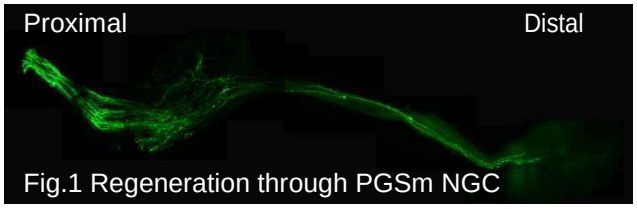

Fig.1 Regeneration through PGSm NGC

RESULTS: Neuronal cell live dead analysis showed PGSm is a good substrate for neuronal cell adhesion and had no neuronal toxicity. Primary Schwann cell studies similarly confirmed PGSm as a good material for Schwann cell adhesion, and overall *in vitro* data indicated that PGSm was permissive for use as a novel NGC. *Ex vivo* studies showed the DRG adhering to the NGC with neurites extending along the NGC. *In vivo* mouse studies using Thy-1-YFP-H mice showed that over 3 weeks the nerve was able to fully regenerate from the proximal to distal stump (Fig.1). Fluorescent axons imaged from the mice are currently being evaluated.

DISCUSSION & CONCLUSIONS: PGSm has advantageous tuneable properties such as modulus values and degradation rates. It exhibits favourable results *in vitro, ex vivo* and *in vivo*; and is easily structured using UV light-based stereolithography. This makes PGSm a future candidate biomaterial for peripheral nerve regeneration.

P842 Investigating the use of engineered neural tissue to generate peripheral nerve repair constructs populated with neurons

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Following peripheral nerve injury, the motor axons in the distal nerve between the injury site and the muscle degenerate. Recovery of function following proximal injuries is a clinical challenge since neuronal regeneration rate is limited, resulting in muscle atrophy due to the delay, even where the 'gold standard' autograft is used. Much research focuses on improving repair conduits that mimic the autograft and promote host neurite regeneration, whereas here we propose to improve long gap repair by populating constructs with neurons. With a ready-to-implant construct populated with glial cells supporting neurons with elongated neurites, the gap between proximal stump and muscle could potentially be reconnected promptly. Immediate innervation of the muscle would help reduce atrophy as regeneration progresses. The overall aim here therefore is to prepare a construct containing neurons with elongated neurites with a view to rapid bridging of a nerve injury site and restoration of innervation to muscle. The objective of this initial study was to investigate the potential for using Engineered Neural Tissue (EngNT) as the basis for establishing robust constructs containing functional neurons with elongated aligned neurites.

EngNT is formed from simultaneous self-alignment of Schwann cells and collagen fibrils in a tethered gel, followed by stabilisation with plastic compression resulting in an anisotropic tissue-like structure. Various types of neurons including NG108-15 cell line and primary rat motor neurons, were maintained in co-culture with EngNT at different densities for 1, 4 and 7 days. Neurite growth was assessed by immunostaining for ßIII-tubulin followed by fluorescence microscopy and image analysis. The results showed that NG108-15 neurites aligned parallel to Schwann cells, with 85% of neurites exhibiting less than 30 degree deviation from the alignment axis. Neurite length increased with time in culture, with some neurites reaching approximately 428 µm after 7 days. Neurite growth from neurons in EngNT was longer than neurite growth in control cultures without Schwann cells. Moreover, the neurite length of NG108-15 cells in EngNT was considerably greater than reported previously using other culture conditions. These results indicate that EngNT may be an appropriate substrate for generating long neurites in vitro with a view to generating therapeutic constructs containing long functional neurons.

P843 Interleukin-6 conjugated chitosan scaffolds for promoting peripheral nerve regeneration

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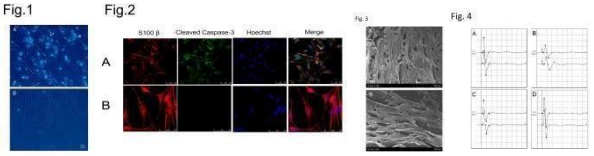
Peripheral nerve defects, especially for injuries greater than a few millimetres, the surgical intervention is often required. Implantation of an autologous nerve graft is recognized as the ”gold standard” strategy of peripheral nerve repair, although it is associated with donor site morbidity and is limited in its availability. To overcome this problem, tissue engineered nerve grafts (TENGs) represent a promising alternative to autologous nerve grafts used for peripheral nerve repair, and have become a focus in research of peripheral nerve regeneration.

Interleukin-6 (IL-6) is an endogenous cytokine not only in the activation of the immune system but also in the regulation of metabolic, regenerative, and neural processes. Here, we construct a novel TENG that is featured by a controlled release drug delivery system through incorporation of IL-6 into a nerve conduit by using genipin as the crosslinking agent to fabricate of chitosan (CS)–genipin (GP)–IL-6 nerve conduits.

The aim of this study was to investigate the effect of CS–GP–IL-6 nerve conduits extracts in vitro and also to evaluate the nerve regeneration outcome and functional recovery by using the TENG to bridge 10mm sciatic nerve defect in rats. We found that the cell morphology of DRG neurons cultured in CS–GP–IL-6 nerve conduits extracts had no obvious change in their cell morphology as compared to untreated cells, while primary DRG neurons pre-treated with 80 ng/ml of TNF-α cultured in plain neuronal medium demonstrated some morphological features of apoptosis, as characterized by the cytoplasmic blebbing and vacuolization (Fig. 1). Immunocytochemistry provided further evidence that Schwann cells obviously expressed apoptosis-related factors, cleaved caspase-3 following treatment with 80 ng/ml TNF-α, while the cells cultured in CS–GP–IL-6 nerve conduits extracts didn’t express this apoptotic marker, confirming that IL-6 has anti-apoptotic effect in primary Schwann cells (Fig. 2). Moreover, the morphology of CS–GP–IL-6 nerve conduits was observed by scanning electron microscope. The sponge-like inner layer of conduits could allow the exchange of nutrition and fluids, and afford a massive space for the storage of the released IL-6 (Fig. 3). Twelve weeks after nerve grafting, The CMAP amplitude ratio and MCV value showed no significant difference between TENG and autograft groups, but CAMP amplitude ratio was significantly larger in either TENG or autograft group than in scaffold group（$P<0.05$）(Fig. 4).

Collectively, CS–GP–IL-6 nerve conduits had an integrated system for continuous release of IL-6, CS–GP–IL-6 nerve conduits extracts has anti-apoptotic effect in vitro, also the outcome and functional recovery of constructed TENG were significantly greater than that in scaffold group, thus holding promise for peripheral nerve repair applications.



P844 Development of acellular allogeneic nerve grafts for peripheral nerve reconstruction

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Introduction: Severe peripheral nerve injuries, characterised by nerve gap defects greater than 1 - 2 cm, are currently repaired using autografts or synthetic nerve guides to bridge the gap. However, such techniques have a limited efficacy and functional recovery is seldom achieved. There is often insufficient autograft material for major reconstruction, and synthetic nerve guides lack native extracellular matrix architecture and composition. We hypothesise that an acellular nerve will support axon regeneration across a defect through the provision of a native microenvironment, particularly endoneurial tubes. Acellular porcine peripheral nerves have previously been shown to promote the regeneration of axons across a defect, both *in vitro* and *in vivo*, and support seeded Schwann cells. The aim of this research is to develop a physically compatible, non-immunogenic allogeneic nerve graft capable of supporting Schwann cells and axon extension.

Methods: Human femoral nerves were decellularised using a combination of hypotonic buffers, low concentration sodium dodecyl sulphate (0.1 %; w/v), nuclease enzymes and protease inhibitors. Total DNA from native and acellular (n=9) nerve was extracted and quantified spectrophotometrically. Biocompatibility of acellular nerve was assessed using a contact cytotoxicity assay (n=3) with L929, BHK and RN22 cells. The architecture and collagen, fat and myelin content of acellular nerve were assessed histologically. Collagen, fat and glycosaminoglycans were quantified using standard biochemical assays. Specific ECM components, including laminin, fibronectin and collagen type 4 were located using monoclonal antibodies.

Results: The average DNA content of native and acellular human femoral nerves was 346.2 ± 53.8 and 30.5 ± 7.4 $ng.mg^{-1}$ respectively. Histological staining demonstrated acellular nerves to have retained a native architecture and distribution of collagen fibres. Important ECM components, including fat, collagen type 4, laminin and fibronectin were still present following decellularisation. The decellularisation process was also shown to remove myelin. Acellular nerves were found to be biocompatible with L929, BHK and RN22 cells.

Discussion: An optimised process for the decellularisation of human femoral nerves has been developed to produce biocompatible, acellular grafts up to approximately 6 cm in length. Acellular nerves retained native histioarchitecture and endoneurial structures, which are essential for providing topographic guidance and promoting Schwann cell migration and axon extension. In addition, important basal lamina components were preserved. Specific ECM molecules, including collagen type 4 and laminin, are essential for regulating cell adhesion and growth, and promoting Schwann cell-axon interactions. The removal of myelin may also facilitate regeneration, as remaining axon and myelin debris are thought to inhibit axonal growth. Current results indicate acellular human nerves have clinical potential to promote axon regeneration across a defect.

P845 A novel, versatile method for the development of immunomodulatory synthetic grafts for in situ vascular tissue regeneration

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In-situ vascular regeneration is based on the implantation of biodegradable synthetic grafts that act as a temporary substrate for blood cell infiltration and mature tissue formation. Bioactivation of acellular synthetic grafts which can modulate macrophage polarization is a promising, off-the-shelf approach to enhance functional tissue regeneration *in situ*. Macrophages are indeed believed to determine the outcome of the implantation procedure depending on their phenotype[1]. In this respect, functional tissue regeneration was proven *in vitro* and *in vivo* for synthetic materials functionalized with interleukin 4 (IL-4)[2-4], a cytokine responsible of the activation of the wound healing macrophage phenotype. However, the proposed approaches are based on burst or gradual release of IL-4 within a limited period of time (few hours-few days)[2-4]. Here, we propose a new, versatile method for the development of immunomodulatory materials based on non-covalent IL-4 functionalization via a supramolecular approach. Particularly, we synthesized a UPy-modified peptide, i.e. the UPy-heparin binding peptide (UPy-HBP), and mixed in and matched to UPy–modified polymers via UPy-UPy interactions (dimerization of self- complementary quadruple hydrogen bondings)[5]. The UPy-HBP modified supramolecular polymer was further functionalized with heparin, which can click to the UPy-HBP, and IL-4, which is known to bind to heparin via its heparin binding domain[6]. For primary human macrophages cultured on 2D materials, differences in cell morphology were observed over time, with elongated M(IL-4)- type macrophages[7] displayed on IL4 functionalized films only. Multiplex ELISA revealed immunomodulatory properties of the IL-4 functionalized material over time, with a decrease in secretion levels of some pro-inflammatory cytokines and the increase in production of anti- inflammatory cytokines at day 7 (Fig.1), confirmed by the promotion of anti-inflammatory macrophages shown via immunofluorescence analysis. For 3D electrospun scaffolds, successful heparin complexation and retained IL-4 were confirmed via heparin-FITC immunofluorescence analysis and ELISA, respectively. The heparin–based holds a great deal of promise for *in vivo* applications, and for the future development of multi-functionalized materials with heparin binding growth factors, in the attempt to mimic the plethora of signals involved in the inflammatory response *in vivo.*

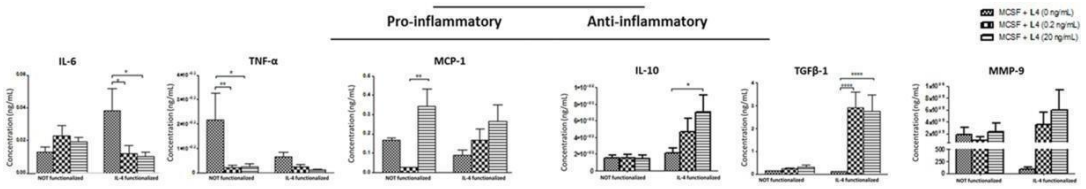

Fig.1. Protein production quantified via Multiplex ELISA. Secretion levels of pro- and anti-inflammatory cytokines for monocyte-derived macrophages treated for 7 days with MCSF on functionalized materials (CE-UPy-PCL) and materials functionalized (CE-UPy-PCL+UPy-HBP) with different doses of IL-4, i.e. 0, 0.2 and 20 ng/mL.

P846 The bone healing process benefits from a naïve immune cell milieu - a humanized mouse model study

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Aims

Bone healing in itself is a highly successful process resulting in fully regenerated bone (restitutio ad integrum). However, in around 5-10% of patient cases, bone healing is delayed or fails, resulting in a non-union. The healing process is highly complex with consecutive, partly overlapping stages that involve multiple cells and signalling factors, which interact in a tightly regulated symphony. Previously it was shown that specific immune cells subsets have a negative effect on the bone healing process. Specifically cells of the adaptive immune system are characterised as unfavourable. These cells do accumulate during aging. In this study the influence of an aged immune system has been investigated in view of the effect on the bone healing capacity. In order to increase the validity of our animal models the attempt was made to create a mouse with an aged human immune cell setting.

Methods

Immune aging was analysed in 12, 52 and 102 weeks old C57BL/6 mice using flow cytometry. To test human immune cells in mice, NOD scid gamma-/- (NSG) mice were transferred with mononuclear blood cells (PBMC) from donors with a high effector T cell count and a high naïve T cell count respectively. An osteotomy was performed using an external fixation system (RISystem) and a gap of 0.7mm. Healing was evaluated with µCT, flow cytometry and histology. In vitro analysis about the cross reactivity of cytokines between mouse and man supported this experiment.

Results

The immune cell composition in mice changed during aging in accordance with the human aging immune system. The memory T cell population increases while the naïve T cell pool diminishes: CD44+CD62L+/- T-cells are more frequent in 102 week old mice compared to the 52 week and 12 week old animals ($p<0.05$). Transferring naïve human PBMC into mice lacking their own immune system showed an increased bone volume in tissue volume (BV/TV) compared to sham animals. The newly formed bone structures showed thicker structures but less trabeculae number when immune cells were impacting on the healing process. Cell culture experiments showed that the pro- and anti-inflammatory cytokine composition is essential for the osteogenic differentiation of mesenchymal stromal cells.

Impact

The immune cell composition is requisite for the bone mineralization during fracture healing. This result supports the fact that immune cells are essential for the regenerative process of bone healing. The age specific changes in the immune cell composition has a severe impact on the bone healing capacity in aged. Aged individuals are in a chronic stage of inflammation (inflammaging) that negatively influences bone healing. These results highlights the necessity for further research to unravel the bone healing process in consideration of the immune system and also proves that the field of osteoimmunology could possibly lead to new therapeutic approaches especially for the aged population.

P847 Reconstruction of mandibular bone with the contribution of Gelpord3D constructs in pig

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Reconstruction of bone by autologous bone transplant has been established as the **"**gold standard**"** due to the osteogenic properties of the transplanted material. However, bone grafting is only applicable to relatively small defects. The objective of this work is to evaluate the efficiency of a biomaterial (nanocrystalline carbonated hydroxyapatite reinforced agarose hydrogel) as a scaffold and/or construct (scaffold plus cells) for bone regeneration in critical mandibular defects. The GELPOR3D shaping method can be used to create what are described as ceramic reinforced hydrogels where the structural components maintain both their original microstructural and textural properties and thus their functionality. Using this method, nanohydroxyapatite/agarose (80/20%) scaffolds containing undifferentiated mesenchymal cells of adipose tissue (MSCAT) or osteogenic differentiated cells (O-MSCAT) were prepared. Histological (von Kossa, alkaline phosphatase) and immunohistochemical techniques with antibodies specific for RUNX2, OCT3/4, Nanog, and osteopontin were used for characterize the cells. For in vivo studies, we created (n=6) four critical mandibular defects (35 mm x 15 mm) (2 hemiarcade) on which the scaffold plus cells was implemented (total number of defects = 24). The animals were sacrificed after 6 months. The samples obtained were decalcified and processed for extracellular matrix (ECM) examination using scanning electron microscopy, micro-CT techniques, PCR and immunological staining (osteopontin, osteocalcin, RUNX-2 and Collagen I and III). Data were analyzed using statistical package version 22.0 SPSS. MicroCT analysis showed an increase of ~20% in the size of the defect without scaffolds. In contrast, when scaffolds and MSCAT or O-MSCATS constructs were implanted, we obtained a more than 50 % diminution in defect size. Furthermore, evaluation of ECM area showed a 40 % increase in bone regenerated area. In conclusion, nanohydroxyapatite/agarose scaffolds containing undifferentiated mesenchymal cells offer a promising approach for repairing critical mandibular defects.

Keywords. Critical bone defects, scaffold, construct and tissue engineering.

ACKNOWLEDGEMENTS: This work was supported by grants from IMIDEF-2014.

P849 Anabolic effects of bioactive proteins and extracellular vesicles derived from notochordal cell-conditioned medium

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Low back pain has been related to intervertebral disc (IVD) degeneration. During maturation and early degeneration of the IVD, notochordal cells (NCs) are gradually replaced by chondrocyte- like cells (CLCs) in the nucleus pulposus (NP), suggesting that NCs play a role in maintaining tissue health. Affirmatively, NC-conditioned medium (NCCM) exerts regenerative effects on CLC proliferation and extracellular matrix (ECM) production. The aim of this study was to identify NC-secreted substances that stimulate IVD regeneration.

Mass spectrometry was performed on whole porcine, canine and human NCCM, which was generated by culturing healthy porcine, canine, and human NC-rich NP tissue for 4 days in plain culture medium. As a first step to elucidate which bioactive substances are responsible for the effects of NCCM, porcine and canine NCCM were separated in a soluble (NCCM-S; peptides and proteins) and pelletable (NCCM-P; protein aggregates and extracellular vesicles (EVs)) fraction by ultracentrifugation, and tested *in vitro* on bovine CLCs and canine CLCs, respectively. Main readout parameters were extracellular matrix production/degradation and cell proliferation. In a follow-up study, EVs and proteins derived from porcine NCCM were separated through qEV size exclusion columns and (separately) ultracentrifugated. Thereafter, to characterize EVs, the EV fraction was labelled with PKH67, floated in sucrose gradients and quantitatively analysed by high-resolution flow cytometry. The separate effects of the porcine NCCM-derived EVs and proteins were studied on micro-aggregates containing CLCs from degenerated canine IVDs *in vitro*.

Mass spectrometry of whole porcine, canine, and human NCCM identified 149, 170, and 217 proteins, respectively, with 66 proteins in common. Mainly ECM-related proteins were identified, but also organelle-derived and membrane-bound vesicle proteins. In both bovine and canine CLCs, NCCM-S exerted a more pronounced anabolic effect than NCCM-P. While the effect of porcine NCCM-P on bovine CLCs was negligible, canine NCCM-P moderately enhanced GAG and collagen type II deposition by canine CLCs. Furthermore, porcine NCCM contained a considerable amount of EVs. While the porcine NCCM-derived proteins and EVs did not influence the DNA content of canine CLC micro-aggregates, they both increased the GAG content.

Instead of a single regenerative NCCM candidate (growth factor), we found that porcine and canine NCCM exerted their anabolic effects mainly through soluble factors. However, also pelletable NCCM factors - containing both protein aggregates and EVs - showed moderate regenerative potential. Porcine NCCM contained a considerable amount of EVs, which (when applied without interfering protein aggregates that were present in NCCM-P) also exerted anabolic effects on canine CLCs. Thus, the effect of the abundantly present EVs in NCCM should not be overlooked and should also be tested at human CLCs in the future. It remains to be determined how proteins may interfere with the biologic functions of the NCCM-derived EVs.

SIGNIFICANCE: Since the present study indicates that both NCCM-derived proteins and EVs showed regenerative potential, the combination of factors present in NCCM may be more important than the application of separate factors.

P850 The regenerative effects of notochordal cell matrix on nucleus pulposus cells

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Low back pain has been related to degeneration of the intervertebral disc (IVD). Since dogs experience back pain and IVD degeneration with similar characteristics as humans, they are considered a suitable animal model for human IVD degeneration. Current treatments for IVD disease do not lead to repair, so there is need for regenerative treatments resulting into functional IVD restoration. During IVD degeneration, notochordal cells (NCs) are replaced by chondrocyte-like cells (CLCs). NCs produce soluble factors that induce cell proliferation and extracellular matrix (ECM) deposition in CLCs, but identification of these specific factor(s) appears difficult. A novel approach would be to directly use the matrix of NP tissue rich in NCs, the so-called notochordal cell matrix (NCM), which has already been shown to exert anabolic effects on bovine CLCs. Therefore, as a first step towards translation, the regenerative effects of NCM on canine and human CLCs derived from degenerated IVDs were determined.

NCM was produced from healthy porcine NC-rich NP tissue (n = 6, pooled) by lyophilization and resuspension in basal culture medium (10 mg/mL). The effect of NCM was determined on canine (n = 6) and human (n = 6) CLCs derived from degenerated IVDs cultured in micro-aggregates for 28 days under hypoxic conditions. Main readout parameters were extracellular matrix (ECM) anabolism, catabolism, cell proliferation, and apoptosis.

Generally, canine CLCs responded more pronounced to NCM than human CLCs. NCM significantly increased the glycosaminoglycan (ECM) content of human and canine CLC micro-aggregates compared with controls. Furthermore, NCM induced mild collagen type I and abundant collagen type II deposition in the CLCs. ECM catabolism and apoptosis marker gene expression was decreased by NCM treatment, whereas *CCND1* gene expression (marker for proliferation) was increased in human and canine CLCs. Lastly, NCM significantly induced rapid Smad1 (BMP) and Smad2 (TGF-β) signalling in canine CLCs, whereas it decreased Smad1 and Smad2 signalling in human CLCs.

In conclusion, NCM induced an anabolic, anti-catabolic, anti-apoptotic, and proliferative effect on human and canine CLCs derived from degenerated IVDs. Thus, directly applying notochordal cell matrix could be a promising regenerative treatment for canine and human IVD disease, circumventing the (challenging) identification and application of bioactive NC-secreted substances. The long term effects of NCM are currently being determined in an experimental animal model.

P851 Combining engineered silk composite and genipin-enhanced fibrin hydrogel to repair the annulus fibrosus

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Introduction: Low back pain is often caused by trauma or disc degeneration. Here, we aim for an "inside-out" approach for repairing herniated intervertebral discs (IVD) or injuries of the outer annulus fibrosus (AF) by using a fibrin hydrogel in combination with modified silk. Within this study, we investigated the feasibility of a genipin cross-linked fibrin hydrogel *in vitro* and an *ex vivo* organ culture approach.

Material and Methods: Bovine IVDs were harvested under aseptic conditions and an injury was induced (2mm biopsy punch). Human-based fibrin hydrogel (Tisseel, Baxter) enhanced with genipin was used to fill the cavity [1,2]. A GMP-compliant silk membrane-fleece composite was placed on the hydrogel to close the injury. Subsequently, IVDs were subjected to *in vitro* organ culture for 14 days using three loading regimes: 1) "complex" (0.2MPa compression and 0±2° torsion at 0.2Hz for 8h/day), 2) static diurnal (0.2MPa) and 3) no loading. For complex loading a custom-built two-degree of freedom bioreactor was used. At the end of culture, the discs were inspected for seal failure, height, metabolic activity, cell death (necrosis and apoptosis), DNA, glycosaminoglycan (GAG) and collagen (hydroxyproline) contents. Additionally, cell cytocompatibility of genipin-enhanced fibrin hydrogel was tested *in vitro* with low-passage human mesenchymal stem cells (n=6) and donor matched (n=3) human AF and nucleus pulposus (NP) cells. Cytocompatibility was investigated on day 1, 4, 7 and 14 of monolayer culture (120,000 cells/well, 48-well plate) exposed to increasing genipin concentrations and DMSO only as control. Cell activity (resazurin salt) was determined.

