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REVIEW ARTICLE

UTILIZATION OF STEM CELLS IN CRANIOFACIAL REGENERATION AND REPAIR

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ABSTRACT

The use of craniofacial tissue engineering lies in its ability to assist in the regeneration or *de novo* formation of dental, oral, and craniofacial structures that have been lost due to congenital anomalies, trauma or diseases. As such, several craniofacial structures, such as the mandibular condyle, calvarial bone, cranial suture, and subcutaneous adipose tissue have been engineered from mesenchymal stem cells, growth factor, and/or gene therapy approaches. Earlier, durable materials such as amalgam, composites, and metallic alloys were used for repair purposes. Now, in the era of modern technology, one is able to consider sophisticated biological therapies that utilize mesenchymal stem cells, delivered or internally recruited, to generate craniofacial structures in temporary scaffolding biomaterials. Craniofacial tissue engineering is a very exciting prospect of today's world, and is an opportunity that could possibly revolutionize dentistry as we know it. This review discusses potential clinical applications of stem cells in such aspects while mentioning relevant clinical studies that have marked it suitable for such a use. It also discusses immediate challenges and concerns that need to be addressed. Regeneration and repair of calvarial structures, alveolar bone, cleft lip and palate, TMJ and condyle have been discussed in specific (Mao *et al.,* 2006).

INTRODUCTION

Tissue engineering is an interdisciplinary field that utilizes scaffold matrices to fill a void, to provide structural support and to deliver growth factors and/or cells that have the ability to form tissues within the body upon transplantation. Tissue engineering/regenerative medicine strategies require interaction and integration with tissue and cells through incorporation of appropriate physical and cellular signals. Therefore, inclusion of modifying factors such as biologically active proteins and DNA are critical to success. Currently, simpler procedures are more successful and include using primary chondrocytes for the replacement of damaged cartilage (Kuo *et al.,* 2006) as well as skin cell sheets for damaged skin (Shimizu *et al.,* 2003). However, some more complex tissue reconstructions, like the bladder, have performed well (Howard *et al*., 2008) which produce hope for more complex tissue engineered procedures in the future. Although basic functional tissue engineered strategies have been key, there is still considerable scope for future developments of cell sources, individually tailored cell supports, immune modulation, vascularization, and the predictive abilities of computer and mathematical modelling for more complex materials.

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In this review we introduce some of the components and strategies that are currently in development with greater emphasis on work done in the areas of cleft lip/palate, TMJ and condyle, calvarial structures and alveolar bone.

Types of Stem Cells

1. Embryonic Stem Cells

These pluripotent stem cells are derived from the inner cell mass of a blastocyst. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50–150 cells, capable of utilization. This procedure is currently considered controversial. (Baldwing, 2009 and Thomson *et al*., 1998)

2. Fetal Stem Cells

Primitive stem cells are located in the organs of fetuses of which there are two types: Fetal proper and embryonic. Fetal proper stem cells are obtained from the tissue of the fetus proper, usually after an abortion. Extra embryonic fetal stem cells come from extra embryonic membranes, and are normally not distinguished from adult stem cells (Bongso *et al*., 2005).

3. Adult Stem Cells

These are undifferentiated cells, found throughout the body after development that multiply by cell division to replenish

dying cells. They can be found in juvenile as well as adult animals and human bodies (Moore *et al*., 2013).

4. Hematopoietic stem cells

These are found in the bone marrow and give rise to all other blood cells.

5. Mammary stem cells

They provide the source of cells for growth of the mammary gland during puberty and gestation. Such cells can give rise to both the luminal and myoepithelial cell types of the gland, and have been shown to have the ability to regenerate the entire organ in mice (Liu *et al*., 2005).

6. Intestinal stem cells

These cells divide continuously throughout life and use a complex genetic program to produce the cells that line the surface of both small and large intestines (Van Der Flier and Clevers, 2009). These cells reside near the crypts of Lieberkuhn. They have been suspected as the source of most cancers of the small intestine and colon (Shmelkov *et al*., 2008).

