Utilising DOE to optimise hydrogel composition for tissue engineering

UPTON, A., Tirnoveanu, A., Thapa, G., Zimbitas, G., Mylona, A. and Webb, R.

Utilising DOE to Optimise Hydrogel Composition for Tissue Engineering

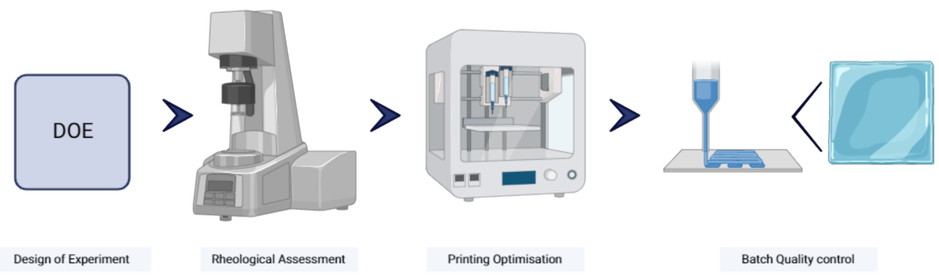
Alice Upton[1], Alice Tirnoveanu[1], Gauri Thapa[1], Georgina Zimbitas[2], Athina Mylona[3] Richard Webb[1]

[1]Stem Cell Research and Bio-Engineering Laboratory, Institute of Medical Sciences, Canterbury Christ Church University, UK
[2]School of Engineering, Technology and Design, Canterbury Christ Church University, UK
[3]Kent and Medway Medical School, UK

INTRODUCTION

Challenges arise when developing a bioink for 3D bioprinting that allows for precise printability, biocompatibility, ideal mechanical properties, and batch-to-batch consistency. Currently there is no standardised protocol to design a functional bioink.

Here, a methodology was designed to optimise hydrogel composition of Gelatin (GEL), Hyaluronic Acid (HA) and Dextran-40 (DEX), utilising the 'Design of Experiment' (DOE) statistical tool and rheological behaviour of each composition.



METHODS AND RESULTS

The 2-level factorial DOE generated 33 samples of GEL (1-5%), HA (0.5-2%), DEX (0.5-2%) at various concentrations, dissolved in cell-culture. As our control Cellink Skin was characterised to establish a target viscosity of 1271.790mPa/s.

The samples from the DOE were subject to an Isothermal Viscosity Test, which determined the viscosity of the samples at a constant temperature of 37oC. The samples were then sheared at a high shear rate of 80 s-1 for 2.5 minutes. Viscosity data was applied to the DOE with non-cell laden bioink components (Figure 1E) Cell laden bioink components (Figure 1A).

The DOE identified that HA is the dominant influencer on viscosity in both bioink components non-cell laden and cell laden (Figure 1B and 1F). HA increased the viscosity of the bioink. DEX also had a significant influence on the viscosity of the bioink as an individual component and combined with GEL, causing the viscosity to decrease (Figure 2).

Optimising the final concentrations of each component utilising the DOE established the bioink composition of 5% GEL, 2% HA and 0.5% DEX with non-cell laden and cell laden samples. With the addition of cells (1x106/ml) the viscosity also decreased by approximately 200 mP/s.

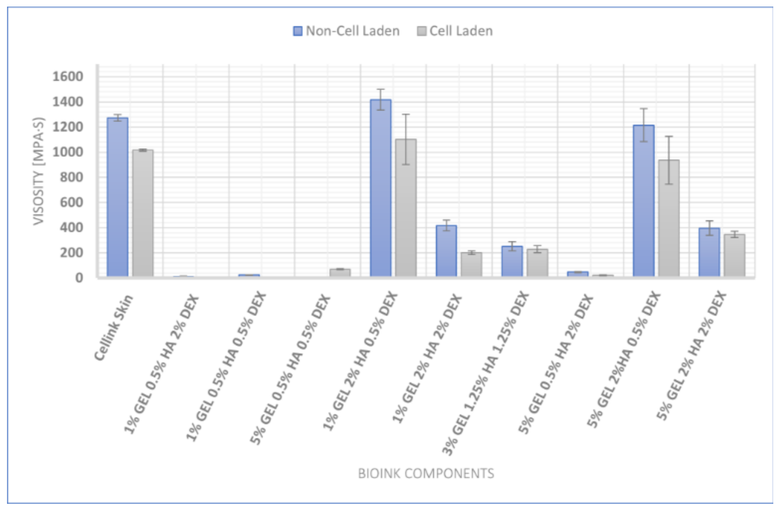


Figure 2 - Viscosity of Each Sample with various conentration (%) of GEL, HA and DEX - Viscosity of experimental condition generated by the DOE exposed to high shear rates using the Isothermal Viscosity Test at 37°C.