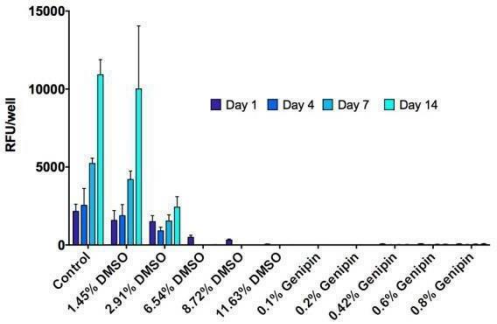

Figure 1: *Cell activity tested on a growth control and genipin samples, additionally samples with the necessary amount of DMSO used to dissolve genipin were tested as controls as well (mean ± SD).*

Results: Macroscopic inspection revealed that the silk seal was not displaced throughout the organ culture period. Metabolic activity, DNA/GAG content and disc height of the repaired discs did not differ significantly from the injured IVDs. The Exception was the higher DNA content under static loading for the repaired discs compared to the injured IVDs (p-value $\leq$ 0.004). *In vitro* experiments on the cellular level showed barely any cell activity for the cells treated with genipin.

Discussion: The combination of genipin-enhanced fibrin hydrogel and silk composite formed a tight failure free AF injury closure. Nevertheless, our *in vitro* assays on primary hMSC and hAF and hNP cells showed a clear cytotoxic effect of genipin. The challenge now will be to find a less toxic but still mechanically load bearing hydrogel for AF repair.

P852 Glucose uptake of human intervertebral disc cells in 3d culture monitored in real-time

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Introduction: Glucose uptake is a crucial parameter for all cells. However, in intervertebral disc (IVD) cells that are exclusively supplied with glucose by passive diffusion, it might be of special importance [1, 2]. We hypothesized that due to the location of the different cell types, i.e. the nucleus pulposus cells (NPC) in the center of the disc, the annulus fibrosus cells (AFC) and the cartilaginous endplate cells (EPC), the glucose consumption rate should be highest with cells being closer to blood vessel supply and the cells in the center (NPC) would require the lowest glucose consumption rate. Here, this hypothesis was tested using clinical primary IVD cells isolated from tissue in real-time.

Materials and Methods: Human primary AFC, NPC and EPC were isolated using a mild two-step digestion protocol with pronase and collagenase 2. Cells were expanded in 2D and were resuspended at passage 2 in 1.2% alginate at a density of 4 Mio cells/ml. 30 beads were added to a T-25 culture flask that was equipped with a glucose sensor (C-CIT Sensors AG, Switzerland) and cultured in a hyperglycaemic condition (DMEM supplemented with 4.5g/l glucose and 10% FCS) under agitation (10rpm). Glucose concentration, proportional to current in nA, was recorded over seven days and media was replaced after 3-4 days. Cell activity (resazurin red assay) was determined on day 1 and on day 7. Further, DNA (Hoechst assay) and glycosaminoglycan (GAG) (dimethylmethylene blue assay) content were determined.

Results: Glucose *in vitro* could be successfully monitored in real-time, Fig 1A. Glucose uptake was derived from current (nA) by performing linear regression. Slopes of least-square fitted linear regression were used for comparison among cell types. Before the media change, all cell types had a similar glucose consumption rate. However, after media change EPC presented a higher glucose consumption than AFC and NPC, Fig 1B.

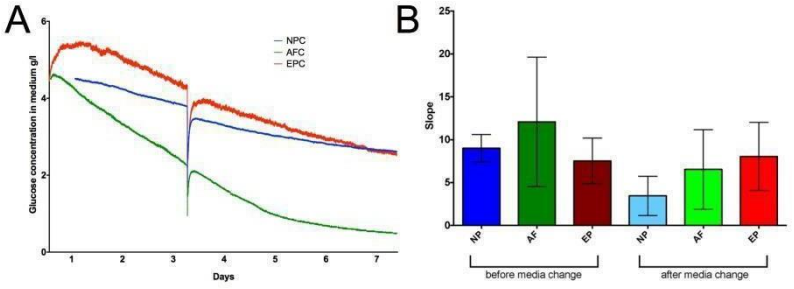

***Figure 1:** A Glucose consumption monitored in real-time over seven days for human AFC, NPC and EPC. **B** Slopes of current before and after media change of 5 experiments ± SD.*

Discussion: There seems a tendency in glucose consumption between the different cell types. This could be explained by their location close to the nutrient transport route, i.e. the capillary system of the vertebrae.

P855 The therapeutic effect of human bone marrow-derived clonal mesenchymal stem cells on scar formation in spinal cord injury

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Aims: This study aimed at evaluating the therapeutic effect on scar formation of human bone marrow-derived clonal mesenchymal stem cells (hcMSCs) homogeneously isolated by using a subfractionation culturing method, in comparison with the non-clonal MSCs (hMSCs), in a rat spinal cord injury (SCI).

Method: The SCI was made using a vascular clip at the T9 level. These cells were transplanted into the lesion site 3 days after injury. A functional test was performed over 4 weeks employing a BBB score. Rats were sacrificed for histological analysis at 3 days, 1 week and 4 weeks following injury.

Results: The transplantation of hcMSCs and hMSCs significantly reduced lesion size and fluid-filled cavity at 4 week in comparison with the PBS control ($p<0.01$). Transplantation of hcMSCs revealed more axons reserved than that of hMSCs in the lesion epicenter filled with non-neuronal tissues. In addition, hcMSCs and hMSCs clearly decreased the inflammatory reaction and intraparenchymal haemorrhaging, compared with those of the PBS group. Interestingly, hcMSCs largely reduced the Col IV expression level, one of the markers of fibrotic scars.

Conclusion: The hcMSCs yielded therapeutic effects more than equal to those of hMSCs on the SCI. Both hcMSCs and hMSC created an increase of axon regeneration and reduced scar formation around the SCI lesion. We speculate that hcMSCs could be of higher value than hMSCs for treating SCI based on the current findings and their practical advantages of a homogeneous nature.

P858 Effect of exogenous stimulation of the BMP antagonists Gremlin and Noggin on primary human osteoblasts and mesenchymal stem cells

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Introduction: The standard treatment for intervertebral disc (IVD) degeneration is discectomy followed by spinal fusion. Clinical observations indicate that partial removal of the IVD during discectomy can lead to a failure of bone formation. One possible explanation for this phenomenon could be a secretion of BMP antagonists by IVD cells. The bone morphogenic protein (BMP) pathway plays a crucial role in bone turnover by inducing osteogenesis. BMP antagonists like Gremlin (GREM1) and Noggin (NOG) are able to inhibit osteogenesis [1]. We hypothesized that human primary osteoblasts (OB) or primary human mesenchymal stem cells (MSC) stimulated with NOG and GREM1 show an inhibition of the osteogenic phenotype.

Methods: OB/MSC were stimulated with 10 or 100 ng/mL NOG and GREM1 respectively that were added to osteogenic medium. The control groups were stimulated with osteogenic medium (positive control) and with control medium, α-MEM + 10% FCS (negative control). After 21 days of culture in hypoxic conditions, matrix mineralization of OB and MSC monolayers were stained with Alizarin red (ALZR). ALZR was quantified by absorbance and normalized to cell activity (Resazurin assay). Furthermore was the ALP content measured at day 10 and 21 of experiment (N=3).

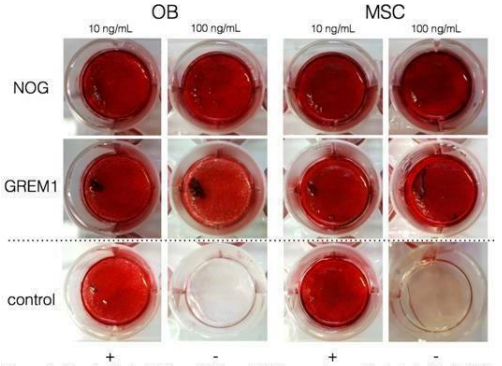

Figure 1: Alizarin Red staining of OB and MSC monolayer stimulated with 10/100 ng/mL GREM and NOG respectively. The control groups were stimulated with osteogenic and control medium. The cells were cultured for 21 days in hypoxic conditions.

Results**:** After 21 days, a significant increase of ALZR staining of mineralized matrix was observed in the OB culture in osteogenic medium (0.341 ± 0.040 mean ± SEM) compared to OB grown in control medium (0.056 ± 0.009) (M-W test: P = 0.0286). OB stimulated with 100 ng/mL of NOG and GREM1 showed a significantly higher mineralization than the negative controls (NOG 0.820 ± 0.336 (Friedman test: P = 0.0124) and GREM 0.659± 0.180, Friedman test: P = 0.0393). The same trend was observed in stimulated MSC (Fig.1). The ALP activity measured at day 10 and 21 in MSC and OB stimulated with 100 ng/mL NOG showed a trend to be higher than the positive control.

Discussion: In this study we investigated the effect of BMP antagonists, i.e. NOG and GREM1 on OB and MSC. Opposite to our expectation, the exogenous stimulation of OB and MSC with GREM1 and NOG did not lead to an inhibition of the osteogenic phenotype and so to a reduction of matrix mineralization. The calcium deposition in OB and MSC monolayer, were stimulated with the addition of the BMP antagonists.

P859 Formation of calcium deposits in primary human osteoblasts in the presence of intervertebral disc cells

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Introduction: Today's standard treatment strategy for intervertebral disc (IVD) degeneration is discectomy followed by spinal fusion. Clinical observation showed that partial IVD tissue removal could lead to a failure of spinal ossification [1]. It was previously shown that human IVD cells inhibit ossification of human mesenchymal stem cells (MSC) [2]. Hence, we hypothesized that human primary osteoblasts (OB) co-cultured with IVD cells would show similar inhibitory effects in ossification as previously demonstrated for MSC.

Methods: OB, isolated from patients (N=6) undergoing total knee replacement, were seeded at a density of 10'000 cells/cm^2. IVD cells, as nucleus pulposus cells (NPC), annulus fibrosus cells (AFC) and cartilaginous endplate cells (CEPC), isolated from patients undergoing spinal surgery (i.e. NPC, AFC and EPC), were encapsulated in 1.2 % alginate beads (~30 µm in ø , at 4M cells/mL, ~ 75'000 cells/bead) and co-cultured via inserts (PET, 0.4 µm pore size) with OB in the lower part as monolayer. Dose response of inhibition was investigated by co-culturing six, nine and twelve NPC, AFC or EPC beads, respectively. The experimental groups were stimulated with osteogenic medium. Additionally, three controls were cultured: OB monolayer with osteogenic medium (± empty beads) and with control medium (α-MEM + 10% FCS). To quantify ossification, matrix mineralization of OB was visualized after 21 days by Alizarin red (ALZR) staining and was quantified by absorbance. Furthermore, was the ALP activity measured at day 10 and 21.

Results**:** After 21 days, the OB culture in osteogenic medium (positive control) showed a statistically higher ALZR staining (0.843 ± 0.224, mean ± SEM) than the negative control (p-value = 0.001). OB cultured with NPC, AFC and EPC showed a trend towards decreasing calcium deposition compared to the positive control (6 beads NPC: 0.476 ± 0.101, AFC: 0.461 ± 0.066, CEPC: 0.471 ± 0.052) (Fig.1). The ALP activity showed a trend to be lower with increasing bead number in every cell type. However, no significant difference to the positive control was detected.

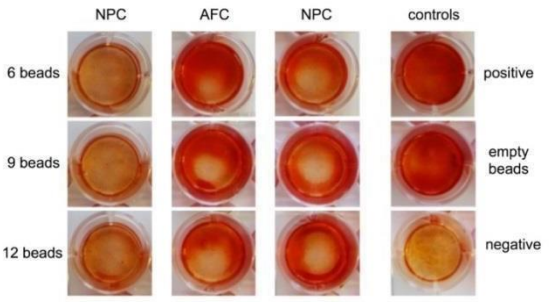

Figure 1. A: Alizarin red staining of osteoblast monolayers co-cultured with 6,9 or 12 NPC, AFC or CEPC respectively, cultivated for 21 days in hypoxic conditions.

Discussion: In this study we could show a tendency that mineralization of primary OB could be inhibited by direct exposure to IVD cells. Like observed in clinics, IVD cells seem to directly influence OB, by apparent inhibition of ossification. Since also empty beads seem to influence the mineralization of OB it is possible, that the alginate or the inserts played a role in matrix mineralization of the OB monolayer.

P860 Intradiscal application of a pcla-peg-pcla hydrogel loaded with celecoxib for the treatment of back pain: A translational approach

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Introduction: Chronic low back pain is a common clinical problem in both the human and canine population. Inflammatory mediators such as prostaglandin E2 (PGE2) play a key role in IVD degeneration in both species, causing structural changes of the IVD and low back pain. Current pharmaceutical treatment often consists of oral anti-inflammatory drugs to alleviate pain. In severe cases, surgery can be performed. Novel treatments for degenerative disc diseases focus on local application of sustained released drug formulations. Injectable formulations that enable local sustained release of anti-inflammatory drugs aim at decreasing inflammation and thereby inhibiting degeneration and pain. The aim of this study was to determine safety and feasibility of intradiscal application of a PCLA-PEG-PCLA hydrogel releasing celecoxib, a COX-2inhibitor.

Materials and methods: Biocompatibility was evaluated after subcutaneous injection in mice and safety of intradiscal injection of the hydrogel was evaluated in experimental dogs with early spontaneous intervertebral disc (IVD) degeneration. COX-2 protein expression was determined in IVD samples surgically obtained from canine patients. Client-owned dogs with low back pain were diagnosed by MRI with degenerative lumbosacral stenosis marked by mild to moderate IVD degeneration and protrusion. The dogs were surgical candidates but were offered intradiscal injection. The PCLA-PEG-PCLA thermogel, loaded with 0.013mg/ml celecoxib, was percutaneously injected into the nucleus pulposus of L7-S1 under fluoroscopy guidance. Follow-up consisted of clinical examination, owner questionnaires, and objective gait analysis by measurement of ground reaction forces (GRFs) using force plate analysis at 6 weeks, and 3 and 6 months after injection. MRI was repeated after 3 months and included T2- and T1-weighted images and T2-mapping.

Results: Biocompatibility of the hydrogel was confirmed after subcutaneous and intradiscal injection. COX-2 expression was increased in IVD samples surgically obtained from canine patients indicating a role of COX-2 in clinical IVD disease. Ten client-owned dogs with chronic low back pain related to IVD degeneration received an intradiscal injection with the celecoxib- loaded hydrogel. None of the dogs showed adverse reactions after intradiscal injection. Follow up MRI showed no worsening of the IVD degeneration and the hydrogel did not influence MRI signal at long term follow up.Clinical improvement was achieved by reduction of low back pain in 9/10 dogs, as was shown by clinical examination and owner questionnaires. GRFs did not significantly change throughout the follow up period. In 3/10 dogs low back pain recurred at 3 months and they subsequently underwent standard-of-care surgical treatment.

Conclusion: This study showed the safety and feasibility of intradiscal injections with a thermoresponsive hydrogel loaded with celecoxib. Ongoing studies concentrate on the long term clinical follow up of these patients. Future studies will determine the optimal loading dose of celecoxib for clinical efficacy. In this setup, the dog can be used as a model for the development of novel treatment modalities in both canine and human patients with chronic low back pain.

P861 Novel knitted titanium nucleus implant: potential role for tissue engineering

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Nowadays low-back pain is confounding factor being associated with the inter-vertebral disc degeneration and high risk of trauma. Arthroplasty is gaining the popularity for treatment of low-back pain caused by degenerative disc disease (DDD), as the most widely accepted treatment, spinal fusion shows limitation. Compared to a total or partial disc replacement, nucleus replacement represents the promising approach being less invasive with less surgical risk. However, currently marketed devices showed a high risk of implant expulsion because of the incomplete integration of the surrounding bone (*i.e*. osseo integration) and cartilage with the material implanted. Therefore this study aims at characterizing the novel knitted titanium nucleus implant *in vitro* which has been designed to optimally restore the biomechanics and so the anatomy.

Medical grade Ti6Al4V made knitted titanium implant showed excellent damping features, visco-elastic properties with high specific strength. Unique knitted macro-pore structure has been designed to have optimum cell ingrowth. The potential application of the knitted titanium scaffold in tissue engineering was evaluated *in vitro* with respect to the cytocompatibility by culturing the Human bone marrow derived mesenchymal stromal cells (SCP1 cell line) and Human primary chondrocytes on materials surface, which together are expected to promote periprosthetic tissue reconstruction. The study is mainly focused on the evaluation of cell reaction to the unique knitted structured implant.

In vitro study evidences the suitability of the knitted titanium scaffold and proved to be cytocompatible. No evidence for adverse/toxic effects on cell viability was observed. We also observed the spreading pattern and cell migration on the scaffold surface. Implants surface properties primarily influence the initial cell response at the cell - metal interface. Based on the foregoing considerations, hydrophilic degree of wettability (contact angle: 84.3) of the knitted titanium implant ultimately stroked a biological response favouring cell adhesion and proliferation. Further studies will address the bio-functionalization of the implant to improve the longevity thereby enhancing the cell-metal interaction.

P862 decellularized ecm coated microparticles release neurotrophic factors to support dental stem cells differentiation for spinal cord regeneration

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Introduction: Traumatic spinal cord injuries (SCI) cause devastating neurological deficits and disabilities [1]. Initial trauma leads to immediate disruption of neural tissue by axon shearing, blood vessel rupture and cell death. Subsequently a cascade of secondary events occurs composed of ischemic injury, inflammation, cell death, demyelination of axonal tracts and the creation of a glial scar, forming a physical and chemical barrier [2]. Cell transplantation to replace damaged cells, provide trophic support and promote functional recovery is a favourable strategy for SCI [3]. Stem cells derived from dental tissues are an attractive source due to accessible supply, high proliferation rate and the potential for autologous transplantation [4]. The objective of the present study was to evaluate the suitability of surface modified PLGA microparticles to provide structural support and controlled release of glial derived neurotrophic factor (GDNF) to enhance differentiation of dental stem cells from apical papilla (SCAPs).

Materials and methods: Microparticles (MPs) were manufactured from PLGA (50:50, MW:53kDa) and an in house produced Triblock copolymer (PLGA-PEG-PLGA) using a double emulsion process. Encapsulation of GDNF was facilitated by inclusion of sodium acetate. Microparticles were coated with extracellular matrix (ECM) hydrogels derived from bone and spinal cord. Surface modification effects were examined by Time of flight secondary ion mass spectrometry (ToF-SIMS), cell viability assays and qPCR.

Results and discussion: ToF-SIMS analysis of normalized ion intensities for protein adhesion showed a significant regression in PLGA characteristic peaks when bone and spinal cord ECM were applied to the MP surface. A correspondingly higher intensity of CN- (m/z 26) and CNO- (m/z 41.99) ions, representative of the peptide backbone in proteins, reflected the increased adherence of ECM proteins on the MP surface. Successful application of ECM coatings affected cell behaviour, with greater cell viability on coated particles observed compared to non-coated particles after 7 days of culture. GDNF activity post encapsulation and release was verified with a neurite outgrowth assay whereby controlled release of GDNF had equivalent effect to exogenous addition of GDNF. SCAPs cultured with GDNF encapsulated MPs coated with spinal cord ECM demonstrated upregulated expression of neural markers including β III tubulin and neuron-specific enolase, indicating induction of neural differentiation of SCAPs.

Conclusions: Polymeric microparticles coated with spinal cord ECM hydrogels promoted dental stem cell viability. Furthermore, release of GDNF from microparticles promoted neural differentiation of the dental stem cells. Co-delivery of microparticles to provide controlled release of factors may facilitate stem cell utilisation in the treatment of spinal cord injury.

P863 Generation of a bioengineered substitute of the human ureter using transdifferentiated cells and nanostructured biomaterials

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Background: Generation of an efficient substitute of the human ureter is an important challenge of current tissue engineering due to two main factors: the difficultness for culturing the different types of ureter cells and the need of developing a fully functional biomaterial. In the present work, we have generated a novel substitute of the human ureter based on nanostructured biomaterials and transdifferentiated mesenchymal stem cells.

Methods: We first generated primary cell cultures of human umbilical cord Wharton's jelly mesenchymal stem cells (HWJSC) and primary cultures of human ureter fibroblasts using enzymatic digestion. These cells were expanded using basic culture medium. Then, a bioengineered substitute of the ureter lamina propria using fibrin-agarose biomaterials with the fibroblasts cultured within. After 24h, the HWJSC were subcultured on top of the lamina propria substitutes as an alternative cell source for the epithelial cells, using an epithelial conditioning medium in order to induce epithelial differentiation. Results were analysed after 7 and 14 days using histological and immunohistochemical analyses using the human native ureter as a control.

Results: The histological analysis showed that the HWJSC cultured on top of the lamina propria substitute were able to for a stratified epithelial-like layer partially resembling the structure of the native urothelium. The lamina propria was rich in fibroblast cells showing proliferation signs. Immunohistochemical analyses showed that the epithelium of the control human native ureter was highly positive for pancytokeratin, cytokeratins CK7, CK8 and CK13 and uroplakin 3. The bioengineered ureter substitute was slightly positive for all these markers except uroplakin 3, with expression of these markers being higher after 14 days of development and lower at day 7.

Discussion and conclusions: These results suggest that nanostructured fibrin-agarose biomaterials may be adequate scaffolds for ureter tissue engineering, supporting appropriate growth and development of the stromal fibroblasts and the generation of an epithelial substitute on top. This epithelial substitute showed partial signs of differentiation, but the expression of specific markers was low as compared to native controls. In summary, these data support the use of these bioartificial ureter substitutes and warrant the need of carrying out in vivo experiments in laboratory animals.

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P864 Immunohistochemical identification of major histocompatibility complex antigens in transdifferentiated epithelial cells

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Background: Mesenchymal stem cells (MSC) are involved in modulating immune response and tissue repair at in vitro an in vivo levels. Several studies have demonstrated that MSC may inhibit lymphocyte proliferation and regulatory T and B cells induction, showing great interest for the treatment and prevention of tissue and organ rejection. However, the immunomodulatory effects of MSC transdifferentiated to other cell types have not been investigated to the date. In this work, we have transdifferentiated adipose (ADSC), dental pulp (DPSC) and Wharton's jelly stem cells (HWJSC) to epithelial-like differentiated cells in order to evaluate their differentiation and immunomodulatory potential for tissue engineering protocols.

Methods: First, we generated a bioengineered substitute of a human stroma tissue by using primary fibroblasts cultured in plastic-compressed hydrogels. Then, primary cultures of ADSC, DPSC andWHJSC were placed on top of the stromal substitutes in order to induce epithelial-like cell transdifferentiation. For this, EGF-enriched conditioning medium was added every two days and air-liquid culture technique was applied for 14 days. To evaluate the transdifferentiation potential of MSC, pancytokeratin and cytokeratin 19 (CK19) expression was evaluated, whereas the immune phenotype of these bioengineered tissue substitutes was tested by immunohistochemistry for HLA-I and HLA-II surface antigens.

