Auricular reconstruction: where are we now? A critical literature review

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INTRODUCTION

External ear deformities can be inherited in the form of microtia and anotia, or acquired in the case of trauma, burns or following excision of skin cancers [1,2]. The resulting cosmetic appearance can have a significant impact on psychosocial well-being as well as affecting sound localisation and hearing [3]. Additionally, functional issues can arise such as difficulty wearing glasses with a potential to impact the global development of children. Quality of life indicators as well as psychosocial state can be improved with auricular reconstruction or prostheses [4].

BACKGROUND

Embryological Pinna Development and Microtia

The pinna forms from both the mesoderm and ectoderm of the first and second branchial arches. The six Hillocks of His form on these two arches around the first branchial cleft and join at around week eight of gestation. By week twelve, the pinna is usually formed and by week twenty has migrated from the original position at the lower neck to eye level as the mandible enlarges. Microtia affects 0.8-4.2 per 10,000 births, is unilateral in around 80% of cases and affects males in the ratio of 1.5:1. Microtia is believed to result from cell death on the first two arches with various causes proposed, such as maternal illness, teratogens, genetic defects and syndromes [5].

Aetiology of Microtia

Congenital microtia has been linked to at least 50 genes and Homobox A2 (HOXA2) being shown to be linked with syndromic microtia [5,6], chromodomain helicase DNA binding protein-7 (CHD7) being linked to CHARGE syndrome [7], Chromatin Licensing and DNA replication Factor-1 (CDT1) [8], Origin recognition complex-4 (ORC4), Origin recognition complex-6 (ORC6) and Cell division control protein-6 (CDC6) linked to Meier-Gorlin Syndrome [9], Mediator of RNA polymerase II transcription subunit-2 (MED2) linked to Opitz-Kaveggia syndrome [10], Twist family BHLH transcription factor-1 (TWIST1) linked to Saethre-Chotzen syndrome and Origin recognition complex-1 (ORC1) linked to small ears and syndromic microtia [11,12]. Recognised associated abnormalities of microtia include spinal deformity, renal dysplasia, cleft lip and palate, congenital heart disease and eye malformations [11].

Current Treatment Options for Auricular Reconstruction

For skin cancers of the pinna surgical treatment options include Mohs surgery, wide local excision or even pinnectomy leaving patients with obvious outer ear deformities [13] (note Figures 1 and 2). Depending on the amount of tissue loss, reconstructive options include flap techniques and autologous cartilage grafting. Microtia can be managed with silicone prostheses and successful cases utilising osseo-integrated screws have been described with good cosmetic outcomes [14]. Figure 3. Other treatment options for microtia include synthetic implants and autologous costal cartilage grafts, the latter being considered the gold standard [1,4]. This involves obtaining three to four costal cartilage sections for direct reconstruction of the pinna. This multi-stage procedure was pioneered by Brent [15,16] and Nagata [17] showing good cosmetic outcomes and long-term durability. Nevertheless, this technique relies on surgical artistic expertise with highly variable outcomes [18]. The literature reports increased pinna stiffness compared to native auricular cartilage [2,15]. Issues regarding rejection as well as long-term sustainability have also been highlighted with changes in shape and size noticed over time [1]. However, the major limitation is the significant donor site morbidity and need for multiple operations. Chest wall pain and clicking are most commonly reported but complications may include pneumothorax, atelectasis, pleural effusions, thoracic scoliosis, seromas and significant scarring [1]. This highlights the need for developing new strategies involving tissue regeneration and novel technologies.

Tissue Engineering Options for Auricular Reconstruction

Auricular reconstruction has been an exciting area of regenerative medicine which gained public interest following Vacanti's work in 1997 in which bovine chondrocytes were seeded onto a synthetic scaffold and implanted into nude mice [20]. More recent studies have endeavoured to use human subjects. Yanaga et al injected cultured ear chondrocytes subcutaneously into the human abdomen and later harvested the cartilage for pinna sculpturing and human implantation [21]. More recently Zhou et al successfully implanted bioengineered auricles onto five children in China [22]. Despite these recent advancements autologous costal cartilage remains the gold-standard treatment for microtia [1,4].