To ensure consistent quality of the bioinks between batches, 10 batches were subject to the Isothermal Viscosity test in triplicates. The overall average of the 10 batches produced viscosity of 1271.338 mPa/s, with a deviation of 50.296 mPa/s, which is less than a 5% variation across batches (Figure 3).

To establish that the properties of the bioink are consistent, a frequency sweep was performed from 100 – 0.1 rad/s. to evaluate the time-dependent viscoelastic properties of the bioink batches (Figure 4). Using the Loss factor to describe the properties of each batch, this test showed consistent properties that do not deviate less than 0.1 Pa.

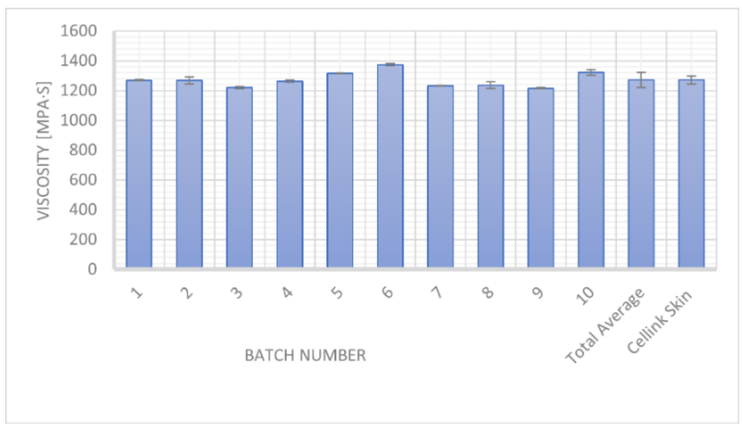


Figure 3 - Batch quality testing of the final composition of bioink - Displays the quality of each sample of 5% GEL 2% HA and 0.5% DEX

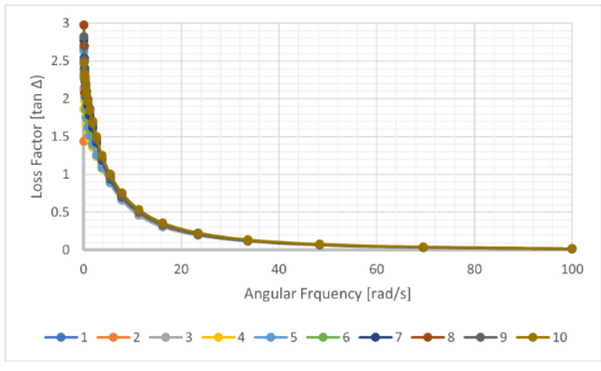


Figure 4 - Frequency Sweep of 10 Batches of Bioink - Displays the loss factor of each batch of bioink of 5% GEL, 2% HA and 0.5% DEX.