Results: Our results demonstrate that the three alternative cell sources used in this work -ADSC, DPSC and WHJSC- could be efficiently used for the development of several human tissues. In the three cases, a well-differentiated epithelium-like structure was formed on top of the stromal substitute. This epithelial-like layer was positive for pancytokeratin and CK19, although the expression levels were lower than those of control native tissues. In contrast, expression of the HLA markers analysed here was very low or negative in all these epithelial substitutes.

Discussion and Conclusions: Numerous studies have demonstrated the immunomodulatory properties of MSC, and the advantages of these cells in cell therapy and tissue engineering, including the possibility of generating heterotypical tissue substitutes containing these cells. Our results show for the first time that transdifferentiated MSC could be devoid of the major immune antigens, at least ex vivo. Therefore, we hypothesize that artificial tissues generated with these alternative cell sources transdifferentiated to epithelial cells could be used for the replacement of the human skin, cornea or oral mucosa with low risk of HLA-mediated immune rejection. Future studies in laboratory animals should confirm this hypothesis.

Acknowledgements: Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica, Ministry of Economy and Competitiveness (Instituto de Salud Carlos III), grants FIS PI14/955 and FIS PI15/2048 (co-financed by ERDF-FEDER, European Union) and by SAS_PI-0386-2014, Junta de Andalucia, Spain.

P865 Formation of libraries of chemically functionalised and topographically textured microparticles to screen stem cell-particle interactions

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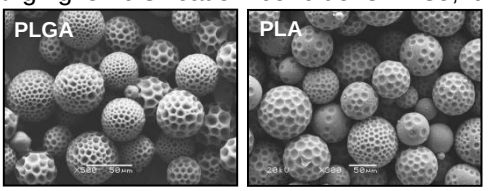
Understanding biomaterial-cell interactions is important in order to establish the basis for successful tissue engineering and consequently improve the capacity for tissue repair. Material properties including topography, chemistry and stiffness have been demonstrated to individually have an impact on mammalian cell fate. The aim of this work is to study this concept on large libraries of 3D materials which combine all these properties by fabricating a series of chemically modified textured microparticulate architectures.

Non-porous smooth microparticles based on the biodegradable polymers poly(lactic acid) (PLA) and poly(lactic acid-co-glycolic acid) 85:15 (PLGA), were fabricated by a solvent evaporation oil-in-water single emulsion technique. Polymer dissolved in dichloromethane was emulsified in the aqueous phase in the presence of polyvinylalcohol (PVA, 98% hydrolysed) as a stabiliser (1% w/v) at 600 rpm. To produce textured microparticles, topographies were induced by incorporating fusidic acid (FA) in the organic phase at different polymer/FA ratios. Emulsification was performed as described above and dimpled particles were obtained after FA release in PBS over 7 days. Both polymer and drug concentration in the initial mixture was shown to significantly influence the final particle and dimple size distribution. Under these conditions, the average particle diameter diminished from 50 µm to 20 µm when polymer concentration was decreased from 10% to 2% w/v, although these can be easily tuned by changing emulsification conditions. Also, dimple diameter varied with the largest dimples observed on the smallest particles. In addition, dimple formation was observed independently of polymer type (See Figure).



Next, chemistries which have previously demonstrated significant capabilities in modulating cell response, will be incorporated on these surfaces following a ‘grafting from’ approach. Firstly, aminolysis of superficial polymer chains was induced by limited exposure to ethylenediamine in order to enhance the particles’ surface reactivity whilst retaining particle morphology. Secondly, the free amine groups now available on the surface served as ‘anchors’ for the desired polymerisation initiators carrying appropriate functionalities, i.e. α-bromoisobutyryl bromide. The aminolysis extent and initiator immobilisation were assessed by the 2,4,6-trinitrobenzene sulfonic acid assay and FTIR spectroscopy. Monomers of interest will be subsequently polymerised from the activated surfaces and the influence of these topo-chemical combinations on cardiomyocyte maturation and mesenchymal stem cell differentiation in 3D will be evaluated. Importantly, functionalities of a very dissimilar nature can be introduced following a common approach reflecting the versatility of the selected methodology and compatibility with high throughput technologies. Moreover, this procedure retains bulk material properties and the topographical features whilst changing the outermost surface characteristics. In conclusion, these materials present a platform for new biomaterials discovery in 3D.

P866 Effect of substrate topography on osteogenic potential of mouse iPS-cell derived embryoid bodies out growing cells

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Purpose:

The purpose of this study was to investigate the effect of culture on polystyrene substrates of differing topographies on the osteogenic potential of embryoid bodies out growing cells.

Materials and methods:

Polystyrene substrates were sandblasted with 25-, 50-, and 150-μm Aluminum Oxide particles to obtain topographies with an average Sa value of 0.6, 1.2, and 1.8 μm, respectively. Embryoid bodies derived from mouse iPS cells were formed according to the protocol of Nakatsuji and Suemori. The embryoid bodies were seeded on the substrates and left for 5 days. They were then examined over the next 16 days by SEM, immunocytofluorescence (ICF) for vinculin, and quantitative RT-PCR (qRT-PCR) using primers for RUNX-2 and Collagen typeI.

Results:

The results of ICF and SEM revealed that the surface roughness of the substrates had caused the cells to elongate. Vinculin staining demonstrated how the Sa value affected mode of cellular attachment to the substrate. The results showed that the flat surface, resulted in random distribution of focal adhesion points, but rough surfaces resulted in focal adhesion points which were smaller but more concentrated on the podia of the cells. The results of qRT-PCR revealed that runx-2 expression was highest on day 8 on surfaces with an Sa value of 1.2 μm and on day 16 on both the 0.6- and 1.2-μm substrates. Expression of Collagen type I on day 8 was highest on the 0.6- followed by the 1.2-μm substrates.

Conclusions:

Surface topography affects cell shape and early osteogenic gene expression in the out growing cells, particularly on substrates with an Sa value of 1.2 μm.

P867 A comparative study of human plasma fibronectin potentialization of the osteogenic activity of BMPs (-2, -6 and -7) onto hydroxyapatite coatings

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Design of new osteoinductive biomaterials to recapitulate an optimized physiological environment capable of recruiting stem cells and instructing their fate towards the osteoblastic lineage has become a priority in orthopedic surgery. This work aims at evaluating the bioactivity of BMP combined with human plasma fibronectin (FN/BMP) delivered in solution or coated onto titanium-hydroxyapatite (TiHA) surfaces. Herein, we focus on the comparison of *in vitro* osteogenic efficacy in mouse C2C12 pre-osteoblasts of three BMP members, namely: BMP-2, BMP-6 and BMP-7. In parallel, we evaluated the molecular binding strength between each BMP with FN using the Surface Plasmon Resonance (SPR) technology. The affinity of BMPs for FN fundamentally differs depending on the BMP considered. Indeed, combining FN with BMP-2 on TiHA surfaces potentiates the burst of gene-mediated osteogenic induction, while it prolongs the osteogenic activity of BMP-6 and surprisingly annihilates the BMP-7 one. These results correlate with FN/BMP affinity for TiHA, since BMP-6 > BMP-2 > BMP-7. Finally, by analyzing the osteogenic activity in the peri-implant environment, we showed that osteoinductive paracrine effects were significantly decreased upon (FN/BMP-6), as opposed to (FN/BMP-2) coatings. Altogether, our results support the use of FN/BMP-6 to develop a biomimetic microenvironment capable to induce osteogenic activity under physiological conditions, with minimum side effects.

P868 Modulation of gene expression in human adipose-derived stem cells seeded on PLGA or biomimetic nanocomposite: comparison of 2D films and 3D electrospun meshes - the real comparison

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Objective: Tissue engineering of materials seeded with stem cells is confronted with finding the appropriate cues to trigger the intended stem cell differentiation. In this regard, three-dimensional environments offered to stem cells are often compared to their two-dimensional culture behavior in the plastic culture dish. Here, we compare the gene expression pattern of stem cells seeded in a 3D electrospun mesh compared to cells seeded on a 2D film – of exactly the samematerial.

Materials and Methods: Electrospun meshes of poly-lactic-co-glycolic acid (PLGA) or nanocomposite disks of PLGA and amorphous calcium phosphate nanoparticles (PLGA/a-CaP) were seeded with ASCs and cultured either in DMEM or in osteogenic medium (OM). Corresponding two-dimensional films were also seeded with ASCs. After two weeks, minimum stem cell criteria markers as well as typical markers for osteogenesis, endothelial cell differentiation, adipogenesis and chondrogenesis were analyzed by quantitative real-time PCR.

Results: While stem cell markers were overall only slightly affected, osteogenic genes were upregulated when 3D meshes were compared to 2D films, especially osteocalcin. As for angiogenesis, CD31 was upregulated under all conditions. Adipogenic differentiation as assessed by PPAR-□-2 revealed an upregulation for the PLGA/DMEM system, while a clear downregulation was found for the PLGA/aCaP/OM system.

Conclusions: When 3D electrospun fiber meshes are seeded with ASCs and their differentiation is compared to 2D films, only few genes are affected with the same trend (CD105, osteocalcin and CD31). Otherwise, gene expression for a 2D/3D change is highly dependent on the material composition as well as the culture medium. The beneficial three-dimensional environment for stem cells found in many studies has therefore not to be interpreted by the third dimension alone and should carefully be compared to 2D films fabricated of the same material.

P869 Human pluripotent stem cells cultured on recombinant human vitronectin-grafted hydrogels by adjusting surface charge

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Human pluripotent stem cells (hPSCs) are promising cell source for regenerative medicine and drug discovery. The development of chemically defined biomaterials is necessary for culturing hPSCs for clinical applications without xenogenic contaminants. We have developed the biomaterials for hPSCs culture to maintain their pluripotency, such as (a) dishes coated with recombinant vitronectin and (b) dishes immobilized with oligopeptides derived from extracellular matrices (ECMs). We investigate the effect of the elasticity of the synthetic dishes on the pluripotency fate and proliferation of hPSCs in this study. We developed polyvinylalcohol-co-itaconic acid (PVA-IA) films grafted with recombinant human vitronectin (rhVN) to evaluate the physical effect of elasticity of hydrogels grafted with biologically active nanosegments from the pluripotency and proliferation fates of hESCs (WA09). The PVA-IA hydrogels were prepared with different elasticities ranging from 10.3 to 30.4 kPa storage moduli by controlling the crosslinking time with glutaraldehyde. Subsequently, rhVN was grafted on PVA-IA hydrogels with or without poly-L-lysine main chains. We designed to adjust the surface charge of PVA-IA hydrogels by grafting poly-L-lysine, which were evaluated by zeta potential. This study investigates the optimal elasticity of PVA-IA hydrogels grafted with rhVN that was prepared with much less concentration (5 μg/ml) compared to the concentration of oligovitronectin (500 μg/ml) that was generally used for the expansion of hPSCs for a long period of hPSC culture (10-20 passages) under xeno-free condition in previous study. hPSCs cultured on PVA-IA hydrogels grafted with rhVN were evaluated from pluripotent protein expression by immunostaining, embryoid body (EB) formation, and teratoma formation after 10 and 20 passages.

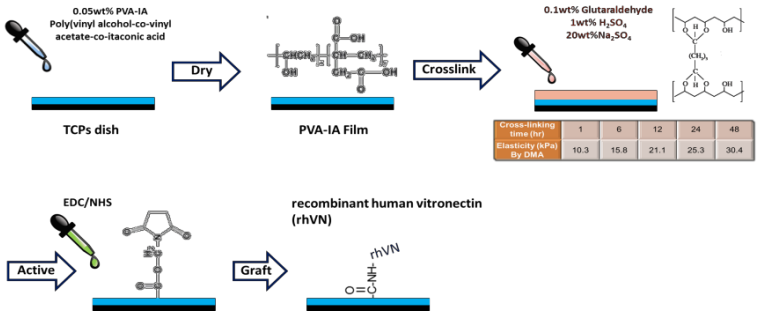

Fig. 1 Preparation of PVA-IA hydrogel dishes grafted with recombinant human vitronectin (rhVN) with different elasticity.

P870 Pore alignment regulates MSC differentiation in solubilized extracellular matrix derived scaffolds

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INTRODUCTION: Articular cartilage (AC) is a soft tissue lining the ends of the bones in synovial joints. Even minor lesions can cause pain, impaired mobility and result in sub-chondral bone damage if untreated; subsequently progressing to osteoarthritis, a disease affecting millions of adults worldwide. Moreover, injuries affecting AC and the underlying bone show poor long term endogenous regeneration capacity. Despite previous attempts to repair osteochondral defects, its regeneration still remains a significant clinical challenge. This motivates the development of new tissue engineering (TE) strategies to regenerate this complex interface. In recent years there has been increased interest in the use of decellularized Extracellular Matrix (ECM) derived scaffolds to induce repair of musculoskeletal tissues. These matrices contain structural and functional proteins that may facilitate repair native-specific tissues such as AC and bone. Structural features such as scaffold pore alignment have also been shown to influence cell infiltration, differentiation and matrix deposition, however such factors have not been explored for ECM derived scaffolds. The objective of this study is to develop a novel bilayered solubilized ECM derived scaffold with controlled porosity alignment for osteochondral TE. MATERIALS AND METHODS: Articular Cartilage (AC) and growth plate (GP), were harvested from porcine knee joints, solubilized (sECM) and freeze-dried in two different conditions to produce scaffolds containing random or aligned porosity by controlling directional freezing. Scaffolds were seeded with bone marrow derived Mesenchymal Stem Cells (BMSCs) and cultured for 28 days in chondrogenic differentiation media, osteogenic differentiation media or expansion media. RESULTS: In chondrogenic conditions, the highest levels of sGAG and collagen deposition occurred in the sECM scaffolds with aligned porosity. Of the two sECM, the highest values of sGAG occurred in AC sECM. On the other hand, the highest levels of calcium deposition occurred in scaffolds with random porosity. Of the two sECM, the highest level of mineralisation occurred in GP sECM. In osteogenic conditions, all groups showed low levels of sGAG and collagen deposition but high levels of calcium deposition, with no significant difference across the groups. In expansion media low levels of sGAG and calcium were deposited. DISCUSSION: The results of this study demonstrated that pore alignment affects the capacity of different solubilised ECM materials to support either osteogenesis or chondrogenesis of MSCs. The next phase of this study will explore the assembly of sECM as a bi-layer scaffold for osteochondral tissue engineering.

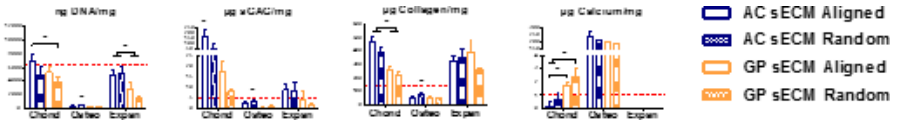

Figure 1. Biochemical analysis after 28 days in chondrogenic, osteogenic and expansion media. Red dash line representing levels at day. N=3, *=p<0.05 compared within the same media condition.

P871 Myocardial differentiation of human adipose stem cells on elastin surfaces

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Extracellular mechanical stimuli contribute to the stem cell differentiation fate and cell maturation. In this study, we attempt to obtain cardiomyocyte-like cells from adipose stem cells (ASCs) by using mechanical and paracrine stimuli. In order to achieve a viscoelastic structure, natural elastin was selected as the source material for the synthesis of 20-40 µm sized microspheres. Human ASCs isolated from waste lipoaspiration material were expanded in culture, and characterized by colony forming unit-fibroblast, tri-lineage differentiation, and flow cytometry assays. Characterized ASCs were cultured in the presence of elastin-based microspheres in 2D and 3D culture environment. In order to uncouple the effect of gravitational force and the effect of elasticity from the microsphere surface; cells were cultured under microgravity conditions in Synthecon® bioreactor system that was initially designed by NASA. Differentiation was induced with 5-azacytidine in cardiomyocyte growth DMEM-F12/IMDM medium containing B27, EGF, bFGF, cardiothrophin and thrombin. For the preparation of elastin microspheres, pure α-elastin from bovine neck ligament was degraded in an acidic solution. By this method, the non-soluble natural elastin was entirely dissolved in an aqueous solution. The degraded protein was later crosslinked to form hydrogel microspheres in a water-in-oil emulsion system. Microsphere characterization: The mean microsphere diameter was measured with AxioVision software, and ImageJ software (NIH) was used to count microspheres from evaluating phase-contrast micrographs. Differentiation experiments: Immunohistochemistry analysis of the cell-aggregates revealed expression of the cardiac-specific transcription factor Nkx-2.5. Further IHC assays for GATA4, connexin43 and cardiac troponin I were also carried out. Varying conditions of 3D microgravity cultures were performed and showed promising RT-PCR results positive for cardiovascular-related markers.

P873 cellularization of an esophagus substitute by hadsc sheets

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The Oesophagus Tissue Engineering Project was born of the clinical need to develop oesophageal reconstruction strategy more effective than those used routinely. Following a review of the literature Dr. Luc concluded that the materials for the manufacture of a surrogate must have mechanical properties and a similar organization to those of the extracellular matrix (ECM) of a native esophagus. In this context, the esophagus properties of other species were analysed and the porcine model selected due to similarities of structure and mechanical properties. An optimized protocol of the pig's esophagus decellularization has been developed aiming at demonstrating the feasibility of this strategy. The decellularized matrix (DM) recellelularization by autologous cells would favour better healing and prevent the formation of the fibrous tissue responsible for closing the light of the esophagus. The main objective of this work is to demonstrate the ability of pig DMs to be cellularized, in tubular form, by human ADSCs cultured in sheets according to the method of "tissue engineering by self-assembly" (TESA) on their internal (lumen) and external faces. Secondary objectives are to verify the amount of residual DNA in the DM, to characterize the quantity of the remaining GAGs, and to compare the effect of the last rinse of decellularization on the biocompatibility of DM by an attachment test, and cytotoxicity tests.

Twenty-five pig esophagus were decellularized by a dynamic method combining sodium azide and deoxycholic acid. Human ADSCs were extracted and cultivated in DMEM-HAM (3:1, 10% FBS) medium and seeded on DM for adhesion tests, and Neutral Red and MTT tests (n=3). Human ADSCs were also cultivated in DMEM-HAM (3:1, 20% FCS) with 5% ascorbic acid to obtain cell sheets after 51 days. They were wrapped around the tubular DM (on the internal wall 14 days and then the external surface) and cultivated 35 days (n=3). Samples were harvested at D7, D14, D20 and D28 until D35 for histological analyses (HES staining).

The concentration of residual DNA (50 ± 18 ng / mg of dry tissue) validates the effectiveness of decellularization. The study of DM biocompatibility (attachment, RN and MTT) stressed the importance of the final rinse with culture medium: impregnating DM with molecules (growth factors SFV, nutrients) promotes adherence and survival of ADSCs. The sheets of ADSCs have adhered to the outer face of DM in which migration was observed. It seems to be preferably at the remnants of the adventitia. Adhesion was not observed on the inner surface (lumen) of the DM.

In conclusion, we have demonstrated the ability of the oesophageal substitute to be colonized by human ADSCs. New DM seeding will be performed with labelled ADSCs (tdTomato) to verify their migration within the DM. In addition, a Live-Dead test could be performed to demonstrate the viability of cells *in vitro* on DM. The renewal of the culture medium can be improved by cultivating dynamically the DM seeded with sheets of ADSCs (in a flow simulation bench).

P874 Investigating the alignment of cellulose nanowhiskers and its potential to encourage myogenic differentiation of stem cells

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Skeletal muscle has a high capacity for self-regeneration. It is constantly repairing itself as we grow yet it has limitations. Muscle loss due to accidents or disease results in scar tissue formation which restricts the original function of the tissue. The use of a substrate that can encourage stem cells to undergo myogenic differentiation could repair the muscle's functionality.

It is proposed that aligned cellulose nanowhiskers (CNWs) could be functional in inducing myogenic differentiation of stem cells. The combination of the aligned topography and adjustable stiffness in the polyelectrolyte layers could act as mechanical stimuli for myogenic expression.

Through the partial acid hydrolysis of tunicin cellulose with sulphuric acid, CNWs with dimensions of around 5 nm in diameter and lengths ranging from nanometres to microns can be produced. The use of sulphuric acid leaves a slight negative charge on the CNWs which enable the building of polyelectrolyte layers with positively charged chitosan, creating a layered substrate. CNWs can be spin coated resulting in a radially aligned top layer suitable for cell culture. Stem cells were cultured on a range of CNWs substrates with different numbers of polyelectrolyte layers. Alignment and myogenic expression was investigated using bright field imaging and immunofluorescence staining respectively.

Alignment of the CNWs was confirmed using atomic force microscopy. Cells were initially observed for end-to-end alignment to the topography of the spin coated top layer and further stained for the presence of myogenin, a muscle specific transcription factor that indicates that there is potential for myogenic differentiation to occur.

The aligned topography and use of the different number of layers shows potential in driving stem cell differentiation triggered by physical stimuli. The use of CNW polyelectrolyte layers in directing stem cells towards myogenic differentiation shows promise as a first step in aiding muscle repair. The application of the CNW substrate gives a basis for further investigation in to establishing optimal conditions for myogenic expression in stem cells in vitro.

P875 MicroRNA expression of osteogenically differentiated human adipose stem cells under hypoxic and normoxic culture conditions

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Mesenchymal stem cells (MSCs) receive great interest in cellular therapies including bone regeneration applications. Their contribution to bone fracture repair depends on their proliferation capacity, osteogenic differentiation and induction of neovascularization. There is increasing evidence that hypoxic conditioning improves these properties through the regulation of a large number of transcription factors and signaling pathways. The aim of this study was to comparatively evaluate the effects of hypoxic (2% oxygen) and normoxic (20% oxygen) conditions on the in-vitro osteogenic differentiation of human adipose derived MSC cultures, in the miRNA expression level. Abdominal adipose tissues of adult patients were collected from elective operations under ethical approval. MSCs were isolated from the adipose tissue and expanded. The cells were then immunophenotypically characterized by flow cytometry. Osteogenic differentiation was carried out in DMEM-LG supplemented with 10% FBS, Penstrep, 10 nM β- glycerophosphate, 0.1 µM dexamethasone, and 0.2 mM ascorbic acid at 37°C, 5% CO_2, 95% humidity, and either at ~2% or ~20% oxygen for upto 28 days. MSCs were collected at predetermined time points, total RNA, then mRNA isolated, and finally cDNA syntesized. The samples were examined for human miRNAs which are known to regulate the osteoblastic phenotype. Osteogenic cultures in hypoxic and normoxic conditions were also analysed using histochemistry (Von Kossa, Alizarin red) and immunohistochemistry (Alkaline phosphatase, Osteonectin, Osteopontin, Osteocalcin). Adipose MSCs in hypoxic cultures demonstrated enhanced self-renewal and proliferation capacity. Eventually, differentially expressed miRNAs provided additional information to identify the changes of osteogenic differentiated human adipose MSCs invitro, under hypoxic and normoxic culture conditions at the molecular level.