7. Mesenchymal stem cells (MSCs)

These cells are of stromal origin and may differentiate into a variety of tissues. MSCs have been isolated from placenta, adipose tissue, lung, bone marrow and blood, Wharton's jelly from the umbilical cord, (Secco *et al*., 2006) and teeth.

8. Endothelial stem cells

These cells are a rare and controversial group with the ability to differentiate into endothelial cells, the cells that line blood vessels (Jiang *et al*., 2002).

9. Neural stem cells

The existence of these cells in the adult brain has been postulated following the discovery that the process of neurogenesis, the birth of new neurons, continues into adulthood, as seen in rats (Kennea *et al*., 2002).

10. Olfactory adult stem cells

Olfactory adult stem cells are harvested from the human olfactory mucosa cells, which are found in the lining of the nose and are involved in the sense of smell (Preynat-Seauve and Krause, 2002).

11. Neural crest stem cells

These cells can generate neurons, Schwann cells, myofibroblast, chondrocytes and melanocytes (Lakshmipathy and Verfaillie , 2005).

12. Testicular cells

Multipotent stem cells with a claimed equivalency to embryonic stem cells have been derived from spermatogonial progenitor cells found in the testicles of laboratory mice. The extracted stem cells are known as human adult germline stem cells (GSC) (Conrad *et al*., 2008).

13. Amniotic Stem Cells

Amniotic stem cells are multipotent and can differentiate in different cell lines. Use of stem cells from amniotic fluid overcomes the ethical objections to using human embryos as a source of cells (De Coppi *et al*., 2007).

14. Cord blood derived Stem Cells

These are a type of multipotent stem cell arising from mammalian cord blood (Hong *et al*., 2005).

15. Induced pluripotent Stem Cells

Induced pluripotent stem cells (also known as iPS cells or iPSCs) are a type of pluripotent stem cell that are generated directly from adult cells. Pluripotent stem cells hold great promise in the field of regenerative medicine. Because they can propagate indefinitely, as well as give rise to every other cell type in the body (such as neurons, heart, pancreatic, and liver cells), they represent a single source of cells that could be used to replace those lost to damage or disease (Yu *et al*., 2007).

Engineering of Complex Oral Tissues

Two main approaches are utilized in this area to produce engineered tissue. First, scaffolding can be used as a cell support device upon which cells are seeded *in vitro*; cells are then encouraged to lay down matrix to produce the foundations of a tissue for transplantation. The second approach involves using the scaffold as a growth factor/drug delivery device. This strategy involves the scaffold being combined with growth factors, so upon implantation, cells from the body are recruited to the scaffold site and form tissue upon and throughout the matrices. These two approaches are not mutually exclusive and can be easily combined.

The manner in which a cell type and scaffolding are combined should be carefully matched for purpose as it has been demonstrated that composition, topography and architecture of scaffolds are able to interact and influence cell behavior. Scaffold architecture has been shown to modify the response of cells and subsequent tissue formation, as demonstrated by the generation of mineralization fronts in specific regions of scaffolds (Ripamonti and Soluble, 2004) Nano to microscale topography has been demonstrated to affect cell behavior by modification of their cytoskeleton arrangements (Meredith *et al*., 2007). Furthermore, different cell types react to different materials; for example, different scaffold materials produce different levels of glycos-amino glycans in tissue engineered cartilage (Freed *et al*., 1993). The source of cells is also an important choice for scaffolds, as is the type of culture (Francioli *et al*., 2007). There are a range of cell types that can now be combined with scaffolds to produce tissue engineered constructs.

Potential Clinical Applications with Review of Clinical Studies

Craniofacial osseous deficiencies can also arise from infection, trauma, congenital malformations and progressive deforming skeletal diseases. Transplantation of a bone marrow stromal cell population that contains skeletal stem cells may provide a

promising alternative approach for reconstruction of craniofacial defects by circumventing many of the limitations of auto and allografting methods. The potential application of stem cells mostly lies in making cells and tissues for medical therapies. Dental stem cells can be an innovative approach to the therapy of dental tissue engineering, treating diseases like periodontitis and dental caries, improving dental pulp healing and in the regeneration of craniofacial bone and teeth. Mesenchymal cells are also known as bone marrow derived stem cells that can differentiate into a variety of cell types. As such, the dental pulp is a rich source of mesenchymal stem cells. These cells are multipotent stromal cells that can differentiate into cells that could be used to repair different tissues and generate bone (Gandia *et al*., 2008; Graziano *et al*., 2008 and Bueno *et al*., 2008).