The basic requirements for tissue engineering are cells, growth factors and a scaffold (Figure 4 [23]) [19,23]. Zhou et al describes the process of creating a bio-engineered pinna. A Computed Tomography (CT) scan is taken of the unaffected ear, a mirror image created, a biodegradable scaffold 3D bio-printed and chondrocytes seeded onto this construct. Chondrocytes are harvested from the microtia cartilage so as to minimise donor site morbidity. After a period of cell expansion in vitro, the auricle is implanted [22].

Scaffolds

Scaffolds have previously been used in an array of tissue engineering constructs ranging from bone [25], tendon [26] and cartilage [27] constructs. More recently the use of a combination of polymer scaffold which can support a secondary hydrogel scaffold have been utilised in auricular tissue engineering [21,22]. Primarily the scaffold should not initiate an immune response as a whole or in its breakdown metabolites, whilst retaining mechanical integrity of the engineered tissue to resemble that of native tissue [23,24]. An inverse relationship with new tissue formation is seen, as ultimately the scaffold would degrade to be replaced by a mechanically stable, functional tissue.

Cellular component of implant

Early tissue engineering attempts for auricular cartilage utilised chondrocytes [31] with limited results due to the limited expansion capacity of mature chondrocytes when compared to ovine models [32]. More recently, numerous papers have employed the use of tissue-specific mesenchymal stem cells such as: chondroprogenitors [33], adipose derived mesenchymal stem cells [34] and bone marrow derived mesenchymal stem cells [35]. Therefore, due to the increased population doubling capacity of the progenitor cells when compared to chondrocytes, the progenitor cells have become the favoured cell choice for tissue engineering constructs.

Progenitor/stem cell differentiation

Adult stem cells (mesenchymal stem cells)/progenitor cells were first identified by Till and McCulloch in 1961 [36]. The cells were capable of producing multilineage, haematopoietic colonies within the spleen. The term mesenchymal stem cells (MSC) was coined in 1991 by Caplan [37] building on the earlier works of Friedenstein et al in 1987 [38]. However, the non-haemopoietic stem cell capable of multilineage differentiation did not attract global interest until the multilineage works of Pittenger et al in 1999 [39]. Since the original identification of the MSC differentiation, potential has expanded and is shown in Figure 5 [23]. Since the original identification of the bone marrow derived MSC/Progenitor cells, MSC/Progenitor cells have also been identified in: tendon [40], articular cartilage [41], auricular cartilage [42], tracheal cartilage [43], ligament [44], adipose [45] and muscle tissue [46].

Once the progenitor/stem cell has been isolated and expanded the need to differentiate the stem cells towards the tissue of interest needs to be initiated. The differentiation towards a chondrocyte “like” lineage has been investigated for over 30 years with a wide range of opinions as to not only the best growth factor but also the use of mechanotransduction. Members of the transforming growth factor beta (TGFβ), namely TGFβ-1 [47], TGFβ-2 [48] and TGFβ-3 [49] have all been utilised in chondrogenic differentiation up to 28-56 days. Also, fibroblastic growth factor -18 (FGF-18) [50] and more recently bone morphogenic protein -9 (BMP-9) [51] have all been used to differentiate progenitor and stem cells towards a chondrocyte “like” lineage. Recently, the use of mechanotransduction by means of transmission of external forces to induce differentiation has become more relevant in the reduction of growth factor use and minimising growth factor induced adverse effects such as ossification or even tumour genesis which has been observed with insulin-like growth factor -1 (IGF-1) [52].

Mechanotransduction has been shown to induce differentiation and an array of cellular response to different forces such as sheer [53] induced chondrocyte regeneration, stretch [54] resulting in upregulation of elastin (ligament) and compression [55] in osteogenesis have been observed. The choice of force, growth factor or combination of stimuli should not be overlooked when tissue engineering a construct for implantation. Figure 6.