P877 Platelet-rich plasma modulates mesenchymal cells chondrogenesis in collagen-hyaluronic acid scaffolds according to concentration

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Purpose: Upon induction with specific growth factors, bone marrow-derived mesenchymal stromal cells (BMMSC) can differentiate into chondrocytes and synthesize cartilage extracellular matrix. However, the development of a tissue substitute that could restore articular cartilage defects is still a perspective. In order to achieve that goal, biomaterials act as scaffolds for 3D cell culture, providing biological, biochemical and mechanical cues to promote BMMSC

differentiation. Additionally, several growth factors with important roles in chondrogenesis are

present in plateletrich plasma (PRP). Therefore, the purpose of this work was to investigate whether PRP could augment BMMSC chondrogenic differentiation seeded on collagen-hyaluronic acid scaffolds (CHyA).

Methods and Materials: Human BMMSC were cultivated in 2D and in 3D (seeded on CHyA scaffolds) in the presence of a basal or a chondrogenic medium (with TGFβ) supplemented with fetal bovine serum (FBS) or PRP. The human BMMSC were commercially acquired. The CHyA scaffolds were fabricated by a freeze-drying mechanism of a collagen type I and hyaluronic acid suspension. The PRP was obtained by double-centrifugation of peripheral blood collected from healthy donors, and activated with calcium chloride.

Results: PRP at the concentration of 2.5% had similar effects on cellular growth compared to 10% FBS, while 10% PRP resulted in the maximum induction of BMMSC proliferation. Both in 2D and 3D, 2.5% PRP stimulated the expression of chondrogenic genes, while 10% PRP related most to a hypertrophic/osteogenic phenotype. Moreover, in 3D, the chondrogenic medium supplemented with 2.5% PRP resulted in greater sulphated glycosaminoglycan synthesis and scaffolds mechanical properties.

Conclusion: The results corroborate the hypothesis of PRP chondrogenic potential, but highlights that it can be differently modulated according to concentration. Future studies are still needed to verify if the substitution of FBS to PRP *in vitro* will further improve clinical outcomes of cartilage cell therapies and tissue engineering.

P878 Growth and differentiation of adipose-derived stem cells on Ti-doped diamond-like carbon layers

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Diamond-like carbon (DLC) layers, used as prostheses coatings are biocompatible, hard, smooth, chemically inert, but they often delaminate. Doping with Cr, Ti, F or Si decreases material internal stress and improves mechanical properties. Ti-doped DLC layers, prepared by a dual pulsed laser deposition, have been tested to assess their ability to stimulate growth and differentiation of adipose-derived stem cells into osteoblasts. According to XPS, DLC contained 0, 0.9, 1.8, 2.9, 6.4, and 9.5 % Ti. The DLC layers were seeded with adipose-derived stem cells at the density of 25 000 cells/cm^2 in 1.5 mL of DMEM with 10% FS and 10 µg/mL FGF2, and cultured for 21 days. From day 4, β-glycerolphosphate, L-glutamine, ascorbic acid, vitamin D3, and dexamethazone were added into the cell culture medium. Medium was replaced twice a week. Metabolic activity (resazurin assay) was measured on days 1, 7, 14, 21, alkaline phosphatase activity, on days 7 and 14. The cells were stained for type I collagen and osteocalcin. We observed higher cell metabolic activity on glass and PS on day 1. A positive effect of higher Ti concentration in DLC on cell growth was observed on day 7 and 21. On all DLC samples, the ALP activity was increased compared to PS and Glass on both time intervals. The intensity of fluorescence staining of osteocalcin positively correlated with Ti concentration in DLC on day 21. On day 14, only weak diffuse staining of collagen was present, however, on day 21, a dense network of collagen fibres was observed on all samples. Ti-doped DLC layers with the higher Ti content are promising materials for bone replacements.

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P879 Pluripotency and differentiation ability of human adipose-derived stem cells cultured in 2D and 3D culture

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Human adult stem cells, such as human adipose-derived stem cells, are considered to be an attractive source of stem cells than human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). This is because human adult stem cells do not generate the ethical concerns that accompany in hESCs. Although human adipose-derived stem cells (hADSCs) are promising for use in regenerative medicine, their lower expansion ability (aging problem) due to the lower pluripotency of hADSCs compared with hESCs and hiPSCs is a critical issue. We found that the pluripotency gene expression of Oct4, Sox2, and Nanog in hADSCs after cultivation on TCPS dramatically decreased compared to those in the cells in stromal vascular fraction (SVF) as well as the cells in 3-D culture. There are high pluripotent stem cells in SVFs and 3-D culture, although SVF has more heterogeneous population compared to hADSCs cultured on tissue culture polystyrene (TCPS) dishes. There are two theories to explain this phenomenon, one is “Elite model” and the other is “Stochastic model”. Elite model indicates there contains high pluripotent stem cells in SVF and in 3-D culture where high pluripotency stem cells were decreased after cultivation on TCPS (2-D culture). Stochastic model explains that the pluripotency of the same stem cells changes depending on their microenvironment where hADSCs in floating conditions (3-D culture) express high pluripotency gene expression. We evaluated whether hADSCs can be explained by “Stochastic model” or “Elite model” by hADSCs culture in 2-D culture and 3-D culture sequentially. Precisely, we evaluated the difference of gene expression of the cells when hADSCs were cultured in the 2-D condition (TCPS) and in 3-D condition (Ultra low dish) sequentially (Fig. 1). We found that pluripotent gene expression in 3-D culture condition was higher than that in 2-D culture condition. Furthermore, we investigated the pluripotency and differentiation ability on the different substrate (Matrigel, Synthemax, CellSart) after culture in 2-D and 3-D culture (Fig. 2).

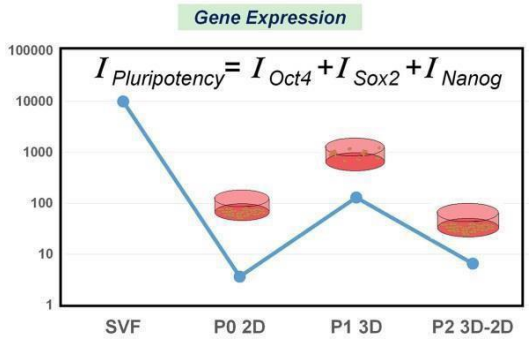

Fig. 1 Pluripotent gene expression of hADSCs cultured in SVF, 2-D, and 3-D conditions sequentially.

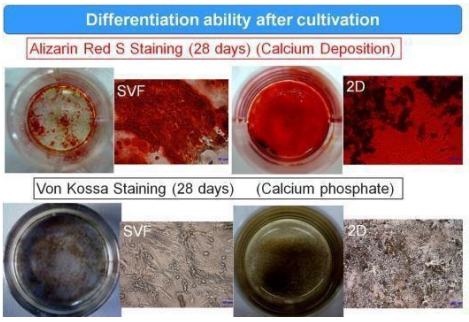

Fig. 2 Differentiation ability of hADSCs in SVF and after culture in 2-D condition.

P880 In situ and in vivo monitoring of biological response to titanium dental implant through measuring electrochemical parameters

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Purpose: This study was to *in situ* and *in vivo* monitor the biological response, in terms of electrochemical parameters, to titanium dental implant.

Materials and methods: The BMP-2 was immobilized to titanium dental implant through using a natural cross-linker genipin. The screw-type titanium dental implants, including the sandblasted with large grit and acid-etched (SLA) implants with and without BMP-2 immobilization, were implanted into the femur of New Zealand rabbits. A self-designed electrochemical cell was used to periodically measure the electrochemical parameters, including the impedance and polarization resistance.

Results: The BMP-2-immobilized SLA implants showed higher *in situ* and *in vivo* electrochemical impedance and polarization resistance after implantation in one hour, which was mainly ascribed to the faster blood coagulation on the BMP-2-immobilized SLA surfaces vs. SLA only surfaces. After six weeks, the BMP-2-immobilized SLA implants showed faster osseointegration than the untreated SLA implants, leading to a significantly higher electrochemical impedance and polarization resistance. The abovementioned results have been also confirmed by the *in vitro* biological analyses.

Conclusions: The *in situ* and *in vivo* biological response to the SLA titanium dental implant can be monitored periodically through measuring the electrochemical parameters. The BMP-2 immobilization on SLA titanium dental implant further increased the osseointegration.

P882 The effect of degradation of ternary composite scaffolds on differentiation of human bone marrow mesenchymal stem cells

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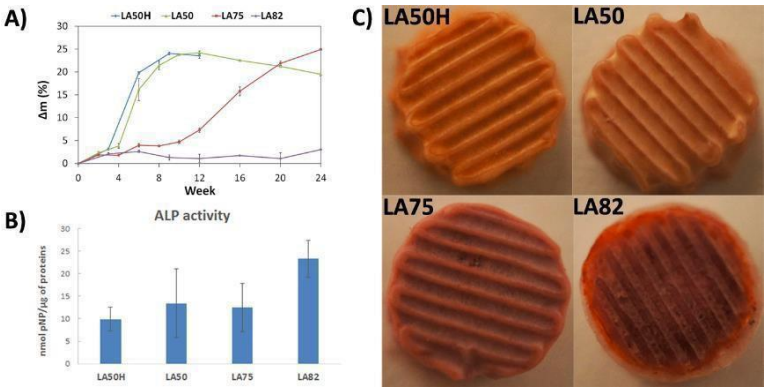
Introduction: In one of its approaches, tissue engineering utilizes biodegradable scaffolds as temporary matrices for cell growth and differentiation. Degradation of the scaffolds should match regeneration of the damaged tissue. In our laboratory, we developed a series of ternary composite scaffolds made of poly(ε-caprolactone) (PCL), poly(lactide-*co*-glycolide) (PLGA) and bioactive tricalcium phosphate (TCP). By varying hydrolysis rate of the PLGA phase, we tailored degradation rates of the scaffolds to match regeneration of bone. However, during degradation in simulated body fluid (SBF), we observed that deposition of calcium phosphates occurred only after the PLGA was cleared from the composites.

The aim of this study was to assess the effect of degradation of the ternary scaffolds on differentiation of human bone marrow mesenchymal stem cells (hMSCs).

Materials and methods: Scaffolds with various degradation rates (LA50H, LA50, LA75 and LA82; mass loss in SBF depicted in figure A) were fabricated by means of additive manufacturing. HMSCs were cultured in expansion and osteogenic media. MTS assay was performed at day 1, 4 and 7 to assess cell growth. ALP and micro-BCA assays were conducted to determine ALP activity at day 7. Alizarin Red staining was carried out after 28 days of osteogenic differentiation to visualize mineralization of extracellular matrix.

Results: After 7 days of culture, we observed the lowest hMSCs metabolic activity, thus proliferation, in the slowly degrading scaffolds LA82. This finding was confirmed by total protein concentration. Conversely to the proliferation, the highest ALP activity was measured in scaffolds LA82, and the lowest on the fastest degrading scaffolds LA50H (B). Moreover, poor mineralization was observed on fast degrading scaffolds LA50H and LA50 (C).



Conclusion: Based on the preliminary data, it seems that degradation products of ternary composite scaffolds PCL/PLGA/TCP may delay osteogenic differentiation of hMSCs.

P883 Direct reprogramming of fibroblasts into integration-free, induced neurons using mRNA delivery

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Converting somatic cells into induced neurons (iNs) directly without inducing pluripotency has clinical significance for treatment of central nerve diseases. In general, viruses are used to deliver the reprogramming factors into somatic cells for direct reprogramming. However, generating iNs for human therapeutic purpose requires new gene-delivery systems that do not integrate transgenes into the genome. Here, we demonstrated that graphene oxide-polyethylenimine (GO-PEI) is an effective and safe system for mRNA delivery for direct reprogramming into iNs. GO-PEI exhibited low toxicity to cells and elevated mRNA delivery efficiency, which induced direct conversion of fibroblasts into iNs without integrating the factors into the genome. In addition, in vivo transduction of reprogramming factors with GO-PEI into the brain of Parkinson's disease mice generated iNs and alleviated the disease symptoms. Taken together, the GO-PEI delivery system can be used to produce iNs and develop direct cell reprogramming-based therapies for neurodegenerative diseases.

P884 Characterization of clonal colony formation process of human induced pluripotent stem cells based on kinetic analysis

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Human induced pluripotent stem cells (hiPSCs) can self-renew and differentiate into diverse cell types, thus providing a platform for industrial and clinical applications. However, heterogeneity and functional variability within stem cell populations are fully elucidated leading to inefficiencies in differentiation and concerns regarding reproducibility. In this study, we assessed the growth and development of colonies of hiPSCs based on a kinetic analysis and discusses the status of this growth form in relation to that of colony formation at a clonal level. To this end, we first compared the differences between two commercially available culture media (StemFit AK02 and mTeSR1) on the plates coated with laminin E8 fragments (iMatrix-511). When the hiPSCs dissociated into single cells was plated in either StemFit AK02 or mTeSR1 at a density of 2.5 x 10^3 cell/cm^2, with the addition of 10 μM ROCK inhibitor, the number of live cells in StemFit AK02 after 24 h were significantly higher than in mTeSR1. Moreover, StemFit AK02 culture 120 h after plating, the total cell numbers was significantly higher in mTeSR1 culture, showing more efficient clonally derived colony formation. We tested whether there was an effect of cell survival, division, and migration in the manner of colony formation, and found that survival and proliferation are highly correlated with the formation of clonal colonies. The time-lapse observation demonstrated that the hiPSC in the mTeSR1 exhibited the higher migratory ability compared with that of cells in StemFit AK02 within the first 24 h post-plating. In contrast, the proliferative ability of hiPSCs was significantly higher in the StemFit AK02 than in the mTeSR1. If the cells in mTeSR1 fail to migrate within this time period, then colony formation is unlikely. We confirmed that the StemFit AK02 facilitated colony formation through the survival and proliferation of single cells rather than cell–cell contact formation through migration. These results indicated that the StemFit AK02 improved the clonal expansion of hiPSCs in such a way of maintaining a higher proliferative population and hindering encounter between cells in the process of colony formation. The results obtained in this study demonstrate that the behavioural understanding of individual cells can give inclusive information about the states of cell population in a clonal culture system.

P885 Gelatin based biocoposite film for in vitro development of respiratory epithelium

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Introduction:

The respiratory tissues injuries, infections, and inflammation may lead to degeneration and fibrosis of the respiratory epithelium with serious consequences for the patients. However, cell based therapies aiming at regeneration of organised respiratory epithelium is still a difficult task due to the complex morphology and low proliferation capacity of epithelial basal cells. There is therefore a need for new approaches to allow easier application of cell based therapies for respiratory epithelium regeneration. Naturally-derived substrates are attractive systems for tissue engineering, due the fact of being the building blocks of the extracellular matrix hence they have been widely used in composite form in various tissue engineering applications.

Methodology and results:

In the current study, it is proposed that it is possible to control stem cell differentiation by modulating the microenvironment of stem cells culture using the gelatin based film scaffold and controlled release of growth factors encapsulated within the films. The bone marrow derived mesenchymal stem cells (BM-MSCs) were cultured on gelatin and gelatin-hyaluronic acid composite films encapsulated with keratinocyte growth factor and fibroblast growth factor 10 in HAM-F12 media with 10µM Retinoic acid. These gelatin based films show excellent controlled release of soluble growth factors up- to four weeks. The MSCs- start expression of Pan cytokeratin and CK-18; the epithelium markers for respiratory epithelium and also reduced expression of vimentin; a marker for MSCs in three weeks culture. In addition the MSCs acquired a cobblestone shaped morphology after being cultured in airway epithelial cell growth medium for two weeks. However, the presence of goblet cell marker mucin or tight junction protein (ZO1) was not observed after three weeks of air-liquid culture.

Conclusions & future studies:

We report the use of, a modular gelatin-based film release system as a platform for fabrication of engineered respiratory epithelium tissues for directional release of growth factors to attain precise epithelium differentiation in MSCs culture. Further optimisation of in vitro culture microenvironment are underway for the development of organised multi-type respiratory epithelium development by modifying the culture conditions

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Declaration: There is no conflict of interest to declare.

P886 The design and production of chemical nanoarrays to control mesenchymal stem cell adhesion and differentiation using polymer pen lithography

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Introduction

It is well established that cells sense and respond to changes in material chemistry, topography and stiffness. In addition, recent advances have proven that cells can sense and respond to stimulus at the sub-micron/nano scale. Here we report on the use of polymer pen lithography, to produce large area chemical nano-arrays, that are designed to control the spatially defined interaction, at the nano-scale, between selected chemical groups (-NH_2 and -CO_2H), and human mesenchymal stem cells (hMSC). The data presented defines the ability of selected chemical groups to control initial integrin binding, focal contact formation and subsequent MSC differentiation and phenotype. A definitive relationship between spatial orientation of integrins/focal contacts and presenting chemical group is presented.

Materials & Methods

16-mercaptohexadecanoic acid (MHA) or 11-amino-1-undecanethiol (AUT) was patterned by PPL with formation of square arrays on large-area gold surfaces (2 cm x 2 cm). Each array consisted of a feature size (modified area) 300 nm ± 5 nm, with variations in the spatial distribution of adjacent features from 1-3 μm. Arrays passivated with (11-mercaptoundecyl)hexa(ethylene glycol) (m-PEG) enabling controlled interactions with the chemical groups of interest at the point of contact. MSCs were cultured in contact with the surfaces in basal medium for time periods up to 28 days. Levels of adhesion, phenotype expression and differentiation were defined using a combination of reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry.

Results and Conclusion

Data obtained has demonstrated that changes in the nano-scale resolution of presenting -NH_2 and -CO_2H groups is a powerful tool in controlling initial MSC adhesion and subsequent differentiation. Selected MHA/-CO_2H chemical arrays induced hypertrophic cartilage phenotype, clustered cell morphology which was positive for Collagen X and osteocalcin, whilst in contrast AUT/-NH_2 patterned surfaces with identical topography design showed little to no cell adhesion. In addition it was proven that different chemical nanoarrays influenced protein adsorption and confirmation, which had a role in dictating the alignment of cells on the surface. The data proved that both sub-micron deposition of a chemical group has a definitive effect of MSC behaviour *via* control of initial cell attachment mechanisms and potential control of alignment, which is associated with mechanotransduction *via* the organisation of actin fibres within the cell.

Acknowledgment

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P887 Mesenchymal stem cells for osteonecrosis of the femoral headdata from ongoing clinical trial (code: xcel-mt-10-01; eudract: 2010-023998-18)

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Background

Osteonecrosis of the femoral head (ONFH) is a disease characterized by an ischemic event that eventually progresses to collapse of the femoral head which ends up with an end-stage total articular degeneration associated with pain and functional limitation. Current therapeutic strategies lack effective treatments for limiting progression towards femoral head collapse. We are conducting a clinical trial with an advanced therapy medicinal product (ATMP) to assess the effect of "ex-vivo" expanded autologous bone marrow mesenchymal stem cells (MSC) fixed in allogeneic bone tissue (XCEL-MT-OSTEO-ALPHA) in early-stage ONFH in comparison with the standard treatment of isolated core decompression. The working hypothesis proposes that treatment with this tissue engineering product is a valid and useful technique to achieve bone regeneration and avoiding progression to collapse and final arthroplasty.

Materials and Methods

This is a prospective, randomized, two-arms, parallel, single-dose, open-label with blinded assessor, phase I-II clinical trial in which 24 patients affected with osteonecrosis of the femoral head ARCO stage I or II will enter the trial with the primary objective of assessing the feasibility and safety of "ex-vivo" expanded autologous MSC fixed in allogeneic bone tissue (XCEL-MT-OSTEO-ALPHA) under GMP conditions in ONFH. Secondary objectives are to assess the efficacy of the implantation by imaging (magnetic resonance imaging (MRI) and positron emission tomography (PET)) and clinical questionnaires (Visual Analogue Scale for Pain, Short-Form Health Survey (SF-36) and WOMAC Index). Patients are to be randomized to one of the two treatment arms (12 per arm) either to the experimental treatment of core decompression and XCEL-MT-OSTEO-ALPHA or the standard treatment of isolated core decompression. Thereafter, they are followed up for 12 months. Patients randomized to the experimental treatment are to be programmed for bone marrow extraction 3 weeks prior to surgery. Once available, XCEL-MT-OSTEO-ALPHA is mixed with fibrin glue and surgically implanted between the necrotic area and the intact bone.

Results and Conclusions

At present, 20 out of the 24 planned patients have been randomized (10 to each arm). Sixteen of the 20 patients have already finished the one-year follow-up. Three hips evolved to collapse at 1 year follow-up requiring total hip replacement, one hip from the XCEL-MT-OSTEO-ALPHA group and two hips from the isolated core decompression group. These events occurred as a consequence of the illness progression and were assessed as not related to the study medication. No other SAEs have been reported so far. No significant differences in the clinical and radiological results have been observed between both groups. However, in the XCEL-MT-OSTEO-ALPHA group MRI imaging showed revascularization of the necrotic areas and PET studies performed in a sample of patients showed higher signs of osteoblastic activity than in those hips under standard treatment. The anatomopathological study of a femoral head replaced in the XCEL-MT-OSTEO-ALPHA group revealed areas of bone regeneration suggesting the process of regeneration was taking place. Present data suggest that the use of XCEL-MT-OSTEO-ALPHA for the surgical treatment of ONFH is feasible and safe, and it promotes regeneration, thus providing the assessment of a new treatment option for this pathology.

P888 Biological characterization of phosphate-based glass microspheres for stem cell culture and differentiation

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INTRODUCTION: Osteoporosis is a chronic condition of the skeleton that causes the decrease of bone mass and strength, resulting in an increased risk of bone fractures. Due to the greater worldwide incidence of osteoporosis in the ageing population, alternative therapeutic strategies to promote bone regeneration are required. Particularly promising is the combination of stem cells with tailored biomaterial-based devices. Mesenchymal stem cells (MSCs) are multipotent stem cells characterized by the ability to differentiate towards mesodermal- lineages including the bone, and have already been used in the clinic (1). On the other hand, phosphate-based glasses (PBG) constitute promising materials for bone repair applications since they are fully bioresorbable and can display chemical compositions similar to the inorganic part of the bone. PBG microspheres (MS) have been produced which can be injected through a minimally invasive route for in vivo delivery (2). The aim of this work was to characterize the cytocompatibility and osteogenic potential of three PBG formulations prepared as either bulk and porous microspheres, using human bone marrow-derived MSCs.

METHODS: Human bone marrow-derived MSCs were cultured for 21 days in the presence of culture medium conditioned with MS from different compositions of PBG. Non-degradable MS were included as negative control. Cell metabolic activity was measured after 2, 7, 12 and 21 days, while alkaline phosphatase (ALP) activity and alizarin red staining were analysed at d12 and d21, respectively. For the characterization of the PBG-MS degradation, MS were incubated in ultrapure water at 37C for 28 days; the water was refreshed every three days and measurements were done at d1, 3, 7, 14, 21 and 28. Samples of ultrapure water containing the MS degradation products were analysed by Ion chromatography in order to determine the ion release rates from MS.

RESULTS: The results of the metabolic assay indicated that PBG-MS degradation products did not negatively affect the cells in comparison to the culture medium, and supported cell growth over a period of 21 days. The formulations tested also significantly increased ALP activity, and promoted extracellular matrix mineralization in comparison to the non-degradable MS control and culture medium.

CONCLUSIONS: Our results suggest that degradation products from the PBG-MS formulations tested are cytocompatible and suitable for the long-term cell exposure. PBG seemed to promote the hMSCs osteogenic response, confirming PBG-MS as promising candidates for bone regeneration strategies.

