Bone Tissue Engineering in the Maxillofacial Region: The State-of-the-Art Practice and Future Prospects

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Bone reconstruction in the maxillofacial region is a challenging task due to the exclusive anatomical complexity of the tissue, aesthetic requirements and functional demands. The gold standard method for maxillofacial reconstruction is based on autogenous bone grafting, which is associated with certain drawbacks. In this review, we describe recent bone tissue engineering approaches in reconstructive surgery of the maxillofacial region. Proper cell sources, scaffolds, signaling molecules as well as recent bioreactor technology are discussed.

Keywords: Bone; Tissue engineering; Maxillofacial injuries

Introduction

The maxillofacial region, consisting of bone, cartilage, soft tissue, nerves, and blood vessels, is a relatively complex tissue. Critical-size segmental bone defects in this region occur as a result of cancer resection, trauma, congenital malformations and progressive skeletal deformity. Reconstruction of maxillofacial bones is complex due to unique aesthetic requirements and functional demands, which include mastication and expression of emotions. Bone defects in the craniomaxillofacial skeleton have complex three-dimensional (3D) structural characteristics (Figure 1), which make their restoration difficult to achieve. Resection of a tumor mass in the mandible has a marked impact on facial appearance, function and general welfare of patients. Facial bone defects and their associated complex neurovascular structures in the region present a major challenge to achieve a satisfactory reconstruction.

Several methods have been used for bone reconstruction in the maxillofacial region, which include autogenous bone grafts, allografts, alloplasts and xenografts (1). Autogenous bone grafts are considered the gold standard to which other materials are compared. However, autogenous bone grafts are associated with certain disadvantages such as limited availability for the reconstruction of large defects, difficulty obtaining the required shape and donor site morbidity (2). Autogenous bone graft induces osteoconduction, osteoinduction and osteogenesis. Osteoconduction occurs when the bone graft material acts as a template for new bone growth perpetuated by the native bone (3). Osteoinduction involves stimulating osteoprogenitor cells to differentiate to osteoblasts, which then begin new bone formation. The most widely studied osteoinductive cell mediators are bone morphogenetic proteins (BMPs), which can be found in large concentrations in cortical bone (4). Osteogenesis occurs when vital osteoblasts originating from the bone graft material contribute to new bone growth. Although autologous bone grafts have long been considered the gold standard treatment for large segmental bone defects, there are three major limitations with the use of an autogenous free bone graft namely poor osseointegration and excessive resorption when the defect is larger than 6 to 9 cm^3, insufficient blood supply to the graft and the surrounding tissues due to irradiation scarring and infection, and donor site morbidity (5).

Specifically, autogenous bone grafts and bone marrow components have been used for facial bone reconstruction. However, they have drawbacks such as limited availability and the reported morbidity associated with harvesting of bone grafts. Biomaterials, despite their availability, have a high failure rate due to poor vascularity, limited mechanical properties and deficiency in complete osseointegration with the surrounding native bone. These limitations have inspired a search for innovative techniques for bone bioengineering and developing more reliable biomaterials. Alternative options include bone allografts from cadavers, which are also associated with some disadvantages (6).

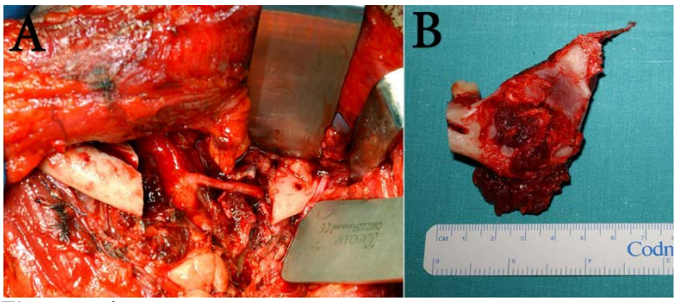

Figure 1. (A) A residual continuity defect following the resection of a mandibular tumor for reconstruction; (B) The shape of the resected segment that requires replacement.