3D Printing

3D printing uses Computer Aided Design (CAD) to generate complex structures by building from the bottom up, layer by layer using biocompatible materials [19]. 3D printing has a huge potential in medicine demonstrated by recent studies [22]. Compared to autologous costal cartilage ear reconstruction, 3D printing can reduce operating time, donor site morbidity, obtain reproducible results and reduce rejection rates [2,19].

The Question

If the tissue-engineered ear is considered far more superior cosmetically with reduced patient morbidity, why is autologous costal cartilage grafting still considered gold-standard? This review will critically analyse recent and breakthrough research in the field of regenerative medicine for the outer ear, considering gaps in current literature, potential for future impact and the next steps.

METHODS

PubMed (MEDLINE) and Cochrane databases were searched using the following key terms: regenerative medicine; tissue engineering; 3D printing; biofabrication; auricular reconstruction; auricular cartilage; chondrocyte, outer ear; pinna. Exclusion criteria were articles not in English and articles not published within the last ten years. Inclusion criteria were studies implanting tissue-engineered auricles into animal or human subjects. Results were filtered by publication dates within the past ten years. The search results were exported to an Excel spreadsheet and duplicate titles were removed. Titles were screened and removed if not relevant. Abstracts were then screened and taken forward for full text review if appropriate. Studies implanting tissue-engineered auricles into human or animal subjects were taken forward for the final list.

In addition, a breakthrough study from 2009 [21] was included which was not identified during the literature search using the above terms. This study was referenced in a number of relevant studies, meets the search criteria and was considered a significant advancement in this field at the time. Figure 7.

RESULTS

Eight studies from the past ten years in which reconstructed auricles were implanted into either animal or human subjects were selected from the literature review (Table II) [19,21,22,57–61]. Only two studies investigated human implantation, highlighting the need for further human studies [17, 18]. Table III shows a critical analysis of these studies, including suggestions for study improvements.

DISCUSSION

The pinna is a prominent facial feature well recognised to affect self-esteem when appearance deviates from the perceived norm. Johns et al showed that correction of external ear deformities can reduce anxiety and depression in children and improve social skills [62].

From the literature it is clear that auricular reconstruction is a complex task owing to the intricate 3-dimensional structure. Table I summarises the strengths and limitations of the three current treatment options and the tissue-engineering option. Autologous costal cartilage grafting has been considered the gold-standard for over fifty years [56]. However, the vast heterogeneity in outcome as well as the operative burden of undergoing multiple staged procedures over years opens up an opportunity for novel methods using regenerative medicine and emerging technologies.

In comparison to autologous costal cartilage grafting, the tissue-engineered approach comes at a much lesser cost to the patient in terms of donor site morbidity, number of operations required and time spent in hospital, a very important consideration for paediatric patients in full-time education [21]. Another major benefit is that medical imaging, in the form of Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), can be used with a Computer Aided Design (CAD) programme to create a patient-specific scaffold based on the contralateral ear, hence improving cosmetic outcome and patient or parental satisfaction.

The use of 3D printing in the medical field has increased recently as the cost declines [63]. Liao et al describe how this technology can be used to create a scaffold not only encompassing the outer structures of the auricle as we see it but also the inner structure containing pores for chondrocyte seeding [19]. This study focusses on the use of 3D printing for creating pinna scaffolds but this technology has also been used for surgical simulation training, pre-operative planning and patient education purposes [4]. Barriers to increasing the use of 3D printing within medicine include financial cost, lack of evidence-base and approval [56]. There does not seem to be a consensus about the best biodegradable material as of yet in terms of bioactivity, structure, rejection and absorption [19].