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P890 Design and in vitro testing of a biofunctionalised polycaprolactone nanofibrous scaffold capable of recruiting endothelial progenitor cells

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Background: Endothelial progenitor cells (EPCs) are a promising cell source for the endogenous endothelialisation of vascular tissue engineering constructs due to their mobilization potential, and their ability to differentiate into a stable mature endothelial phenotype. Recruiting EPCs from the blood requires the use of instructive scaffolds biofunctionalised with specific molecules to enhance their capture and proliferation *in vivo*. TPS peptide (TPSLEQRTVYAK) is specific and selective to blood outgrowth endothelial cells (BOECs) expanded from EPCs[1]. In the current study, we aim to immobilize cysteine-containing-TPS peptide into jet-sprayed polycaprolactone (PCL) anisotropic nanofibrous scaffolds using the sulfhydryl cross-linker maleimide with heterobifunctionality.

Methods: BOECs were isolated from blood of healthy donors using selective plating, and their phenotype was confirmed by FACS analysis. PCL nanofibrous scaffolds were prepared by jet spraying technique at 2000RPM [2]. Surface activation of PCL scaffolds was achieved by incubation in 20% ethylenediamine (EDA) for 1hr at 40^{o}C with shaking. Scaffolds were then incubated with maleimide (PEG-NHS-Prop-Mal,1mg/ml) for 2hrs at room temperature, followed by an overnight incubation with 1mg/ml of the peptide in sodium bicarbonate solution (50mM; pH9). The efficiency of crosslinking was estimated using a FITC-tagged-TPS peptide, and by Ellman's assay. Cell capture assays were performed to test the ability of the modified scaffolds to capture BOECs. BOECs were incubated with the modified scaffolds for 1hr at 37C, then were stained with DAPI, and the number of adhered cells per cm^2 was calculated. The assay was also performed on A549, and human valve endothelial and interstitial cells. Cell capturing ability under flow conditions was tested by incubating the scaffolds with a 30ml suspension of BOECs in a tube rotator set to 15RPM. Number of adhered cells was calculated at 1,3,7 and 14 days.

Results: Isolated BOECs were positive for CD31, VE cadherin and CD34, and were negative for CD14 and CD45. Scaffolds modified with the FITC-tagged-TPS peptide showed homogenous fluorescent staining throughout the scaffold. The concentration of unreacted cysteine in the peptide solution was significantly reduced after crosslinking ($p<0.05$, $n=3$). TPS-modified-PCL scaffolds were able to specifically increase the adhesion of BOECs by approximately 6 fold when compared to untreated PCL ($p=0.02$,$n=3$). Modified scaffolds did not induce the adhesion of the other tested cell types ($n=3$). Our preliminary data showed that the modified scaffolds were able to capture BOECs under flow in a tube rotator, with increasing cell numbers at longer incubation periods.

Conclusion: In this study, we were able to immobilize TPS into jet sprayed PCL nanofibrous scaffolds through a controlled highly-specific sulfhydryl crosslinking approach. The results showed that this construct is able to specifically attract BOECs under static and flow conditions, which is a promising step towards the development of an acellular instructive implant for *in situ* vascular tissue engineering applications.

P891 Investigating the fate and location of therapeutic magnetic nanoparticle tagged stem cells post implantation in an ovine critical sized defect model

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The ovine critical size defect model provides a strong basis for testing bone tissue engineered constructs for use in the treatment of non-unions and severe trauma. This model has been applied across a number of preclinical studies with emphasis on stem cell based therapies for bone repair. Key to ensuring successful outcomes is understanding the fate of therapeutic cell in terms of identity, viability and bio-distribution post implantation. This study aims to evaluate such parameters in an ovine critical sized defect model in conjunction with a novel biomagnetic (MNPs) remote control technology, MICA. MICA (Magnetic Ion Channel Activation) has been shown to enhance osteogenic differentiation of mesenchymal stem cells *in vivo* and *in vitro* via remote magnetic nanoparticle mediated activation of the TREK-1 ion channel.

Methodology involved the isolation and expansion of autologous STRO-4 positive MSCs. MSCs (P1) were initially labelled with a fluorescent lipophilic dye (DII) and TREK-1 functionalised MNPS prior to encapsulation within a naturally derived bone extracellular matrix gel (ECM). Encapsulated cells ($5x10^6$) were implanted within a critical sized defect (0.8x1.5cm) in the medial femoral condyle of a sheep. Implanted cells within the condyle were stimulated by an external custom built magnetic array (1hr/day) until sacrifice. Sheep were sacrificed at either 2 days or 7 days post initial surgery where ECM was harvested and processed for analysis. The harvested construct was divided into 3 equal parts with the top and middle sections snap frozen for cryosectioning and mechanical properties of the bottom section evaluated.

Whole mount flourescent imaging showed that cells within the harvested gels were present at both day 2 and day 7 with DII labelled cells clearly visible throughout the construct. Viability of implanted cells post impantation was assessed using LDH (lactase dehydrogenase) staining which demonstrated 50% viability across all experimental groups. MNPs localisation was confirmed on viable cells by Prussian blue staining and dextran immunohistochemisty. Immunohistochemistry further revealed varying levels of osteogenic differentiation at these early time points across groups. Serum CRP (c-reactive protein) levels were measured by ELISA with no obvious increase in CRP levels observed as a result of MNP implanations. This implies that there were no adverse effects related to localised MNP delivery. Finally a sharp increase in the stiffness of all gels was measured post implantiaon when compared to *in vitro* controls.

To conclude, this study demonstrates the short term maintenance, safety and feasibility of MNP tagged MSCs which have been used to control and manipulate cell fate in cell therapy applications.

P894 Effect of hyaluronan supplemented culture media on human mesenchymal stem cell chondrogenesis

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Introduction: Multiaxial load in vitro has been shown to induce chondrogenesis of human mesenchymal stem cells (hMSCs) by increased synthesis and activation of endogenous TGF-β. The presence of hyaluronan (HA) in synovial fluid has an impact on the elastoviscosity of joint synovial fluid and consequently on the friction coefficient of the opposing articular surfaces. This important biological function will also potentially change the amount of mechanically activated TGF-β, with an impact on the hMSC response in vivo. Therefore, a medium containing HA may better recapitulate the rheological and biological features of the synovial fluid present in the patients' intra-articular environment. Thus, the aim of the present study is to improve the composition of a standard chondrogenic media by assessing the influence of HA supplemented culture media on the chondrogenesis of hMSCs.

Methods: hMSCs isolated from bone marrow were suspended at P3 in a fibrinogen-thrombin-solution, seeded evenly throughout porous polyurethane scaffolds and cultured in four different media. Control medium was serum free basal medium containing DMEM high glucose supplemented with 1% ITS+, 1% Pen/Strep, 1% non-essential amino acid, 50 µg/ml ascorbate- 2-phosphate, 5 µM EACA, 10^{-7}M dexamethasone (HA- TGFβ-). This media was further supplemented with 10 ng/mL TGF-β1 (HA- TGFβ+) or with 0.2% 1800 kDa HA (HA+ TGFβ-) or with both (HA+ TGFβ+). After 14 and 28 days of culture total RNA was extracted from the constructs and TaqMan reverse transcription and Real-time PCR were performed. A panel of human genes associated with chondrogenic markers (Collagen II, Collagen X, Aggrecan and hCD44) was investigated. After 14 and 28 days of culture, the constructs were digested with proteinase K and total DNA content and sulphated glycosaminoglycans (GAG) were measured spectrofluorometrically. Total GAG content of the culture media was also measured to assess the release of matrix molecules from the constructs into the media.

Results & discussion: Gene expression among the groups was comparable, with the exception of a clear downregulation of the hypertrophic marker Collagen X in the presence of HA (HA+ TGFβ+) compared to (HA- TGFβ+). Total GAG synthesized in samples supplemented with HA alone was consistently higher if compared with the control medium that was not supplemented (HA- TGFβ-). Is also noteworthy to highlight that in the early days of the culture there was no detectable level of GAG in any sample not supplemented with HA. Conversely there was significant GAG production within the first week in all samples supplemented with HA, both in the presence and absence of active TGFβ. These results demonstrate that the addition of HA to medium had a positive effect on the intrinsic capacity of hMSCs to produce ECM, especially in the early days of the culture. Additionally, exogenous HA could prevent the undesired upregulation of the hypertrophic marker Collagen X that is normally induced by active TGFβ. Therefore a better control of chondrogenic differentiation of hMSCs may be also achieved through the development of an appropriate culture media that more accurately represents synovial fluid.

P895 Deciphering osteogenic differentiation of bone progenitor cells on novel dental implant surfaces

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Introduction: Implant material and surface properties, including hydrophilicity and nanostructures, have been identified to promote osseointegration of implants, key to a stable long-term bone anchorage. However, most of the studies on osseointegration do not consider the impact of blood coagulation on top of the implant materials, which is however the first response of the body during surgery of the patient. While titanium and to some extent titanium alloys have been the "gold-standard" for dental implant systems, in case of thin gingival biotype or buccal bone loss a greyish region may appear through the gingiva. Therefore ceramics of yttrium-stabilized zirconia were reported to be promising alternatives to titanium. Unfortunately, current materials and surface modifications fail to recreate the osseointegration potential of titanium surfaces.

In this study an in vitro model was applied to study the early interaction with human whole blood and state-of-the-art zirconia (ZLA) and titanium (SLA® SLActive®) surfaces and how this influences osteogenic fate decisions of human primary bone and progenitor cells (HBCs).

Methods: Microstructured hydrophobic (SLA®) or hydrophilic (SLActive®) titanium and zirconia (ZLA) surfaces were incubated with freshly taken, partially heparinized human whole blood (ethical approval; EKSG 12/111). Coagulation was analyzed by SEM, immunohistochemistry, and ELISA. HBCs were then cultured on top of the blood-incubated surfaces and analysed for osteogenic differentiation after 28d as described before[1].

Results: Most pronounced blood coagulation was observed for ZLA, reflected by highest concentrations of fibrin on top of the surfaces and significantly increased levels of molecular markers of coagulation, e.g. platelet factor 4, in the supernatants of incubation. On the contrary lowest levels of fibrin or markers of the coagulation were obtained for SLA®. HBC mineralisation showed significantly higher levels of Ca^{2+} on top of blood incubated surfaces. Irrespective of prior treatment of the samples highest levels of Ca^{2+} were obtained on top of SLActive® surfaces. Furthermore higher levels of Ca^{2+} were found after culture on top of ZLA surfaces compared to SLA, but lower levels compared to SLActive. Levels of TGF-β, known to be key to HBC fate decisions, bound to the fibrin matrix were analysed via ELISA after enzymatic digestion of the fibrin and a correlation between material and observed factor concentration was obtained. Levels of TGF-β were lowest for SLActive and highest for ZLA.

Conclusion: The advanced in vitro model for osseointegration, including a blood incubation step prior to HBC culture, mimics the in vivo situation during implantation and allows to study the mechanisms guiding HBC fate decisions and to investigate the osseointegration of novel materials. In ongoing studies further cytokines bound to the fibrin matrix are quantified applying multiplex immunoassays. Ultimately, we envision to analyse these multifaceted data via a partial least square analysis approach[2] in order to identify key parameters responsible for the material dependent osseointegration

P897 Matrix interactions controlling osteogenesis from pluripotent stem cells

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It is well-established that the commitment and differentiation of stem cells can be influenced by both chemical as well as mechanical signals provided by the niche and the extracellular environment that they interact with. It has been established recently that matrix stiffness plays a key role in regulating osteoblast differentiation from mesenchymal stem cells. However, the regulation of osteogenic differentiation from pluripotent stem cells by defined growth factors and matrices of different stiffnesses requires further investigation. In this study, we have used the *in vitro* differentiation of pluripotent mouse Embryonic Stem Cells (mESCs) to osteoblasts and chondrocytes, to investigate the interplay between BMP signalling, matrix stiffness and Rho/ROCK signalling on chondro-osteo differentiation.

We have shown earlier that differentiation of mESCs through a step-wise protocol involving primitive streak/mesoderm specification and FGF-2-dependent mesoderm enrichment, yields a population of precursors that have both chondrogenic and osteogenic potentials when cultured in specific media. Our current studies now show that addition of BMP-4 at discrete intervals during differentiation induces the osteoblast lineage at the expense of the chondrocyte lineage, as determined by histochemical staining and molecular marker gene expression for lineage-specific markers. The addition of a ROCK inhibitor enhanced the BMP-4 effect, suggesting an important additional role of cell spreading and cell-matrix interactions. To test this, mESC differentiation was carried out on polyacrylamide gel substrates of low (2KPa) and high (50KPa) stiffness. Cells cultured on 2KPa maintained chondrocyte differentiation whereas osteogenic differentiation was downregulated. In contrast, cells on 50KPa substrate maintained both osteogenic and chondrogenic potentials to the same extent as on tissue culture plastic. Preliminary findings also suggest that inhibition of ROCK reverses the cell rounding phenotype induced by seeding on 2KPa substrates, although subsequent effects on differentiation are not yet clear. These results suggest that mESC-derived chondro-osteo lineage commitment is regulated by a potential cross- talk between chemical and mechanical signals.

P898 Investigating co-culture of periosteal and bone marrow mesenchymal stem cells onto bone scaffolds for critical size bone defect repair

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Critical size bone defects are currently repaired using autologous bone grafting, however risks include insufficient graft volume and donor site morbidity. A new approach for surgical repair is 'hybrid grafting', where a bone scaffold is mixed with autologous mesenchymal stem cells (MSCs) that can be contained to the defect site by a barrier membrane. This study investigates the *in vitro* 2D culture characteristics of human donor matched P MSCs and BM MSCs and 3D co-culture of P and BM MSCs on bone scaffolds.

Matched P and BM were harvested from healthy donors, MSC frequency was quantified and MSCs were grown in tissue culture. MSC phenotype was assessed using flow cytometry, osteogenic and adipogenic differentiation potential was evaluated. P and BM-MSCs were stained with CSFE and Yellow Cell Trace, respectively and mixed at ratios of 2:1, 1:1 and 1:2 with P and BM only as controls, followed by culture with a bone scaffold.

P-MSCs were found at significantly higher frequency compared to BM-MSC (average 5200 versus 6 MSC per $1x10^6$ nucleated cells, respectively). P cultures were CD90, CD73 and CD105 positive, but CD14, CD34 and CD45 negative, as were BM cultures. During long-term culture, P-MSC continued to divide up to 20 population doublings (PD), with faster growth rates up to the first passage (1.8 days/PD) compared to later passages (3.8 days/PD). BM-MSC had fast growth rates up to the first passage (2.6 days/PD) after which growth plateaued. BM and P-MSC were SUSD2 positive, however P-MSC were CD146 negative. Compared to BM-MSC, P-MSC showed similar osteogenic but lower adipogenic potential. SEM confirmed cell attachment to bone scaffolds. Confocal microscopy could distinguish between P and BM-MSCs in co-culture and staining was confirmed to be cytoplasmic as overlaid with ToPro3 nuclei staining. In addition, flow cytometry showed the stained cells were positive for the respective stains as well as CD73+ and CD45-.

In conclusion, P-MSCs had greater proliferative potential with similar osteogenic but lower adipogenic potential in comparison to donor-matched BM-MSCs. In 3D culture, stained MSCs were shown to attach to bone scaffolds and confocal microscopy and flow cytometry can be used to distinguish between the two MSC types. Further investigation is needed into the effect of co-culture on proliferation rates and osteogenic potential.

P899 *In vitro* differentiation of rat omentum-derived mesenchymal stem cells into parathyroid-like cells for the treatment of permanent hypoparathyroidism

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The omentum is considered as a source of stem cells and easily harvested in large quantities without serious complication, visible scar, and tissue contamination. Since *in vitro* differentiation of omentum-derived mesenchymal stem cells(OMSC) into various cells, here we aim to determine a feasibility of *in vitro* differentiation to parathyroid cell for the treatment of permanent hypoparathyroidism by autologous transplantation. The omentum were harvested in Sprague-Dawley rats and stem cells were isolated and expanded. The cells of passage#3 were used to differentiation using the modified Bingham protocol. Our findings reveal that OMSC were expanded over passage#5 and maintained their characteristics. Moreover, successful differentiation by either monolayer or 3D expansion to parathyroid-like cell was identified by immunohistochemistry, qPCR and ELISA. The omentum could be another affordable source of stem cells for autologous transplantation and OMSC could be differentiated into parathyroid-like cells.

P900 The effect of osteogenesis on scaffolds fabricated using melt electrospinning writing using a co-culture model

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IIntroduction: Bone regeneration using a scaffold-based tissue engineering approach involves a spectrum of overlapping processes, which are driven by cell-to-cell, cell-to-extracellular matrix (ECM) and cell-to-biomaterials interactions. Traditionally, the study of osteogenesis potential of tissue-engineered constructs (TECs) in vitro only considers the osteoblasts or mesenchymal cells (MSCs)-to-biomaterials interactions1. However, this poorly recapitulates the process of bone regeneration under physiological environment, whereby cross-talk or coupling of bone forming osteoblasts and bone resorbing osteoclasts is a fundamental requirement for bone remodelling. Hence, in this study, a growth factors free co-culture model, comprising osteoblasts and monocytes/osteoclasts was established to allow for the elucidation of osteoblasts-to-monocytes/osteoclasts interactions in a 3D environment using scaffolds fabricated via melt electrospinning writing technique. This model enables the study of the osteogenesis potential of a TEC taking into consideration osteoblasts-to-monocytes/osteoclasts and cells-to-biomaterials interactions.

Methods & Materials: Scaffolds made of medical-grade polycaprolactone (mPCL) were fabricated by mean of melt electrospinning writing. Subsequently, scaffolds (dimension: 8 x 8 mm, 10 layers) were coated with a thin layer of calcium phosphate (CaP) by means of chemical deposition. Scaffolds with and without CaP coating were seeded with human-derived primary osteoblasts (8 x 104 cells/scaffold) and monocytes (5 x 105 cells/scaffolds) and cultured for up to nine weeks. At time-points, cells were evaluated for alkaline phosphatase (ALP) and tartrate- resistant acid phosphatase (TRAP) activity. Additionally, cell morphology was observed through fluorescence microscopy, matrix mineralization was observed with von Kossa staining and osteoblastic and osteoclastic-related genes expression were analyzed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR).

Results & Discussion: As illustrated in Figure 1, the simultaneous presence of osteoblasts and monocytes/osteoclasts and CaP accelerated cell matrix formation on scaffolds. Quantitative gene expression profile showed similar findings. Whereby, osteoblastic- and osteoclastic-related gene expression was highest in the PCL/CaP co-culture groups compared to other groups. This indicated synergistic effects of soluble factors secreted by cells and solubilized inorganic components from the scaffolds in promoting matrix deposition.

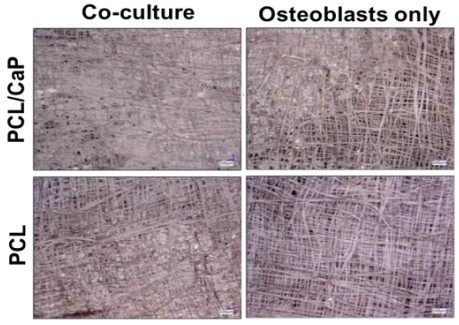

Figure 1 *Microscopy images of PCL and PCL/CaP coated scaffold in osteoblasts only or osteoblasts and monocytes/osteoclasts co-culture after 1 week.*

P901 Osteoconductive scaffolds based on bioactive polysaccharides for the proliferation of human derived dental pulp stem cells

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Bone tissue damages can derive from diseases such as osteogenesis imperfecta, osteoarthritis, osteomyelitis, and osteoporosis, or from traumatic injury, orthopaedic surgeries and primary tumour resection (Porter *et al.* 2009 *Biotechnol Progress*). In particular, non-critical bone defects and voids represent a hot spot for mechanical stresses that can cause fractures, thus it is necessary to accelerate their healing (Jawad *et al.* 2013 *J Orthop Res*). Moreover, spinal fusion, maxillary sinus floor elevation, guided bone regeneration, represent other clinical cases in which it is necessary to provide substitutes for bone tissue regeneration. (Mardas *et al.* 2010 *Clin Oral Impl Res*)

The use of synthetic bone substitutes reduces surgical procedures and the risk of infection or immunogenicity, and decreases the risk of disease transmission (Mottaghitalab *et al* 2015 *J Control Rel*; Porter *et al.* 2009 *Biotechnol Progress*). Moreover, synthetic scaffolds can be loaded with bioactive molecules or cells before implantation. Polysaccharides offer the opportunity to prepare biocompatible bone substitutes with specific morphological and biological characteristics, such as high interconnectivity and enhanced cell adhesion, which could mimic properly the bone tissue and support and enhance bone healing (Woodruff *et al.* 2010 *Prog Polym Sci*; Ward *et al.* 2010 *Oral Dis*)

In this work, tridimensional scaffolds based on alginate, hydroxyapatite (HAp) and derivatives of chitosan were prepared and characterized. The scaffolds were obtained through freeze casting of alginate/HAp hydrogels. Morphological characterization, obtained by means of micro-computed tomography, revealed that scaffold porosity and interconnectivity are compatible with the requirements for the bone tissue regeneration (Turco *et al.* 2009 *Biomacromolecules*). Moreover, the physical-chemical stability displayed by the scaffolds makes them suitable for supporting cell adhesion, colonization and growth. Another important aspect hereby taken in account was the introduction of the chitosan derivatives that showed bioactive properties such as increased osteoblasts proliferation and ALP activity, and increased bone-implant contact in the case of thermosets functionalized with this polysaccharide (Marsich *et al.* 2013 *Acta Biomater*).

The scaffolds presented in this work were successfully used for the growth and the differentiation of human derived dentin pulp stem cells (hDPSCs). Flow cytofluorimetry proved that hDPSCs harvested in our laboratory can be maintained in culture with an undifferentiated phenotype and that can be differentiated in osteoblasts under proper stimuli, as demonstrated by enzymatic and biochemical assays.

Overall, the combination of hDPSCs and polysaccharide based bioactive scaffolds, represents an effective strategy for the preparation of osteoconductive and osteoinductive cell-loaded biomaterials for the treatment of bone defects and for the clinical practice in oral surgery.

P903 Suitability of human muscle-derived stem cells on electrospun PCL scaffolds for skeletal muscle tissue engineering

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Introduction: Electrospun poly-ε-caprolactone (PCL) scaffolds have been used previously for tissue engineering applications. We have evaluated the *in vitro* suitability of an electrospun random-aligned PCL scaffold seeded with primary human skeletal muscle derived cells (hSkMCs) for skeletal muscle tissue engineering.

Methods: Culture expanded hSkMCs (n=5) were seeded at 1 lakh per sq. cm on the scaffold and assessed for cell viability and proliferation, immunophenotyping, histological analysis, immunohistochemistry and gene expression on day 7 and 14.

Results: The average scaffold fiber diameter was 4.42±0.516 µm and scanning electron microscope revealed cells spanning the surface of individual fibres. Live-dead assay on the construct showed 61% viable cells at day 14. The expression of CD56 (satellite cell and myoblast marker) was over 70% on the cell-seeded construct at day 14 and was compared to the monolayer. Immunohistochemistry showed the percentage of CD56 and desmin (myoblast marker) expression on day 14 to be 77% and 23% respectively. Gene expression analysis on day 7 showed an increase in *MYOD1, DESMIN, MYF5* and *Ki67* expression.