Tissue engineering approaches that deliver osteoconductive scaffolds, osteoprogenitor cells and growth factors directly into the bone defects hold great potential for achieving optimal bone healing in difficult cases while eliminating the drawbacks associated with the conventional treatments. Tissue engineering approaches provide powerful tools to achieve long-term satisfactory results enabling customized reconstruction with the support of natural healing processes. Undoubtedly, further advances in tissue engineering are essential to achieve reliable and satisfactory clinical results (7).

Herein, we highlight the recent advances in tissue engineering in an attempt to overcome the clinical challenges in reconstructive surgery of the maxillofacial region.

Stem cells in maxillofacial bone tissue engineering

The cells are among the key components of bone tissue engineering. Proper cell sources for bone regeneration are either differentiated bone cells or pluri/multi potent cells holding the capacity of osteogenic differentiation (8, 9) (Figure 2).

Adult mesenchymal stem cells (MSCs) possess the capacity of self-renewal and the potential for multi-lineage differentiation; therefore, they serve as a suitable source of stem cells for bone tissue engineering. These cells can be isolated from the bone marrow, adipose tissue, umbilical cord blood, dental pulp, etc.; MSCs from these sources have different characteristics and diverse proliferation and differentiation capacities (8, 10). Although clinical applications might be based on their differentiation capacity, it is more dependent on their abundance, frequency, and expansion potential (11). Moreover, MSCs are proven to be immuno-privileged cells (12). They may be available for cell replacement therapy in HLA-incompatible hosts before and after osteogenic differentiation *in vitro*. Several studies have shown adequate engraftment of MSCs after their allogeneic and even xenogenic transplantation *in vivo* (13). It is well-known that MSCs are capable of forming at least 3 cell lineages: osteogenic, chondrogenic and adipogenic. Other lineages such as myogenic, neurogenic and tenogenic may be derived from MSCs as well.

Bone marrow derived mesenchymal stem cells (BMSCs), which are also known as bone marrow derived stromal cells, are the most well-known source of MSCs for use in bone tissue engineering, which can be easily isolated from the iliac crest aspirates, core biopsies and surgical waste (14). Lee *et al.*, reported successful reconstruction of a 15 cm segmental mandibular defect with BMSCs. In their human trial, a central hemangioma was diagnosed in a 14-year-old male patient. The resected jaw was freeze-dried to 7.6×10^{-6} mmHg for 48 hours and was then perforated using surgical burs. Autogenous BMSCs were aspirated, isolated and cultured *in vitro*. They were repositioned in the mandibular defect area. One year post-operatively, the mandible demonstrated excellent clinical and radiographic evidence of bone regeneration (15).

Interestingly, researchers have found a difference between MSCs from long bones and MSCs from the mandible. Human mandibular or maxillary bone marrow stromal cells demonstrate greater cell proliferation, delayed senescence and stronger expression of osteoblastic markers compared to iliac crest-derived marrow cells from the same patients (16). This suggests distinct functions, differentiation potential and osteogenic potential of mandibular vs. long-bone marrow stromal cells. Culture of MSCs of the mandible form more colonies, suggesting a larger colony forming unit (CFU) population (17).

Adipose tissue (AT) is known as one of the richest sources of MSCs (18). Although there is evidence that AT-MSCs are not able to support formation of hematopoietic marrow, they are accepted as a promising source of stem cells in bone tissue engineering, since they have proven to have the capacity of osteogenic differentiation (19, 20). In addition, given the well-documented ability to yield larger numbers of MSCs under local anesthesia, AT may provide a more efficient source of MSCs for research and clinical applications with decreased patient morbidity during cell harvesting (21). Therefore, AT-MSCs could be a good resource and an alternative to BMSCs. Cowan *et al.*, showed that their harvesting technique usually yields ~800 mg of subcutaneous fat tissue and 0.6 mg bone marrow per mouse and therefore, the yield of cells is much higher from fat than from bone marrow. They also described that the proliferation rate of AT-MSCs was substantially higher than that of BMSCs during subsequent *in vitro* expansion. Because AT-MSCs proliferate rapidly in culture, populations can readily reach the high levels needed for clinical application (22).