1. Calvarial Structures

In a study, fat-derived cells were isolated, made to differentiate into osteocytes in osteogenic medium and seeded in poly-L-lactic acid. The results were that a palatally placed bone defect was successfully reconstructed. This experiment demonstrates the feasibility of reconstructing bony defects with fat-derived cells (Conejero *et al*., 2006). Further, a study was designed to evaluate mesenchymal stem cell (MSC)-based alveolar bone regeneration in a canine alveolar saddle defect model. MSCs were loaded onto hydroxyapatite/tricalcium phosphate (HA/TCP) matrices and in the end the conclusion drawn was that autologous and allogenic MSCs have the capacity to regenerate bone within craniofacial defects (Ingeborg *et al*., 2003). Another study, was designed to assess the osteogenic potential and utility of using ASCs to regenerate bone in a rabbit calvarial defect model. As a result, pre implantation osteoinduction of ASCs was seen to enhance the osteogenic capacity. It is assumed that larger defects would likely demonstrate better osteogenic potential (Dudas *et al*., 2006).

There exist several limitations to autologous bone grafts and alloplastic materials. Hence, recognising that new methods of cranioplasties are needed, researchers used autologous adipose derived stem cells seeded in beta tricalcium phosphate granules. The successes of some clinical cases in this regard pave the way for further studies to turn this method into a more reliable treatment regimen (Thesleff *et al*., 2011). Furthermore, there was a 7-year-old girl suffering from widespread severe head injury with multiple bony defects. Autologous ASC's were processed simultaneously and applied to the calvarial defects in a single operative procedure. The postoperative CT-scans were able to demonstrate new bone formation three months after the reconstruction (Lendeckel *et al*., 2004).

2. Alveolar Bone

In a prospective study, adipose derived stem cells were isolated from the subcutaneous fat of lateral thoracic area in four dogs. The undifferentiated stem cells were seeded in Collatamp and transferred into mandibular bone having through and through defects. Similar defects on control groups were filled only with cell free Collatamp. After 6 weeks, biopsies were taken and it was discovered that the former caused more bone to regenerate (Haghighat *et al*., 2011). The defect in a patient, who underwent a hemimaxillectomy due to a large keratocyst, was reconstructed with a microvascular flap using auto ASCs, beta-tricalcium phosphate and bone morphogenetic protein-2. After 8 months of follow-up, the flap had developed mature bone structures and vasculature and was transplanted into the defect area. This was a pioneering clinical case where ectopic bone was produced using autoASCs in microvascular reconstruction surgery and it has, since paved for new clinical trials in the field (Mesimäki *et al*., 2009). The use of tissue-engineered osteogenic material comprising platelet-rich plasma and autologous mesenchymal stem cells was first observed in an alveolar cleft osteoplasty for a young female patient. Within 3 months, regeneration of bone took place and in 6 months there was bridging of the cleft, marking a successful surgery (Hibi *et al*., 2006). In patients that presented with bilateral bone re-absorption of the alveolar ridge distal to the second molar secondary to impaction of the third molar, researchers used a bio-complex constructed from dental pulp stem/ progenitor cells and a collagen sponge scaffold for oral maxilla facial bone tissue repair. Three months after autologous grafting, histological observations clearly demonstrated that the bio-complex used can completely restore human mandible bone defects, indicating that this cell population could be used for the repair and/or regeneration of tissues and organs (Krebsbach *et al*., 2002).