Impact of the investigation: The scaffold supported muscle derived stem cell adhesion, proliferation and phenotype maintenance. The skeletal muscle derived cells were at different stages of lineage commitment over the scaffold and our results suggest that day 7 may be ideal for cell-scaffold transplantation to observe functional skeletal muscle regeneration *in vivo.* This novel strategy could be tested in pre-clinical models for volumetric muscle loss or preventing/repairing incisional hernia which will help in better tissue regeneration and integration against the standard of care.

P904 Hybrid assemblage of gingiva-derived mesenchymal stem cells and nitric oxide releasing microsphere for enhanced differentiation

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Stem cell therapy is an attractive approach to bone tissue regeneration. Nitric oxide (NO) has been reported to facilitate osteogenic differentiation of stem cells. To enhance osteogenic differentiation of gingiva-derived mesenchymal stem cells (GMSCs), our purpose was to design a method for *in situ* delivery of exogenous NO to these cells. Poly(lactic-co-glycolic acid) microspheres were used to deliver polyethylenimine/NONOate to the cells for an extended period under *in vitro* culture conditions. A hybrid aggregate of GMSCs and NO-releasing microspheres was prepared by the hanging drop technique. Results showed a homogeneous arrangement of the stem cells and microspheres in heterospheroids without affecting cell viability. Moreover, the *in situ* delivery of NO within the heterospheroids enhanced osteogenic differentiation indicated by 1.2-fold increase in alkaline phosphatase activity and an approximately 10% increase in alizarin red staining. Thus, delivery of the drugs inducing differentiation of stem cells within 3D system may be one of possible strategies to direct differentiation of a stem cell-based therapeutic agent toward a specific lineage.

P905 Viability of synovial fluid MSCs cultured on electrospun PCL membranes containing microfabricated features

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INTRODUCTION: The generation of well-defined surface topographies on biomaterials is a promising approach to control cell behaviour and fate, potentially leading to enhanced healing in regenerative medicine applications. An interesting strategy is the creation of features designed to mimic specific aspects of the native stem cell niche. These 'synthetic niches' may thus act as microenvironments supporting stem cell populations and/or driving their differentiation. An innovative manufacturing platform developed at the University of Sheffield allows for the fabrication of membranes exhibiting 'synthetic niches' by combining the electrospinning technique and collectors made using advanced manufacturing techniques. This technology has been tested successfully in corneal repair using limbal cells [1]. The aim of this study was to investigate the potential use of this technology in cartilage repair using synovial fluid mesenchymal stromal cells (SF-MSCs).

METHODS: Solutions of polycaprolactone were electrospun directly onto pre-shaped metallic collectors fabricated using selective laser melting (i.e. Three topographies: holes, protrusions, and grid-like shape). SF-MSCs were isolated from bovine synovial fluid taken as described previously [2]. SF-MSCs (passage 3) were cultured on the electrospun membranes for 3 and 7 days. Cell viability was determined by measurement of metabolic activity using Prestoblue ®. Cellular morphology and distribution on the membranes were studied using fluorescence microscopy of SF-MSCs stained with DAPI and FITC.

RESULTS: The topography of the collectors was accurately reproduced on the electrospun membranes, creating three distinct types of surface features. The metabolic activity of SF-MSCs was comparable in all the membranes and increased with time in culture. The cells were observed to colonise the microfabricated features, with differences in fibre orientation potentially affecting cellular alignment.

DISCUSSION & CONCLUSIONS: This preliminary study showed that membranes containing 'synthetic niches' and manufactured using this innovative process are promising candidates for SF-MSCs culture, and thus require further investigation to evaluate their potential use for cartilage repair.

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DISCLOSURES: The authors of this study have nothing to disclose.

P907 A novel real-time migration assay identifies stem cell migration in 3D matrices

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MSCs represent an important source of cells for the repair of a number of damaged tissues. The release of MSCs in vivo is likely to be followed by their active migration driven by multiple signals ranging from growth factors to chemokines secreted by injured cells and/or respondent immune cells. The soluble factors that induce stem cell migration are often identified in vitro using two-dimensional transwell and scratch assays. These assays have a major disadvantage that they grossly oversimplify the complex process of migration, thus lacking the structural architecture. Spheroid based assays, where cluster of cells are grown on gels on a 3-dimensional (3D) platform, are a more physiologically relevant method for studying migration and invasion. Further, the factors that stimulate MSC migration have not been extensively compared. In this study, we first established a novel 3D real-time migration assay and developed an algorithm to quantify the migration. In brief, spheroids derived from CFDA labeled MSCs were seeded on collagen gel matrix in the presence or absence of factors. After 48 hours, 3D spheroid images in multiple stacks were obtained by confocal microscopy and a newly generated algorithm was applied to quantify migration (Fig. 1A, B). The advantage of this system is that the same spheroid can be tracked over time and hence the migration can be directly correlated with time. In the next part, we compared different concentrations of 13 commonly used cytokines, growth and chemotactic factors for their ability to stimulate the migration of bone marrow and synovial MSCs (BM-MSCs; SYN-MSCs). The migration of both BM- and SYN-MSCs was significantly stimulated in the presence of PDGF-BB, BMP2, NGFβ, SDF1α and CCL5. Inflammatory factors IL1β and TNFα stimulated the migration of BM-MSCs but not synovial MSCs. In contrast, MCP1 stimulated the migration of SYN-MSCs but not BM-MSCs (Fig. 1C, D). This in vitro 3D system is a promising method for 3D screening to identify factors that stimulate stem cell migration in different 3D gels. We have also adapted this system to study the migration of stem cells into different scaffolds and sponges (Fig. 2).

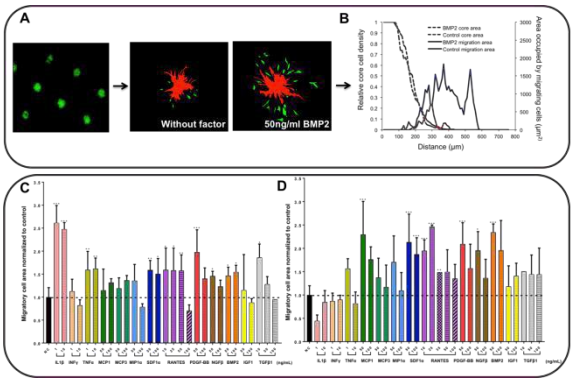

Fig 1. **(A)** Representative images of spheroid based 3D migration assay depicting the chemokinetic effects of BMP2 on BM-MSCs. Fluorescently labeled spheroids were seeded on collagen gel. After 48h of culture with or without BMP2, images were obtained. Core of the spheroid with the sprouting cells are in red and detached migratory cells are in green. **(B)** The algorithm to quantify the migration is depicted in B. **(C & D)** Migration (after 48 hours) of BM-MSCs (C) and SYN-MSCs (D) in response to different factors. n=3. *p<0.05 **p<0.005, ***p<0.0005

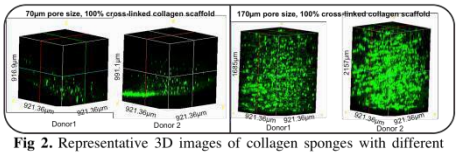

Fig 2. Representative 3D images of collagen sponges with different pore sizes, showing the migration of human BM-MSCs into scaffolds, 10-20 days after seeding

P909 3D matrices based on collagen I and low-sulfated hyaluronan are promising biomaterials supporting bone regeneration

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Motivation

New functional biomaterials based on artificial extracellular matrices (ECM) are engineered to promote bone regeneration. Synthetically sulfated hyaluronan derivative with a low sulfation degree (sHA1) supports osteogenic differentiation of osteoblast precursor cells like human bone marrow stromal cells (hBMSC). The aim of the presented study was to combine the osteoinductive properties of sHA1 with a 3D matrix reflecting a more natural environment for hBMSC. We investigated the response of hBMSC on 3D matrices composed of fibrillar collagen I (col) and sHA1.

Materials & methods

The 3D col networks were prepared from rat tail col and functionalized with sHA1 derivative (degree of sulfation 1, molecular weight 28 kDa). Crosslinking was optionally performed by 1-ethyl-3-[3-dimethyl-aminopropyl]-carbodiimide hydrochloride (EDC) to covalently attach physisorbed sHA1 or increase network stiffness.

Cell morphology, invasion and endogenous ECM formation was studied with immunofluorescence staining and confocal laser scanning microscopy (cLSM). Protein levels of fibronectin among others were analyzed using cell lysates and Western blotting. For characterization of osteogenic differentiation of hBMSC after treatment with osteogenic supplements, TNAP (tissue non-specific alkaline phosphatase) activity and calcium phosphate (CaP) mineral accumulation were determined. Further, cell-induced alterations of network properties (topology and stiffness) were characterized after decellularization by cLSM and colloidal probe force spectroscopy.

Results

All tested substrates revealed as cell compatible: hBMSC invaded the 3D networks, showed a spread morphology and formed well-developed actin stress fibers and elongated filopodia. The cells formed their own ECM (e.g. fibronectin) and actively remodeled the 3D network by enzymes (lysyloxidase, proteases, transglutaminase).

After treatment with osteogenic supplements, hBMSC developed an osteoblast-like phenotype with increased TNAP activity. CaP accumulation was enhanced by increased substrate stiffness. Physisorbed sHA1 had an additional effect on osteogenic differentiation, probably caused by dissolved sHA1 interacting with endogenous ECM proteins or, after cellular uptake, with intracellular targets.

Conclusion

The osteoinductive effect of the 3D networks composed of col I and sHA1 makes them to promising candidates for bone tissue engineering approaches.

P910 Development of completely defined media for hASC-based adipose tissue setup

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Aims

The clinical use of many tissue engineered products is currently prevented by their dependency on animal derived-sera. These sera are associated to high batch-to-batch variations, entail the risk of contaminations and as many constituents are still unknown, they may cover-up actual experimental results [1]. Artificially engineered adipose tissue is still highly needed to replace lost, damaged or burned subcutaneous soft tissue [2]. Additionally adipose tissue test systems could serve to analyze (patho)physiological processes or screen for potential drugs [3]. In the current approach a completely defined adipogenic differentiation as well as a completely defined adipocyte maturation medium was developed to enable the artificial assembly of adipose tissue under chemically defined conditions based on human primary adipose-derived stem cells (hASCs).

Methods

HASCs were expanded and characterized in a serum-free and xeno-free environment. A chemically defined adipogenic differentiation medium was developed by supplementation of growth factors, plasma proteins and trace elements and applied in adipogenic differentiation of hASCs for 14 days. Analogously an adipocyte maturation medium was developed, which was consecutively applied for additional 28 days. Results were compared to serum containing controls. Percentage of differentiated cells was evaluated throughout the complete adipocyte development. Additionally the extent of adipogenic differentiation was analyzed via staining and western blot analysis of accumulated lipids and expressed adipocyte markers like perilipin or detection of released leptin in the cell supernatant.

Results

It was shown that hASCs keep their stem cell characteristics, proliferation capacity, stem cell marker expression and multipotency also after serum-free/xeno-free expansion. The chemically defined medium lead to an efficient adipogenic differentiation of hASCs, which was proven by lipid accumulation of more than two thirds compared to serum-containing controls and high expression of different adipocyte markers like perilipin as well as leptin release. Even after the consecutive long-term maturation of 28 days adipocytes were found to be adherent while cell- specific characteristics were preserved. Throughout the adipocyte developmental period, cells treated with chemically defined media exhibited high viability.

Impact

In the current study, we could implement the efficient in vitro generation of mature adipocytes out of hASCs based on completely defined differentiation and maturation media for the first time. This achievement is of fundamental character for the in vitro setup of adipose tissue under defined conditions and represents an important mile stone for its future use in regenerative medicine. Additionally transfer of results to the setup of other approaches will be of great value for the tissue engineering branch in general.

P912 Umbilical cord wharton's jelly stem cells transdifferentiation to cornea epithelium. A histological and immunohistochemical study

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Background: Umbilical cord Wharton's jelly stem cells (HWJSC) have intrinsic transdifferentiation capabilities to several cell types, including some epithelial cell lineages. In the present work, we investigate the capability of these cells to differentiate to cornea epithelial cells in a three-dimensional model of bioengineered cornea.

Methods: First, human bioengineered cornea stromas were generated in the laboratory by using human corneal stroma keratocytes immersed within nanostructured fibrin-agarose hydrogels. Then, HWJSC cultures were subcultured on top of these stroma substitutes and epithelial differentiation was induced by using specific conditioning medium, air-liquid culture technique and stromal-epithelial interaction. Histological and immunohistochemical analyses were carried out after 1, 2 and 3 weeks of ex vivo development.

Results: Histological analysis showed that HWJSC cultured on the stromal substitute were able to differentiate into corneal epithelial-like cells. At day 7, 5-6 layers of flat cells were found on the stromal substitute, with 8-10 layers at day 14 and more than 15 layers at day 28 of development. Differentiation was not complete, but the histological resemblance with native epithelium was evident. Transdifferentiated epithelium was positive for the marker of cornea epithelium cytokeratin CK3/12 and for the intercellular junctions PKG, ZO1 and CX43, with the highest intensity corresponding to the longest development times. However, global intensity for most markers tended to be lower than control native cornea.

Discussion and conclusions: Our analysis of typical cornea epithelial markers and cell-cell junctions disclosed the presence of intermediate filaments, desmosomal junctions, tight junctions and gap junctions in the bioengineered corneas with a transdifferentiated epithelium, confirming the ability of HWJSC to differentiate into cornea epithelial-like cells. In addition, our findings suggest that bioengineered corneas should be kept in culture for at least 3 weeks for an efficient differentiation process. Differentiation of HWJSC is probably a consequence of adequate epithelial–keratocyte interaction in a three-dimensional artificial tissue and the use of specific air–liquid culture systems, as previously demonstrated. Our findings suggest that HWJSC can be considered an alternative cell source for cornea regeneration and may offer a solution for patients with limbus stem cell deficiency.

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P913 The human tissue-engineered cornea as a model to study the impact of altering the activity of CREB and AKT during corneal wound healing

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Purpose: The cornea is localized at the outer surface of the eye. It is a transparent organ, highly specialized and unique that is continually subjected to abrasive forces and occasional mechanical or chemical trauma because of its anatomical localization. A complete reepithelialization and the reorganization of a mature smooth stratified epithelium are essential in restoring the imaging properties of the cornea. We recently succeeded in tissue-engineering human cornea substitutes that mimic their in vivo counterpart in terms of cell phenotype and tissue architecture. Upon injury, the extracellular matrix (ECM) rapidly changes to promote wound healing through its interactions with membrane-bound integrins. We hypothesize that the remodelling of the ECM occurring during corneal wound healing causes the activation of very specific signal transduction mediators that favour faster closure of the wound by altering the adhesive and migratory properties of the cells surrounding the damaged area. Our goal is to proceed to the pharmacological inhibition or activation of the PI3K/Akt mediators Akt and CREB using the human tissue-engineered cornea (hTECs) as a model.

Methods: The self-assembly approach was used to create hTECs as previously described (Germain et al., Pathobiol. 1999). hTECs were wounded with an 8-mm diameter biopsy punch and deposited on another reconstructed human corneal stroma to allow wound closure on a natural ECM. RNAs and total proteins were prepared from the epithelial cells of wounded and unwounded areas and their gene expression patterns were determined by microarrays. The wounded tissues were then incubated with or without C646 (a CREB inhibitor) or SC79 (an AKT activator). DMSO (the vehicle) was used alone as a negative control. Closure of the wounds was monitored over a period of 5 days to determine whether Akt activation and CREB inhibition will improve closure of the wounds.

Results: Analysis of the microarray data indicates that important alterations in the expression of a few mediators is occurring primarily in the PI3K/Akt pathways in response to the ECM remodeling taking place during wound healing of the hTECs. Pharmacological inhibition of CREB with C646 considerably accelerated the wound closure compared to control. Indeed, this process was even more rapid with the addition of SC-79 to C646. By simultaneously blocking the activation of CREB and enhancing the activation of Akt, we altered the migratory properties of the cells and allowed a more efficient wound healing.

Conclusion: This study demonstrate for the first time that it is possible to accelerate wound healing in vitro using a novel 3D model of human cornea that is very close to the native tissue. Most of all, this experiment validates both the Akt and CREB genes as potential targets on which we may influence to alter the wound healing dynamic of the cornea. This will certainly lead to progress in the clinical field of corneal blindness.

P914 Impact of pharmacological alteration of integrin-dependent signalling pathways on corneal wound healing using a human tissue-engineered cornea as a model

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The cornea is the transparent anterior segment of the eye. Because of its superficial anatomical localization, it is particularly vulnerable to abrasive forces and various traumas, which can lead to significant visual impairments. Upon injury of the corneal epithelium, there is an important remodelling of the underlying extracellular matrix (ECM). This remodelling is perceived by the integrins that recognize the ECM components as their ligand and activate different intracellular signalling pathways, ultimately leading to reepithelialization and reorganization of the injured epithelium, both of which are necessary in order to restore the visual properties of the tissue.

Gene profiling analyses and protein kinases arrays recently carried out in our laboratory demonstrated that expression and activity of a few mediators of integrin-dependent signalling pathways were altered in response to the ECM remodelling taking place during wound healing of the human tissue-engineered corneas (hTECs). Among these mediators, WNK1 seems one of the most promising candidates. As WNK1 is considerably activated through phosphorylation during the corneal wound healing process, we assumed it must play a very important function by promoting corneal wound healing. In this study, we exploited the use of both a pharmacological inhibitor of WNK1 and siRNA suppression of WNK1 expression in wounded and unwounded hTECs in order to investigate the impact of the WNK1 activation status on woundclosure.

The results of this study will contribute to a better understanding of the molecular mechanisms behind corneal wound healing. We believe that altering the activation status of WNK1 mayprove an interesting avenue to accelerate corneal wound closure. We hope our works will identify WNK1 as a new potential therapeutic target in the field of tissue wound healing.

P915 Cornea endothelium differentiation of human umbilical cord, adipose tissue and dental pulp stem cells. A comparative gene expression study

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Background: Generation of bioengineered corneas is highly dependent on the availability of viable cornea tissue, and some cell types such as cornea endothelium are very difficult to expand in culture. For these reasons, alternative cell sources are in need. In the present work, we analysed the endothelial differentiation capability of three types of human mesenchymal cells: umbilical cord Wharton's jelly stem cells (HWJSC), adipose tissue-derived stem cells (ADSC) and dental pulp stem cells (DPSC).

Methods: Primary cell cultures were generated by enzymatic digestion of human biopsies of the umbilical cord, adipose tissue and dental pulp. Cells were kept in basic culture medium. Corneal endothelium differentiation was induced by using a conditioned medium containing hydrocortisone, triiodothyronine, cholera toxin, adenine and epithelial growth factor. The efficiency of the differentiation process was analysed by quantifying mRNA expression for the genes PAX6 and WNT7A (cornea development markers), TJP1, CDH2, CDH12 and several ATPase genes (markers of cornea endothelium). Genes whose expression increased over 25% as compared to basal levels were selected as positively induced.

Results: Induction of the different cell cultures using conditioning media showed positive induction of the gene PAX6 in ADSC and WNT7A in ADSC. TJP1 was induced in HWJSC and CDH12 in HWJSC and DPSC. Analysis of genes encoding for the different ATPases involved in endothelial functions showed that ADSC increased expression of ATP1A1, ATP1A2, ATP1A3, ATP1A4, ATP1B2 and ATP1B3, HWJSC had increased expression of ATP1A2 and ATP1A4 and DPSC showed activation of the genes ATP1A2, ATP1A3, ATP1B2 and ATP1B4.

Discussion and conclusions: These results demonstrate that human mesenchymal stem cells have differentiation capabilities to corneal endothelial cells upon induction with conditioning medium. The two genes specifically related to cornea development (PAX6 and WNT7A) were expressed upon induction in ADSC, but not in the other cell types, suggesting that ADSC have the highest cornea differentiation potential. Although highly specific markers for the corneal endothelium are not available, the expression of N-cadherin, zonula occludens-1 and sodium/potassium ATPase is generally used to identify these cells. In this regard, our results showed that N-cadherin was induced in HWJSC and DPSC, whereas most ATPase genes were induced in HWJSC. Therefore, the three cell types have demonstrated endothelial differentiation potential, with ADSC being probably more efficient for ex vivo cornea endothelium differentiation.

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P916 Deriving an in vitro source of canine corneal stromal stem-like cells to use as potential source for novel treatment strategy for blinding corneal disease

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The cornea is an optically clear tissue permitting light transmission into the eye. Corneal pathology as corneal stromal dystrophy has a prevalence of up to 15% described in 8 different breeds. Cholesterol and phospholipids are deposited in the stroma, similar to Schnyder's dystrophy in humans. Chronic corneal fibrosis is one of the leading causes for visual impairment in veterinary ophthalmology.

We hypothesise that canine corneal stromal stem-like cells (CSSC) can be derived from fresh adult tissue. These cells will provide a unique and valuable resource to the community for studies investigating the pathogenesis of inherited stromal cell dystrophies and the development of novel cell-based therapies for dogs. Here we report on the successful isolation and characterisation of CSSC from healthy dogs and defines the methods to differentiate canine CSSC into keratocytes.

Immunohistochemistry of canine corneas from three donors demonstrated that a small (<5%) but distinct population of CD90 positive cells were distributed in the anterior-mid stroma throughout the limbal and central cornea. There were no regional difference noted (dorsal,ventral,nasal,temporal). This suggested that CSSCs may exist in both the limbal and central canine cornea.

Fifteen canine corneas from 15 young dogs of five different mesocephalic breeds, which were euthanized for reasons unrelated to this project, were used to isolate CSSCs in vitro.

In 10/15 corneas CSSC phenotype could be cultured from both the limbal and central cornea. The CSSCs had a mean maximal passage of P9 (limbal) and P8 (central) over a period of 23.5 days (limbal) and 19.6 days (central) before senescence was reached. The mean population doubling times were 17.54h and 18.03h for the limbal and central CSSC respectively. The cultured limbal and corneal CSSC expressed the following markers: CD90, CD73, CD105, Pax6, N- cadherin, vimentin, but were negative for CD34. Following culture in serum free medium containing ascorbic acid for 14 days the CSSCs from both regions differentiated into keratocytes which expressed ALDH1A3, lumican and keratocan, and were negative for alphaSMA and Pax6. All antibodies were tested for cross-reactivity and specificity to dog proteins using western blot. Limbal and central CSSC differentiated into osteoblasts, adipocytes and chondrocytes as determined by Alizarin red S, van Kossa, oil red O and Alcian blue staining.

Canine corneal stromal stem-like cells of the limbal and central cornea could serve as a source of cells for tissue engineering and future cell therapies for studying and treating sight-threatening corneal diseases as corneal dystrophy and corneal fibrosis.