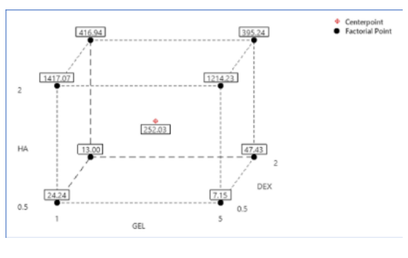


Figure 1A

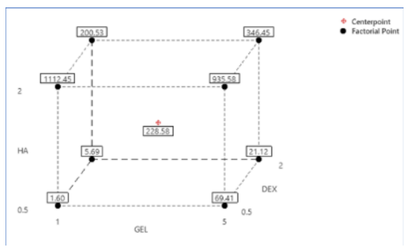


Figure 1E

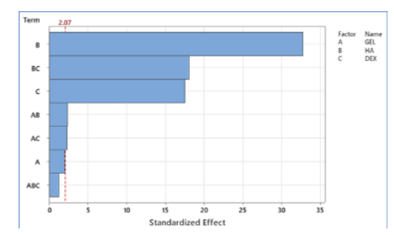


Figure 1B

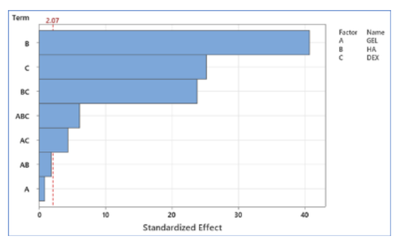


Figure 1F

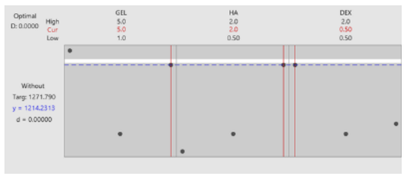


Figure 1C

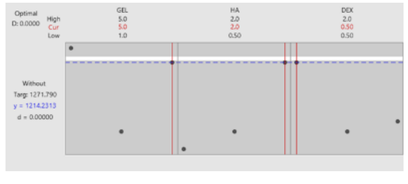


Figure 1G

Figure 1: Statistical analysis of the DOE - Cube Plot for A) Non-cell laden and E) Cell laden bioink components of GEL, HA and DEX - these displays the upper, middle and lower viscoities of each component. **Parteto Chart of the Standardised Effect of B) Non-cell laden and F) Cell laden bioink components of GEL, HA and DEX** - Displays the effects of each component on viscosity. Any component over the threshold of 2.07 has a significant effect on viscoity. **Response Optimisation of C) Non cell-laden and G) Cell-laden bioink components of GEL, HA and DEX -** Displays the optimised viscosity calculated from the DOE.

CONCLUSION

The development of this method to design a bioink has proved to show consistent results. Utilising a commercially available bioink (Cellink Skin) to establish a viscosity baseline for a bioink enabled assumption to be adopted to predict printing parameters which can be applied during the printing process.

Applying the use of a DOE to this methodology removes the time-consuming methods of trial and error, and to accurately design a bioink optimised to a particular target. The use of GEL, HA and DEX provides versatility to this bioink while naturally replicating the cellular microenvironment for soft tissue engineering.

To further establish the bioink properties, additional rheological studies can be performed to understand the behaviour of the material that replicate the stresses during 3D printing. Rheological tests such as stress-strain test to establish if the material is non-Newtonian or Newtonian fluid; Thixotropic behaviour to replicate the time-dependent stresses of the bioink forced through a printing syringe; Temperature Ramp to understand the effects of different temperature on viscosity

ACKNOWLEDGEMENTS

The authors would like to acknowledge the generous contribution of Anton Paar for the MCR 92 Rheometer to complete this work. Also, to acknowledge the financial contribution from NHS, Health Education England Southeast for funding the project, and to ARC/KSS for funding the PhD Scholarship, Faculty of Medicine, Health, and Social Care at Canterbury Christ Church University.

REFERENCES

- Habib, M.A. and Khoda, B., 2022. Rheological analysis of bio-ink for 3D bio-printing processes. Journal of Manufacturing Processes, 76, pp.708-718.
- Amorim, P.A., d'Avila, M.A., Anand, R., Moldenaers, P., Van Puyvelde, P. and Bloemen, V., 2021. Insights on shear rheology of inks for extrusion-based 3D bioprinting. Bioprinting, 22, p.e00129.
- O'Connell, C., Ren, J., Pope, L., Li, Y., Mohandas, A., Blanchard, R., Duchi, S. and Onofrillo, C., 2020. Characterizing bioinks for extrusion bioprinting: printability and rheology. In 3D Bioprinting (pp. 111-133). Humana, New York, NY.
- Nam, S.Y. and Park, S.H., 2018. ECM based bioink for tissue mimetic 3D bioprinting. Biomimetic Medical Materials, pp.335-353.
- Gregory, T., Benhal, P., Scutte, A., Quashie Jr, D., Harrison, K., Cargill, C., Grandison, S., Savitsky, M.J., Ramakrishnan, S. and Ali, J., 2022. Rheological characterization of cell-laden alginate-gelatin hydrogels for 3D biofabrication. Journal of the Mechanical Behavior of Biomedical Materials, 136, p.105474.