3. Cleft Palate and Cleft Lip

Of all the possible craniofacial defects observed in newborns, perhaps the most well-known defect is the cleft palate. Bone marrow cells were used in order to repair alveolar defects in cleft lip/palate. These cells were isolated from the iliac crest but caused morbidity in the donor. Therefore, in order to identify a new alternative source of stem cells with osteogenic potential without conferring morbidity to the donor, researchers have used orbicular oris muscle (OOM) fragments, which are regularly discarded during surgery repair (cheiloplasty) of CLP patients. After appropriate cell culturing, the cells were able to undergo chondrogenic, adipogenic, osteogenic and skeletal muscle differentiation. It was also demonstrated that these cells combined with a collagen membrane led to bone reconstruction. In conclusion, the study suggests that these cells represent a promising source of stem cells for alveolar bone grafting treatment, particularly in young CLP patients (Bueno *et al*., 2008).

4. TMJ and Condyle

The temporomandibular joint is susceptible to diseases and trauma that may ultimately lead to structural degeneration. Current approaches for replacing degenerated mandibular condyles suffer from deficiencies such as donor site morbidity, immunorejection, implant wear and tear, and pathogen transmission. The hypothesis of a particular study was that a human-shaped mandibular condyle can be tissue-engineered from rat mesenchymal stem cells (MSCs) encapsulated in a biocompatible polymer. Eight weeks following *in vivo* implantation of the bilayered osteochondral constructs, mandibular condyles formed *de novo*. Following the success of this study, the approach is being refined for ultimate therapeutic applications (Alhadlaq and Mao, 2003).

Challenges

While advances in tissue engineering have created a wide variety of approaches, an ideal material is yet to be developed.

A matter of concern is the ultimate morphology of the structure produced. So far, no material has been able to exactly replicate the lost tissue structure. However, most researchers believe that with continuing development in the field of tissue engineering, there would be an improvement in the biomaterial scaffold available for cell-based therapeutics and the proper delivery of bioactive molecules. Another one of the main problems of the therapeutic use of stem cells remains the identification of accessible sites within the human body while collecting an adequate amount of stem cells (Gimble and Guilak, 2003). The surgical access to the collection site remains a limiting point, due to the morbidity of the site itself. Further, homologous stem cells transplantations are known to produce pathogen transmission and need immunosupression. Hence, today the use of an autologous stem cell source is considered ideal (d'Aquino *et al*., 2008).

The second major problem is the nature of the cells collected, linked to their functional properties; cells must be able to undergo extensive proliferation in order to repair macroscopic defects and to represent a therapeutic alternative, but this proliferation ability has to follow a pre-determined and repeated scheme. Lastly, delivery of factors that would stimulate stem cells in situ to initiate a process leading to regeneration rather than scar formation, has long been pursued. But success has been limited owing to problems of dosage, lack of full activity of recombinant factors, and inability to sustain a factor's presence for an appropriate length of time. To overcome these problems, 'gene activated matrices' are being investigated that compromise plasmids coding for factors in a variety of delivery vehicles. Today, stem cell based approaches to tissue reconstruction open unpredicted applicative opportunities. Although this discussion has been limited to systems where extensive preclinical and clinical studies have already been conducted, more exciting avenues are in sight in many areas. But enthusiasm over what unquestionably represents a markedly innovative technique with huge therapeutic potential must be balanced against stringent standards of scientific and clinical investigation. In addition, one must remain aware of the wide range of basic and applied issues associated with each system, with the targeted problems, and with the predicted solutions (Bianco and Robey, 2001).

Conclusion

It is evident from the literature that advancements in the use of stem cells has provided a great deal of incentive to be applied clinically. It can differentiate into adipocytes and skeletal muscle; also reforming bone, cementum, dentin, PDL and the TMJ just to name a few. This cell differentiation is essential for tissue reconstruction of different craniofacial defects. It is clear that the potential application of stem cells does improve bone regeneration but the optimum result would be obtained when using two approaches where scaffolding is utilized as cell support device and as growth factor cells. Therefore, looking at the positive aspect of stem cells, this development in biological sciences needs to be implemented clinically to provide a breakthrough in the treatment modalities of regenerative medicine.

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