P917 Contribution of the transcription factors Sp1 and NFI to the preservation of stem cells in tissue-engineered human corneas

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Introduction: Corneal graft restores visual function when visual impairment caused by a corneal disease becomes too severe. However, because of the worldwide shortage of corneal graft tissue (only 1 cornea available for 70 that would be needed) and besides efforts to encourage the cornea donation in all countries, alternative solutions need to be developed. Producing a functional cornea by tissue engineering is among them. Self-renewal of the corneal epithelium through the maintenance of a sub population of corneal stem cells is required to maintain the functionality of such a reconstructed cornea. We previously reported an association between the level of stem cell differentiation and the level to which they express the transcription factors Sp1 and NFI. Our goal is to characterize the impact of different feeder layers on the maintenance of the proliferative properties of human cornea epithelial cells (HCECs) in monolayer cultures. **Methods:** HCECs(n = 3) were isolated from the limbus of a corneal biopsy from living donors and grown in monolayer alone, with irradiated murine fibroblasts (i3T3) or with human fibroblasts (iHFL). The cells were amplified on several passages until they reached replicative senescence. Morphological and growth rate analyses were performed at each passage. Both the expression and DNA binding properties of Sp1 and NFI were evaluated by microarray, Western Blot and gel shift analyses. **Results:** HCECs co-cultured with iHFL were maintained two more passages in culture relative to HCECs co-cultured with i3T3. Both expression and DNA binding of Sp1 remained unchanged between the two feeder layers. On the other hand, expression and DNA binding of NFI were markedly increased at each subsequent passages when co-cultured along with i3T3. These changes also correlated with increased expression of the NFI isoforms NFIA and NFIB. Moreover, the NFIC isoform showed an increased expression at high passages (P7 to P9) when HCECs were grown without any feeder layer. **Conclusion:** The iHFL feeder layer seems to be the most effective in maintaining the proliferative abilities of HCECs in culture. This could be explained by the fact that the expression of NFIB, which is known for its gene's repressor properties, is considerably less abundant in HCECs grown with iHFL. On the other hand, differentiation of cultured cells seems to be associated with an increased expression of NFIC.

P918 Influence of material stiffness on corneal epithelial cell behaviour

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Introduction: Corneal blindness is one of the most common causes of blindness worldwide with limited treatment options. Damage to the corneal epithelium from injury or disease can result in loss of vision and intense pain. In many cases transplantation of a donor epithelium, limbal tissue or anterior segment of the cornea is used to restore vision, however, there is a limited supply of such tissue for these procedures. To engineer an equivalent tissue we must first understand the factors that control the corneal epithelial behaviour. In this study we examined how material stiffness regulates the epithelia behaviour with the aim of determining the optimal conditions for generating new functional tissue.

Methods: Polydimethylsiloxane (PDMS), was used to fabricate substrates with a wide variety of elastic moduli. Primary cultures of corneal epithelial cells were isolated from porcine explants. The cells were seeded at a density of 5,000 cells/cm^2 onto PDMS coated plates of varying stiffness's by using different blends of Sylgard 184 and Sylgard 527. A 0.1% gelatin coating was used to enhance cell adhesion. The quantity of cells and their morphology was analysed using phase-contrast microscopy over several days in culture.

Results: The epithelial cells response to differing substrate stiffness is shown (Fig 1). Cellular proliferation was highest on a substrate of modulus 842kPa while significantly fewer cells were detected on a lower stiffness substrate of modulus of 12kPa. Cells on each group had similar polygonal morphology with the exception of those on the softest PDMS blend (12kPa) which were dendritic.

Conclusions: The results demonstrated that the proliferative capacity and morphology of corneal epithelial cells was affected by substrate stiffness. Future studies will analyse these cells using a combination of western blotting, RT-PCR and immunocytochemical staining. This will give a greater insight into the effect mechanical properties have on corneal epithelial cells. These results have several applications including in the development of biomaterials suitable for transplanting epithelia, in the enhancement of the culture environment for generating functional epithelium in-vitro and in the improvement of engineering corneal epithelial grafts.

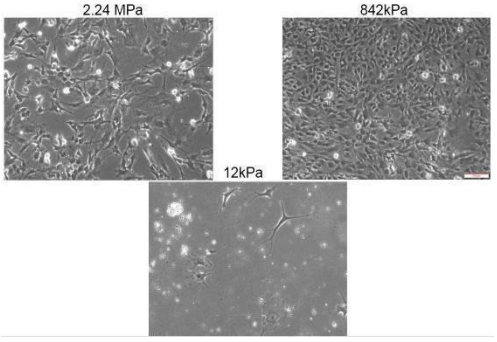

Figure 1 Porcine limbal epithelial cells response to substrate stiffness. Scale bar = 100µm. Lower stiffness's decrease cellular proliferation and induce a dendritic cell shape. Higher stiffness's increase cell proliferation and have polygonal morphology.

P919 Development of a human, 3D, ophthalmic cell culture model for regenerative medicine and drug delivery

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Background: Limitations associated with animal models have encouraged the development of more accurate cell culture models that aim to replicate the growth conditions of native tissue to investigate cell behaviour and assess toxicity.[1] In this study, a 3D *in vitro*, layer-by-layer, ocular model has been developed that mimics the cellular microenvironment and provides offers a screening technique for drug development.

Methods: Human retinal (ARPE-19) and corneal epithelial cells (HCE) were cultured on flat, curved and v-shaped well plates/scaffolds. Cells were characterised for morphology, activity/viability, phenotype and functionality using a combination of microscopy, biochemical assays (i.e. MTS, LDH release, Guava ViaCount), flow cytometry and western blotting. Physical/mechanical characterisation of (human and bovine) vitreous humour and biomimetic substitutes (i.e. sodium hyaluronate, agarose, gelatin, in-house developed hydrogels) were examined using rheometry, texture profile analysis, scanning electron microscopy and *in vitro* diffusion studies (i.e. Franz cells).

Results: Results demonstrate that cell activity and viability were adversely affected by extreme surface geometry (i.e. v-shaped scaffolds) (Figure 1). In contrast, no significant impact on cell functionality or phenotype occurred during culture. 0.9% sodium hyaluronate (in PBS) and a hybrid collagen/sodium hyaluronate hydrogel offers the most promising characteristics for an optimal biomimetic human vitreous.

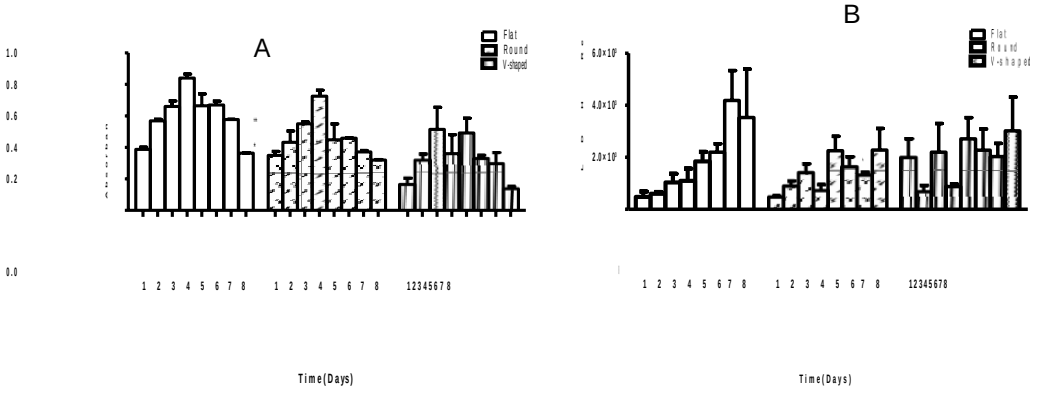

Figure 1. Cell metabolic activity (A) and LDH release (B) of ARPE-19 cells cultured on different shaped well plates. Data are represented as mean ± SEM of three independent repeats (n=3). Significance shown as $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***.

Conclusions: An optimised 3D, in vitro, ophthalmic model consisting of retinal cells, corneal cells and vitreous humour has been successfully developed and may represent a more biologically-relevant model of the human eye than is currently available.

P920 Developing corneal stromal stem cell sheets from an amniotic membrane protein bio-functionalized thermos-responsive polymer substrate for corneal surfacetherapies

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Purpose: To develop thermoresponsive culture surface biofunctionalized with Amniotic Membrane (AM) derived proteins to expand and retrieve Corneal Stromal Derived Stem Cells (CSSC) as a strategy for corneal therapy.

Methodology: The NGMA (N-Isopropyl acrylamide –co- glycidyl methacrylate) was synthesised from its monomers N-Isopropyl acrylamide and Glycidyl methacrylate (GMA) by free radical polymerization. The biofunctionalization was achieved by conjugating with AM protein by epoxy ring opening mechanism of GMA group. The physicochemical properties were characterized by Fourier Transform Infra Red Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and poly acrylamide gel electrophoresis (PAGE). The biofunctionalization was confirmed by culturing CSSC on NGMA-AmPro patterned on non-adherent surface and comparing with cells cultured on gelatine and AM protein. The CSSC was retrieved as cell sheet construct by incubating culture below 10°C for 15 min and transferred to new surface using a gelatin gelsupport.

Results: The NGMA analysed by FTIR confirmed the peaks related to acrylamide and epoxy group. The phase transition of NGMA was determined to be around 28 – 32°C. The presence of a new peak at epoxy region confirmed the protein conjugation. PAGE analysis confirmed the conjugation of AM proteins to NGMA and protein staining of patterned NGMA-AmPro visualized it. CSSC cultured on ¬NGMA-AmPro in comparison with controls confirmed the biofunctionalization. The CSSC monolayer was retrieved by temperature variation and transferred to a new culture surface.

Conclusion: The study showed that NGMA-AmPro substrate is a good platform for culture and retrieval of CSSC as cell sheet constructs. The presence of AM proteins will enhance the wound healing and stem cell properties of CSSCs. Carrier free CSSC cell sheet transfer is a novel promising method in addressing corneal surface disorders by stem cell therapy.

P923 High-throughput development and analysis of prevascularized spheroids for therapeutic cell delivery

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Mesenchymal stem cells (MSC) pre-assembled into aggregates might present enhanced regeneration potential in cell therapies, as compared to single-cells. In multicellular aggregates, cell-cell and cell-matrix interactions are preserved, favouring cell survival, function and retention upon transplantation. The co-incorporation of endothelial cells (EC) in such aggregates (or spheroids), may present additional advantages, by accelerating vascular integration with the host, a key issue for successful engraftment. Here we describe a methodology for the high-throughput production/analysis of MSC spheroids co-incorporating endothelial progenitors and their characterization. Outgrowth EC (OEC) were co-cultured with MSC in nonadhesive microwell arrays, enabling the generation of multiple spheroids that could be directly processed/analysed *in situ* for high-throughput (immuno)histochemistry. In 24h, MSC and OEC (1:1) co-aggregated, forming uniform spheroids that remained metabolically active for up to 21 days. Aggregated cells produced large amounts of extracellular matrix, rich in fibronectin and collagen type IV. OEC segregation occurred, with cells assembling into distinct vascular structures: as aligned cells at peripheral layers and as clusters at the core. In contrast to control MSC spheroids, where ECM deposition was random, in MSC-OEC spheroids, protein fibers aligned at more peripheral layers, co-localizing with OEC, suggesting that OEC-MSC crosstalk modulated 3D niche dynamics. Aggregated cells showed low proliferative activity, but extensive cell migration was observed from spheroids embedded in a fibrin tissue mimic, suggesting that cells should be able to colonize surrounding tissues upon transplantation. This study shaded light on the behaviour of clinically relevant cells pre-assembled into high-throughput generated spheroids that show promise as a tool for therapeutic cell delivery.

P924 Bespoke full-thickness skin as a permanent graft solution

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Skin grafting has been pivotal in the treatment of burns and is generally successful. Despite this, the WHO estimates that 265,000 people die worldwide from burns every year and the CDC estimates up to 10,000 people die per year from burn related infections in the US alone. Skin graft efficacy remains limited for people with severe burns who require repeated grafts from multiple donor sites. Alternative grafting techniques have been developed but, while effective in the short-term are susceptible to rejection, disease transmission, and some are prohibitively fragile. Crucially, these products are not permanent, inevitably requiring autograft replacement. We aim to develop clinically safe methods for growth of autologous full-thickness skin as a permanent graft solution for people with major burns. To achieve this, we have optimised techniques to expand freshly isolated human skin cells as a monolayer in tissue culture flasks before seeding onto a custom-built biosynthetic scaffold for full-thickness skin growth. Firstly, we determined the safest and most effective medium for expanding keratinocytes in a clinical setting and identified small molecules that significantly enhanced their *in vitro* expansion. We delineated the keratinocyte subsets responsible for *in vitro* proliferation and maintenance of full-thickness skin using fluorescence activated cell sorting. In addition, we tracked their molecular profiles in relation to distinct epidermal cell subsets *in vitro* by immunocytochemistry. These populations were primarily $CD29^{+}CD271^{-}$ cells expressing cytokeratin proteins characteristic of undifferentiated basal layer keratinocytes. Finally, we used this knowledge to modify and apply these methods to growth of full-thickness skin on a biosynthetic scaffold. Both dermal and epidermal layers were intact and displayed structures indicative of functional skin including extracellular matrix and stratified epidermis as determined by immunohistochemistry. These results show unprecedented progress towards a permanent autograft solution of lab-grown full-thickness skin for treatment of severe burns.

P925 Surveying cellular and engineered tissue therapies in europe and associated countries in 2014 and 2015

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Cellular and engineered tissue therapies (CETT) are a growing sector for treating various diseases, malignances, and traumatic injuries. This field is not only thriving due to the increase of patients being treated, but also because the scope of clinical conditions, which could be responsive to CETT is broadening as well. As these advances take place it is important to maintain transparency of the treatments effectively carried out, as a basis to coordinate and further develop scientific, clinical and regulatory aspects. Due to this necessity our group has been conducting annual surveys since 2008 on the use of CETT (excluding hematopoietic stem cell treatments for the reconstitution of hematopoiesis) to establish a comprehensive, quantitative map of patients being treated in Europe and associated countries with such therapies. These surveys were supported by several international organizations such as EBMT, ISCT, TERMIS, ISCT, ICRS and IFATS. The reports include the number of treated patients without any reference to clinical outcomes. Data are sorted by indication, cell sources, processes and delivery modes as well as an appendix of active teams. Our presented work displays the results from the surveys for 2014 and 2015 separately and in comparison to one another, as our last published report reviewed the treatments of 2013.

More than 400 groups working in the field were contacted for both years. In 2014 277 teams reported performing CETT on 2054 (778 allogenic and 1276 autologous) patients, where as in 2015 210 teams reported therapies on 3338 (867 allogenic and 2471 autologous) patients.

The reported cell types for the years 2014 / 2015 were respectively mesenchymal stromal cells (MSCs) (56% / 36%), hematopoietic stem cells (26% / 13%), chondrocytes(7% / 14%), dendritic cells (3% / 2%), keratinocytes (3% / 1%), dermal fibroblasts (< 1% / 9%) and others (5% / 25%). These assessments clearly show that even though MSCs are still the predominant cell source used in CETT, there is trend towards an increasing use of differentiated cells, with the two dominant sources being chondrocytes and dermal fibroblasts. This comparison, as well as further evaluations of the collected data, identifies trends and the importance of their investigation in a still rapidly evolving field.

P926 Recombinant human collagen type I hydrogels as superior cell carriers for corneal epithelial stem cells

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PURPOSE: Cultivated limbal epithelial transplantation (CLET) can successfully regenerate the anterior cornea in Limbal Stem Cell Deficiency. The cell carrier most commonly used in CLET is the human amniotic membrane (HAM). The aim of this study is to investigate recombinant human collagen type I (RHCI) hydrogels[1] in ocular tissue engineering, with CLET as the disease model of interest.

METHODS: Ultrathin (<100µm) RHCI hydrogels[1] and HAM were tested for ultrastructure (SEM, TEM, AFM), optical properties (Refractometry - Transparency), physical properties (Rheology), microbial susceptibility (P. aeruginosa challenge) and genotoxicity (VITOTOX). Cell viability and cell proliferation of immortalized corneal epithelial cells cultivated on the different scaffolds were investigated using PrestoBlue, BrdU, and Live cell imaging assays. Composite grafts were generated using primary limbal epithelial stem cells (LESC).

RESULTS: On AFM & EM, RHCI hydrogels consist of randomly arranged collagen fibrils (fig. 1). Optical characterization indicates RHCI is more transparent than HAM. Refractive index of RHCI is closer to that of the cornea (1,37-1,38) compared to that of the HAM. Collagen scaffolds show no genotoxic effect, and are 100-fold more resistant to microbial growth compared to HAM. No significant difference in cell viability and active proliferation rates were noted between the scaffolds and primary LESC formed a confluent cell monolayer on RHCI hydrogels (fig.1).

CONCLUSION: Primary LESCs can be successfully cultivated on RHCI using standardized xeno-free conditions. The favourable optical characteristics, relative microbial resistance, successful composite graft generation, prove that RHCI is a highly promising scaffold for CLET. Fibronectin nano-lithography on RHCI membranes is under investigation to potentially improve cell cultivation. Finally, transplantation in a rabbit model of limbal stem cell deficiency would be necessary to validate the use of RHCI in CLET.

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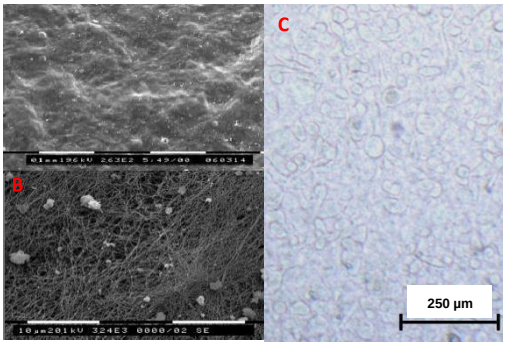
Fig. 1 *SEM image of RHCI hydrogel shows an irregular surface without any fibrillar alignment (A). SEM of the HAM shows irregularly arranged collagen (B). Bright field microscope image of RHCI hydrogel with a confluent layer of human corneal epithelial cells forming a composite graft (C).*



P927 Artificial hypoxia inducible factor-1α (HIF-1α) stabilization enhances cell survival in 2D & 3D models

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Cell survival remains one of the greatest challenges in regenerative medicine. Strategies to increase cell survival following storage/resuscitation and after implantation would greatly enhance clinical translation. Hypoxia (via stabilisation of the hypoxia inducing factor, HIF-1α) has been shown to enhance cellular adaptation to hostile environments. The success of hypoxia preconditioning is, however, curtailed by the effects of a lack of oxygen on cell growth and reperfusion injury following re-oxygenation. Here we investiage if survival maybe increased by stabilisation HIF-1α subunit using HIF1α stabilising mimetics (HSMs), in normoxic conditions prior to implantation or storage, which may activate a number of pro-survival factors in both 2D and 3D models of cell survival. Current studies are underway to understand the pro-survival factors expressed in HIF stabilised cells.

A hypothermic injury model was created, whereby cells (hepatocyte cell line (HepG2) and adipose derived stem cells (ADSCs)) or 3D collagen constructs were exposed to 24 hours at 4oC in perfusion liquid. Cell survival and resuscitation following exposure to the injury were assessed by measuring metabolic activity (Alamar Blue) and proliferation (total DNA); we have shown that HepG2 preserved in organ perfusion liquid with HSMs exhibited significant protection compared with nonpreconditioned cells, and this is dose-dependent as 100μM Co ions and 250μM DMOG showed significant proliferation rate after 24 h at hypothermia (4^{o}C) following resuscitation up to 7 days, Co: 9532.63%±259.84% and DMOG: 10070.53%±526.74% compared to the control 4233.51%±1636.34% ($p \geq 0.05$) , respectively. A similar result was showed in ADMSC (stem cell) lineage when treated in the same way.

In summary, these results have demonstrated that preconditioned cells in HSMs may increase the survival of cells in adverse conditions. Current Studies are underway to understand the pro-survival factors expressed in HIF-1α stabilised cells. The incorporation of HSMs into tissue scaffolds may provide a means to increase cell survival following implantation.

P928 Feasibility of plasma gel as injectable scaffold for hypoparathyroidism cell therapeutics using tonsil-derived mesenchymal stem cells

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Objective: The incidence of hypoparathyroidism has been increasing along with the increasing thyroid surgery. The conventional treatment was oral calcium administration with vitamin D, however, this treatment could not mimic the physiologic effect of the native parathyroid hormone(PTH). We previously reported the possibility of cell therapeutics which was differentiated from the tonsil-derived mesenchymal stem cell (TMSC). For stable hormone release, the ideal scaffold material which facilitates cellular engraftment is mandatory. The plasma gel has been used as augmentation and hemostatic agent in clinical field because plasma has sufficient growth factors and plasma gel is autologous material which has no risk of infection or rejection. The purpose of this study was to evaluate the feasibility of plasma gel as injectable scaffold for hypoparathyroidism cell therapeutics.

Materials and Methods: As in vitro study, rheological evaluation and scanning electron microscope (SEM) analysis were performed on fabricated plasma gel. As animal experiment, 20 male Sprague-Dawley rats were used. The whole animals were divided into two groups according to the type of injected TMSC. After intravenous blood collection, plasma gels were produced by centrifuge and gelation. Two hours after intraperitoneal injection of 5- aminolevulinic acid, rat parathyroid glands were detected under 635 nm fluorescent light and resected. Undifferentiated TMSC (uTMSC group, n = 10) and TMSC differentiated into PTH secreting cells (dTMSC group, n = 10) were administrated at dorsum of the rat by intramuscular injection. Intact PTH, serum calcium and phosphorous levels were measured at pre-and postoperative 1, 3, 7, 10, 14, 21, 28, 42, 56, 84 days. Histological evaluations were performed at 2 months after the injection.

Results: The rheological feature of plasma gel was compatible as gel material. The SEM analysis demonstrated that plasma gel had regular 10-nm sized micropores. On animal experiment, the intact PTH level decreased to non-detectable levels on postoperative 3 day after parathyroidectomy. Serum intact PTH levels began to be detected at 21 to 28 days after cell injection. The mean iPTH levels were 29.22 ± 7.93 pg/mL and 45.73 ± 12.08 and pg/mL in cTMSC and dTMSC groups on postoperative 84 day. The serum calcium levels increased and phosphorus level decreased to maximal level (calcium; 6.435 mg/dL, phosphorus; 12.02 mg/dL) on postoperative 28 day and restored to 7.15 mg/dL and 9.83 mg/ dL on postoperative 84 day. Immunofluorescence microscopy revealed that PTH and CHGA positivity in dTMSC injected area.

Conclusion: Plasma gel is safe autologous material which could be considered as cell therapeutics delivery scaffold.

P930 Optimizing mesenchymal stromal cell therapy using a si-HPMC hydrogel and RGTA molecule for the treatment of severe side effects of pelvic radiotherapy

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Radiation therapy is crucial in the therapeutic arsenal to cure cancers; however, the toxicity of the surrounding healthy tissues remains a substantial limitation. We previously demonstrated, in preclinical and clinical studies, that Mesenchymal Stromal Cells (MSCs) represent a promising strategy to reduce radiation-induced intestinal lesions. However, systemic administration results in low cell engraftment within the irradiated tissue. Here, we propose to use a biocompatible hydrogel (Si-HPMC) for injectable cell delivery using a colonoscope. Moreover, in order to improve host microenvironment, we propose to combine MSCs+Si-HPMC with ReGenerating Agent (RGTA), a heparan sulfate mimetic, able to reconstruct the extracellular matrix scaffold and promote tissue regeneration and recovery of tissue function. First, the rheological parameters of the hydrogel were adapted for the injection through the colonoscope catheter. MSCs are viable within the hydrogel, able to secrete trophic factors and responsive to the inflammatory environment. In a rat model of radiation-induced severe colonic damage, we demonstrated that the combination MSC+Si-HPMC+RGTA improve colonic epithelial structure and function (measured by the technique of Ussing Chambers). We observed a greater and longer (21days) engraftment of MSC when embedded in Si-HPMC. Proliferation/apoptosis assay demonstrated that cells are not proliferating but not apoptotic when embedded in the hydrogel.