A critical analysis of recent studies in which a tissue-engineered auricle was implanted into human or animal subjects has been conducted (Table III). Studies from the past ten years show promise that auricular bioprinting holds real potential for future treatment of pinna deformities. Previous barriers to human application include long term shape and strength instability, sourcing of cells and issues with biosafety including rejection and post-surgical infection. Recent technological advancements have enabled Zhou et al to overcome these issues, demonstrating the first successful implantation of a tissue-engineered ear into five human subjects [22]. This is a landmark study in this field. A combination of CT scanning, 3D printing and in-vitro culturing of microtia chondrocytes onto bio-scaffolds enabled creation of patient-specific auricles modelled on the unaffected ear. A 3 month in-vitro period of neocartilage formation was followed by strict quality analysis prior to human implantation. Three surgical methods were adopted for implantation depending on the patient's anatomy, each detailed in the study. Following implantation, regular follow up showed gradual improvement in definition of the pinna structures (helix, anti-helix, triangular fossa and cavum conchae), particularly from 9 months post-operatively.

The scaffolds used by Zhou et al are composed of a polycaprolactone (PCL) core, covered by polyglycolic acid (PGA) fibres and finally a polylactic acid (PLA) covering. Choice of scaffold material is important in achieving the correct balance between pinna durability and flexibility so as to prevent extrusion [19]. PGA fibres showed good degradation after 3 months in vitro prior to human implantation, reducing risk of host response. The PCL core, which maintained pinna shape, gradually degraded over 24 months as neocartilage formed. Titanium wires have previously been trialled as an alternative but with significant concern for extrusion [64]. This PCL core was shown to improve pinna strength 4-fold compared to scaffolds without. Some literature reports that scaffold degradation takes up to four years, which would suggest that the case followed up by Zhou et al for two and half years has not allowed sufficient time for neocartilage absorption and therefore changes to size and shape [19].

Zhou et al utilise thorough methods of quality assessment throughout the process, such as scanning electron microscopy (SEM) to assess chondrocyte adherence to scaffold, laser scanning to analyse shape changes as well as histological and immunohistochemical analysis of neocartilage pre-implantation, at 6 months and 18 months post-operatively. Neither this study, nor the other seven in this review undertook further investigations to confirm the formation of auricular cartilage rather than hypertrophic chondrocyte condensation leading to ossification of the cartilage tissue. In future studies we recommend the use of alizarin staining to show the absence of calcification as well as using bone markers to show absence of up-regulation [65]. Obvious limitations of Zhou et al include a very small sample size with only one case followed up in detail, short follow up times and a single-centre study. Nevertheless, this is a breakthrough study in the field showing promising results with the use of both bioprinting and tissue-engineering to deliver a personalised patient outcome, with further work ongoing. Table III shows further critical analysis of the study.

The literature search mainly yielded animal studies with just one human study implanting 3D printed bioengineered ears. A common limitation of the current research is small study sizes, ranging from just four to ten subjects where the study size is detailed. Another issue is short end points, with some conclusions drawn after a matter of weeks and long-term outcomes unknown. There are some issues with wound healing and graft acceptance, though it is important to consider the role of patient-specific factors affecting this, just as with the current gold-standard autologous costal cartilage grafting. Notably, the two human studies do not consider the psychosocial impact of recurrent surgeries required to trim the external ear after initial implantation of neocartilage. It is important to recognise the current main barriers to the tissue-engineered auricle (Table I). These include financial cost, lack of evidence and unknown long-term outcomes. Multiple procedures are often still required and the resultant pinna may not possess the elasticity of the native pinna. Finally, the possibility of uncontrolled stem cell differentiation and tumour formation has been raised [1,4,18]. In order to further develop the field of regenerative medicine for auricular reconstruction the next steps should include a large, multi-centred human trial with a substantial follow up time.

CONCLUSION

There is an increasing interest in 3D printing scaffolds and tissue engineering in the management of auricular deformities. Though a recent study has shown promising results this research mainly follows one case [22]. Gold-standard treatment continues to be autologous costal cartilage grafting and though this may one day be overtaken by 3D printing a patient-specific auricular scaffold, larger-scale human clinical trials with longer follow-up periods are needed. Another future possibility to consider is the direct 3D printing of cells to produce a cartilaginous pinna.

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