This combination Si-HPMC+RGTA potentiates the therapeutic effects of MSCs and could be proposed to patients suffering from colonic diseases.

P931 A new tool for direct read-out of transcription factor activity in live cells

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Cells respond to their environment via an intricate cellular signaling network. Insight into this network regulating cell fate is important for controlling stem cell differentiation, understanding diseases such as cancer and osteoarthritis, and defining better regenerative medicine strategies. Changes in cell fate are characterized by changes in gene transcription. These changes are dictated by changes in (master) transcription factor activity. SOX9 is the master transcription factor of cartilage development. Here we present a new method to directly monitor changes in transcription factor activity. We used Fluorescence Recovery After Photobleaching (FRAP) to measure SOX9 protein activity in C20A4 cells as well as in primary human Chondrocytes (hPCs).

C20A4 cells and human primary chondrocytes (hPC) were used. hPC were purchased from Articular Engineering, USA. Chondrocytes were grown on glass coverslips and transfected with SOX9-mGFP or mGFP. Recombinant proteins, WNT3A, DKK1, and sFRP-3 (FRZB), BMP7 (100ng/ml) and IL1β, were obtained from R&D systems and were added 24 h post-transfection at 10 ng/ml. hPC were incubated with the recombinant proteins for 30 minutes or 1 hour for BMP7. FRAP was performed on a Nikon A1 confocal microscope. Results were analyzed using Matlab™ and statistics were performed using Mann-Whitney U tests. To correlate protein mobility with DNA binding and target gene expression, Chromatin immunoprecipitation (ChIP) and qPCR were performed.

In principle, the mobility of the transcription factors is dependent on their binding to their targets and thus their activity. For example: An active transcription factor is transiently bound to DNA, rendering it immobile, whereas an unbound transcription factor will be more highly mobile. We show the direct response of SOX9 to changes in the environment after 30-60 minutes of treatment. In C20/A4 and hPC cells WNT and IL1β significantly increased SOX9 protein mobility and this correlated to a decreased binding of SOX9 to the *ACAN* and *COL2A1* enhancers/ promotors, and decreased gene expression. BMP7 decreased the SOX9 mobility and increased SOX9 DNA binding at these promotor sites. Again, this correlated to an increase in gene expression. In presence of WNT3A, the WNT antagonists DKK1 and FRZB restored the SOX9 mobility to its control level in healthy hPCs. In contrast, neither WNT3A nor its antagonists had any effect on the OA hPCs.

FRAP allows for monitoring transcription factor activity in real-time in primary cells, as compared to conventional readouts that measure changes in target gene expression after hours to days of treatment. Our data show for the first time how external signals directly regulate SOX9 activity and thereby influence cartilage homeostasis.

P932 Role of primary blood outgrow endothelial cells on the revascularization of decellularized porcine organs with a chemically-defined medium in a dynamic perfusion biorreactor

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Blood vessel development occurs by the proliferation, migration and remodelling of endothelial cells from adjacent blood-vessels (angiogenesis) or following differentiation of endothelial progenitor cells (EPCs) form mesodermal precursors (vasculogenesis). Blood outgrowth endothelial cells (BOECs) are a very rare type of circulating progenitor cells (0,05 – 0,2 cells/ml of peripheral blood) endowed with vascular regenerative ability and high proliferative potential. Unlike the other (early) EPCs composed of hematopoietic cells, BOECs are phenotypically true endothelial cells that can promote de novo vessel formation *in vivo*. In this work the authors developed an improved method of BOEC isolation from peripheral blood and in vitro expansion with chemically-defined medium. As a result the cells outgrow in culture earlier and thus have higher proliferative potential. Once expanded the cells were characterized phenotypically (FACS and IF) and functionally (sprouting assay and tube formation). The cells were then seeded on decellularized porcine organs (heart, liver and BioVaSc-TERM®) with the defined medium to assess the revascularization of these organs under perfusion in a bioreactor.

After 4 days it was visible the total repopulation of the blood vessel structures and after 1-2 months the organs were virtually 100% recellularized with visible blood tissue.

Especially in the case of the BioVaSc-TERM® (based on porcine jejunal segments which are decellularized and sterilized and which vascular structures are preserved) this opens the possibility to generate customized tissue models, by including further cell types and also work as a delivery system by connecting it to a patient´s bloodstream after implantation.

Keywords: EPC, BOEC, chemically defined medium, decellularized tissue, BioVaSc-TERM®, recellularization, perfusion bioreactor, vasculogenesis.

P933 Tissue-engineered vascular patches prepared from nanofilms and endothelial cells derived from induced pluripotent stem cells

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Vascular patches prepared from synthetic polymers [e.g. expanded polytetrafluoroethylene (ePTFE), polyethylene terephthalate (Dacron)] are used to reconstruct and repair damaged blood vessels. Unfortunately, these patches have limitations related to thrombus formation and calcification due to blood and tissue incompatibility. Tissue-engineered vascular patches could overcome the problems of synthetic vascular patches. Herein, we describe a tissue-engineered vascular patch prepared from poly(caprolactone) (PCL) and/or poly(dimethylsiloxane) (PDMS) nanofilms, with elastic modulus near values found in arteries, i.e., between 0.1 to 1 MPa [1]. These nanofilms were then seeded with arterial (AELCs) or venous (VELCs) ECs derived from human iPSCs in chemically-defined conditions. The derived ECs were fully characterized at gene, protein and functional levels. The interactions of iPSC-derived ECs with the nanofilms were characterized according to cell adhesion, proliferation, monolayer cell formation, measurement of platelet activation and inflammatory profile against mononuclear cells. The results were compared to nanofilms seeded with somatic ECs (human umbilical vein ECs and human umbilical arterial ECs) or commercial vascular patches seeded with somatic or iPSC-derivedECs.

Our results show that 60-70% of all the cell types adhered to the nanofilms (PDMS and PCL) relatively to TCPS experimental group. Cell proliferation results showed that all cell types proliferated in both nanofilms and vascular patch. The iPSC-derived AELCs and VELCs cultured for 72 h on top of the nanofilms were able to form a monolayer and they expressed VE-Cadherin and ZO-1. Importantly, cells cultured in nanofilms or vascular patches have a pro-inflammatory response after exposure to TNF-α, although with differences. A significant up-regulation of ICAM-1 and E-Selectin mRNA levels was observed for all conditions. However, the up-regulation of E-Selectin mRNA levels in TNF-α-treated AELCs or VELCs were lower than the ones observed in HUVECs or HUAECs. To further confirm the inflammatory response profile of cells cultured in nanofilms or vascular patches we quantified the expression of E-Selectin by flow cytometry. TNF-α-activated AELCs and VELCs cultured in the nanofilms or TCPS expressed negligible values of E-Selectin while TNF- α-activated HUVECs or HUAECs cultured in the nanofilms or TCPS expressed high values of E-Selectin (approximately 50 to 60% of the cells express E-Selectin). Overall, our results suggest that our tissue-engineered vascular patches have a unique inflammatory profile that might be relevant for certain therapeutic uses.

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P934 Human cardiac extracellular matrix coating improves the bioactivity of scaffolds designed to support myocardial regeneration processes

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Objective: Trans-epicardial induction of myocardial regeneration processes is a promising alternative to intramyocardial ATMP delivery, providing stable and sustained presence/release of therapeutic cell products. However, a biosimilar environment within the epicardial scaffold material is needed to facilitate physiologic cell behavior and therapeutic efficacy. We therefore evaluated the capacity of processed human myocardial extracellular matrix (cECM) to modulate the cardio-specific bioactivity of biologic and polymeric carrier material.

Methods: LV myocardium was obtained from non-ischemic heart failure patients undergoing transplantation, decellularized using a non-enzymatic approach and processed to form microparticles or hydrogel (cECM). Particle morphology and protein composition were assessed by laser diffraction imaging, mass spectrometry and immunostaining. Both decellularized amniotic membrane (decellAM) and electrospun Poly-p-dioxanon/Polycaprolacton (70/30) copolymeric Polyetheresterurethane (PEEU) were coated with cECM and the behavior of HL-1 cells, cardiac fibroblasts, iPS-derived cardiomyoyctes, pluripotent stem cells and immune cells (PBMC, monocyte macrophages) seeded on the composite material was studied by MTS test, LDH release, PCR, TUNEL staining, cytokine release and phenotype polarization assays.

Results: Decellularized cECM contained all major components of the native myocardial matrix but also a number of residual obligatory intracellular proteins. The median particle feret diameter was 66 µm (15-157 µm), and liquification/gel formation preserved cECM bioactivity. Proliferation and metabolic activity of cardiomyocyte-like cells were enhanced and apoptosis was reduced by cECM. Compared to non-specific ECM (i.e. Matrigel®, Geltrex®) iPSC and ESC cultured on cECM expressed higher levels of cardiomyogenic transcription factors and mRNA encoding for contractile protein components. Naïve scaffold material only allowed for limited adhesion of cardiomyocyte-like cells, but hECM-coating significantly enhanced adhesion while reducing LDH release. Naïve and LPS-stimulated monocyte secretion of pro-inflammatory cytokines (IL-6, TNFα) was reduced in the presence of hcECM, and macrophage polarization towards the pro-inflammatory M1 state (CD80/CD163/CD206 expression) was inhibited.

Conclusion: Coating of decellAM and PEUU scaffolds with processed human cECM exerts cytoprotective, immunomodulatory and differentiation-guiding effects on cell populations relevant for cardiac regeneration and tissue engineering. cECM may therefore enhance the therapeutic capacity of regeneration-supporting epicardial composite materials.

P938 Modelling muscle disease in the horse using induced plurpotent stem cells

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The current understanding of the genetic and environmental determinants of equine muscle development is, despite considerable research focus, limited. In addition, breeding practices to improve muscle form and function have unintentionally increased the frequency of heritable myopathies in horses, the exact mechanisms of which are relatively unknown. In that regard, the horse offers a physiologically-relevant model for pre-clinical studies on human disease. Improving the understanding of equine myogenesis in both healthy and disease would aid the identification of targets for treatment and ultimately facilitate drug discovery. However, such studies require a robust experimental system, and there is currently a lack of suitable *in vitro* models. To address this need, we used equine induced pluripotent stem cells (iPSCs) to generate functional myotubes *in vitro*.

We first established a protocol for isolating and differentiating equine adult muscle stem cells (satellite cells) into myosin heavy chain (MyHC)-expressing myotubules. To differentiate equine iPSCs into myocytes, we used protocols recently established for mouse and human. Although we were successful in generating cells with myoblast-like morphology, these cells did not express markers of mature skeletal muscle, suggesting only partial differentiation was achieved. We then used an inducible lentiviral MyoD expression system. Using this approach, we successfully differentiated equine iPSCs into multinucleated, MyHC-expressing myofibers that were responsive to stimulation with Ca^{2}+ agonists.

We envision this novel in vitro system will provide a powerful tool for studying equine skeletal muscle development and the molecular pathogenesis of several highly prevalent heritable myopathies. To that end, we are now using CRISPR-Cas9 technology to model equine disease *in vitro* using iPSCs, a first in any veterinary species.

P939 Porous hydroxyapatite scaffold for bone regeneration in canine alveolar sockets

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This study was undertaken to assess bone regeneration using hydroxyapatite (HA) in canine alveolar sockets. The primary focus was comparison of bone regeneration between granular HA (gHA) forms and porous HA (pHA) scaffold. The first molar of both mandibles was extracted in six adult beagle dogs. GHA forms and pHA scaffolds were implanted in extracted alveolar sockets. The alveolar extraction sites were divided randomly using a split-mouth design with three groups: control group (extracted socket only), gHA group (filled with granular HA), and pHA group (filled with porous HA scaffold). The particle size of gHA ranged from 500 μm to 2 mm. PHA scaffold with the first molar root-like shape dimensions were 4 mm in diameter, and 10 mm in length. Computed tomographic (CT) and micro-CT evaluation were performed at baseline, four, and eight weeks post-implantation. New bone formation, mineralization, and connective fibrous tissue formation were evaluated using CT, micro-CT and fluorescence microscopic findings. Evaluation of the osteogenic effect in the gHA and pHA groups showed bone specific surface, bone mineral density, and bone parameters to be significantly higher than that of the control group (p<0.01). Bone volume fraction, bone mineral density, and amount of connective tissue related to disturbing osseointegration of gHA group was higher than in the pHA group. Quantity of new bone formation of pHA group was higher than the gHA group. This study demonstrates that gHA or pHA groups are potentially good bone substitutes for alveolar socket healing. For new bone formation during eight weeks post-implantation, HA with porous scaffold was superior to the granular form ofHA.

Keywords: Alveolar socket, Bone regeneration, Hydroxyapatite, Scaffold.

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P940 Phlorotannin/Polycaprolactone coated endotracheal tube to prevent thracheal stenosis

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Prolonged endotracheal intubation is known as the most common cause of tracheal stenosis. While the incidence of tracheal stenosis has decreased with the use of high volume low pressure cuff, there are still patients suffering from serious airway compromise.

The phlorotannins extracted from *Ecklonia cava* is known to have a variety of biologicalactivites. The objective of this study was to investigate the preventive effect of phlorotannin (PHT)/polycaprolactone (PCL) coated endotracheal tube on endotracheal intubation-induced stenosis in vivo model. The PHT/PCL coated endotracheal tube was developed using a 1.5cm segment of Levin tube (16 French) coated with PCL and then coated with phlorotannin. Drug release analysis of PHT/PCL coated endotracheal tube showed that phlorotannin was continuously release up to 7 days.

In vivo study, grade of trachea stenosis and granulation tissue of the endotracheal tube using endoscopic examination, as well as the collagen deposition and submucosa thickness by histological analysis, was investigated. The PHT/PCL coated endotracheal tube intubation group inhibited grade of trachea stenosis, granulation tissue, collagen deposition and submucosa thickness compared to the PCL coated endotracheal tube intubation group. PHT/PCL coated endotracheal tube intubation group also reduced the mRNA and protein expression of collagen type I, α-SMA and TGF-β1 compared to the PCL coated endotracheal tube intubation group.

Based on these results, the PHT/PCL coated endotracheal tube showed preventive effects of tracheal stenosis caused by endotracheal intubation. Phlorotannin may be considered as a candidate material to be coated at the endotracheal tube to prevent tracheal stenosis.

P941 The transpedicular surgical approach for the development of intervertebral disc targeting regenerative strategies in an ovine model

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Introduction. Low back pain, is a major public health concern that is frequently associated with intervertebral disc degenerative disease (DDD) named discogenic lombalgia. Faced with the limitations of the current therapies, regenerative medicine offers new prospects for the treatment of DDD. Notably, the supplementation of the *Nucleus pulposus* (NP) with regenerative cells possibly associated with biomaterials is considered with a growing interest. However, the lack of validated approaches to inject cells/biomaterials within the intervertebral disc (IVD), particularly in the NP, is one of the major obstacles to the development of IVD regenerative strategies. The pivotal role of *Annulus fibrosus* (AF) in IVD homeostasis makes necessary the development of surgical approaches preserving AF structural integrity. Among these surgical routes, the transpedicular approach (TPA) has been proposed but remains poorly investigated.

Purpose. To investigate the aftermaths of innovative transpedicular approach (TPA) in a sheep model for intervertebral disc (IVD) regenerative strategies.

Methods. Twenty four IVDs from four sheeps were included. TPA and biopsies of the *nucleus pulposus* (NP) were performed in 18 IVDs. Seven discographies were realized to assess the feasibility of contrast agents injections within the IVD using this approach. Six IVDs were used as control. IVDs were then analyzed using MRI, micro-CT scan and histological study. The accuracy of the TPA was measured as the proportion of biopsies and injections within the NP. The consequences of discographies and biopsies after TPA on the vertebra and endplates were observed.

Results. Eighty-three percent of our biopsies or injections were located into the NP. After TPA, osseous fragments were observed within the IVD in 50% of cases. Two cases of (11%) rostral endplate fracture and 5 cases (27%) of cortical breach of pedicle and encroachment into the spinal canal were observed. Two cases of perivertebral venous embolism and 2 cases of backflow through the canal of the TPA inside the vertebra were noticed after injections. Significant damages on the bone structure of the vertebra and on the rostral endplate on which the IVD is inserted were revealed.

Conclusions. TPA induces damages to the endplates and may lead to neurological impairment and leaks of injected materials into the systemic circulation. These consequences must not be ignored before going further with the TPA for IVD regenerative strategies, especially when using stem cells.

P942 Development of a canine intestinal organoid model

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Three-dimensional organ-buds, or organoids, are a novel and organ mimicking *in vitro* model based on LGR5-positive stem cells that shows realistic micro-anatomy. Recently, intestinal organoid cultures from various species including mice and humans have been described. However, canine intestinal crypt-derived organoids are not yet described. Since organoids can be infinitely expanded in culture, remain genetically stable and can be differentiated to all cell types of the intestine, they represent ideal *in vitro* model for intestinal diseases such as inflammatory bowel disease, virus infections, and drug screening. The aim of this study was to establish small intestinal and colon-based organoid culture systems from dogs, followed by molecular and cellular characterization. Fresh cadaveric samples were obtained from duodenum, jejunum and colon, followed by crypt isolation of seven individual dogs. Organoids were propagated in 3D-culture using RPSO1-based expansion media. Differentiation was achieved by removing proliferation inducing components. We established a crypt isolation protocol and kept organoid lines from three areas of the intestine (duodenum, jejunum and colon) from seven dogs in culture during at least 12 passages. The gene-expression levels of stem cell markers (*LGR5*, *CD133*, *ASCL2* and *OLFM4*) were stable in expansion media. After differentiation, expression of the goblet cell marker *MUC2* and Paneth cell marker *NEUROG3* were increased, whereas expression levels of stem cell marker *LGR5* was decreased. This novel robust *in vitro* model of small intestinal and colonic organoids can be applied as an infection model for canine enteric pathogens in the future.

P943 The release of titanium and zirconium in mini pig maxillae from titanium-implants and zirconia implants

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Objectives: The aim of this study is to compare the release of titanium (Ti) and zirconium (Zr) in mini pig maxillae from Ti-implants and ZrO_2-implants.

Methods: Methyl methacrylate embedded Mini pig maxillae with 6 Ti-implants and 4 ZrO_2-implants were analyzed after 12-weeks of implantation. For details see: Clin Oral Implants Res. 2012; 23:281-6. The spatial distribution of Ti and Zr in maxillae near the implants was measured with laser ablation (LA)-inductively coupled plasma (ICP)-mass spectrometry (MS) by making laser ablation on the bone sections (2.5 mm×4 mm) with longitudinal cuts through the implants. From each maxilla two bone slices (about 1 mm thick) adjacent to the implants were measured. The contents of Ti and Zr in these bone slices were determined by ICP-optical emission spectrometry and ICP-MS, respectively.

Results: Increased Ti and Zr intensity could be detected in bone/tissues at a distance of 891±398 µm (mean ± SD) from Ti-implants and 927±404 µm (mean ± SD) from ZrO_2-implants, respectively. The increased intensity was mainly detected in the regions near implant screws. The average Ti content detected in 11 bone slices from samples with Ti-implants was 1.67 mg /kg, which is significantly higher than the Ti content (0.99 mg/kg) detected in 8 slices from samples with ZrO_2-implants. The highest Ti content detected was 2.17 mg/kg. The average Zr content in 4 of 8 bone slices from samples with ZrO_2-implants is 0.59 mg/kg, the other 4 bone slices showed low Zr contents below the detection limit (<0.30 mg/kg), the highest Zr content detected in this group is 0.75 mg/kg. The average Zr content of 8 slices in samples with Ti-implants is 0.71 mg/kg, Zr content in the other 3 bone slices is below detect limit.

Conclusion: After 12-week implantation, increased intensity of Ti and Zr can be detected in bone/tissues near Ti- and ZrO_2-implants. The results show that Ti content released from Ti-implants is higher than the Zr content released from ZrO_2-implants.

P944 Non-union defects show impaired bone formation in the early reparative phase in mouse femoral defect models

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INTRODUCTION: Delayed fracture repair and the formation of non-unions are major issues in orthopaedic surgery. In order to prevent non-healing fractures, there is a need to reliably understand and to detect even small deviations in the healing process. We recently described an *in vivo* micro-CT based approach for healing phase-specific characterization of fracture healing [1, 2]. **AIM:** We now want to assess, whether this longitudinal approach allows inferring of non- union formation prior to radiological manifestation. **METHODS:** Female 20 week-old C57BL/6J mice received a femur defect stabilized with an external fixator (RISystem, Davos, Switzerland; union group, defect size=1.2mm±0.5, n=15; non-union group, defect size= 1.5mm±0.5, n=7). During the healing period the defect area was weekly scanned (vivaCT 40, ScancoMedical, Brüttisellen, Switzerland) to assess structural and dynamic callus parameters. Data were tested for normal distribution followed by ANOVA with Bonferroni correction (significance level $p \leq 0.05$). **RESULTS:** Given the manifestation of non-unions in our model by week 4, we focused on the first 3 weeks of the healing process. In the union group distinct characteristics of the different healing phases were seen (Fig. 1): From week 1-2 a significant 24x increase in bone formation was detected, indicating progression from the inflammation to the reparative phase. This led to a significant gain in bone volume by week 3 (BV_{week3}: 0.8mm^3±0.3, BV_{week2}:0.05mm^3±0.01). Compared to this physiological healing pattern, bone formation in the non-union group was significantly reduced in weeks 2 and 3 by 30% and 55%, respectively, leading to a significantly reduced bone volume by week 3 (-44%). In contrast, bone resorption during the early healing period showed a similar trend for both groups, indicating a dysregulated linkage between formation and resorption activities in the non-union group. **DISCUSSION & CONCLUSIONS:** Using our previously described scanning protocol with subsequent analysis of structural and dynamic callus parameters, we were able to identify bone formation in the early reparative phase as an indicator for subsequent union vs. non-union formation. This standardized approach with early detection of altered reparative activities will allow the precise initialization and longitudinal assessment of potential intervention therapies to counteract non-union formation. **ACKNOWLEDGEMENTS:** The authors acknowledge support from the EU (BIODESIGN FP7-NMP-2012-262948) and the ETHZ Postdoc Fellowship/M.Curie Actions f. People COFUND Program (FEL-25 15-1).

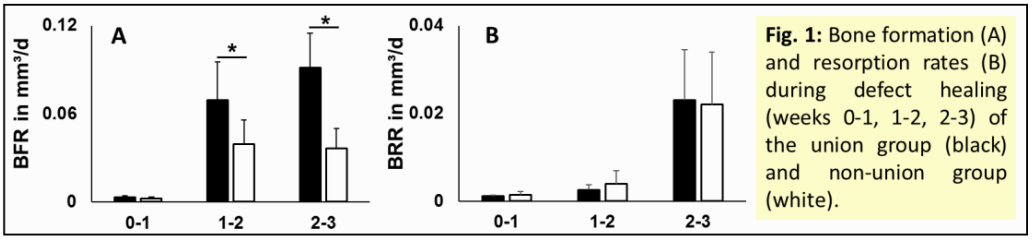

Fig. 1: Bone formation (A) and resorption rates (B) during defect healing (weeks 0-1, 1-2, 2-3) of the union group (black) and non-union group (white).