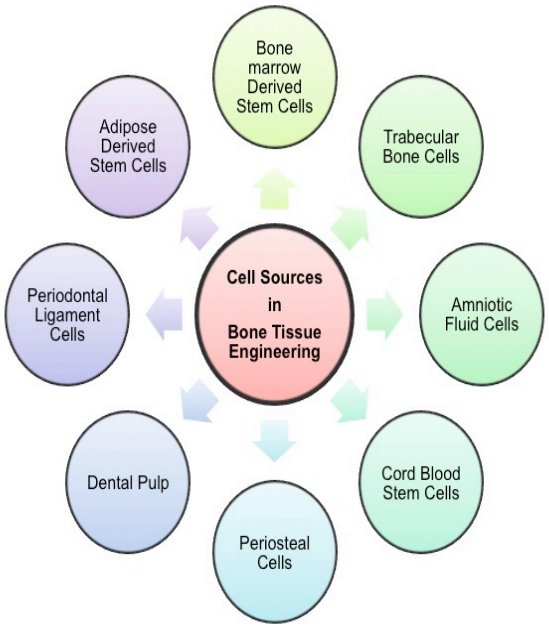

Figure 2. Cell sources for bone tissue engineering

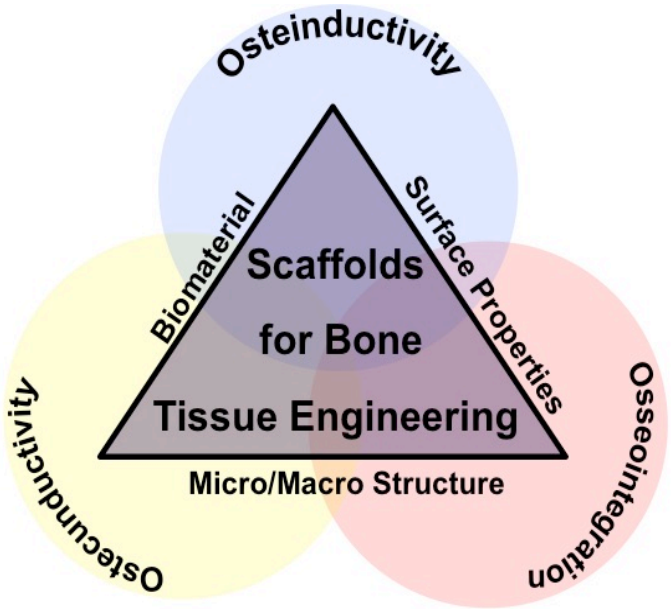

Figure 3. Biomaterials and scaffolds for bone tissue engineering

Recent clinical studies have focused on the use of human AT-MSCs (23). The cells were utilized to replace the lost bone in critical-size calvarial defects in a rat model (24). Some clinical reports focusing on AT-MSCs in the reconstruction of the cranium, maxilla and mandible showed variable rates of success (25). To date, there is a paucity of data defining the mechanisms through which AT-MSCs influence an osseous defect. Whether or not AT-MSCs directly form bone or function as efficient 'factories' to produce potent pro-osteogenic cytokines remains unknown. The reconstruction of large mandibular defects after tumor resection using AT-MSCs, BMP-2, and β-tricalcium phosphate (β-TCP) scaffold combined with computer-aided manufacturing technique has been reported (26). Three patients with recurrent ameloblastomas requiring segmental mandibular resection were included in a study. The reconstructions of the three mandibular defects were successful in bridging the large defects averaging 8.2 cm. Also, the authors reported successful use of AT-MSCs tissue engineered construct to treat a large anterior mandibular defect (27).

Scaffolds and biomaterials in maxillofacial bone tissue engineering

In critical size bone defects, one of the major considerations is to bridge the physical gap. Scaffolds, as a porous structure, should ideally mimic the extracellular environment to encourage cells to attach, migrate, proliferate, differentiate and finally to be replaced with the natural tissue including cells and extracellular matrix (28). Although there are a number of scaffold-free-cell-based therapies, without scaffolding it is hard to imagine that cells could assume aggregate function, induce vascularization, and build a higher-order 3D structure (29). Scaffolds have three features namely material, architecture (micro/macro structure) and surface properties (Figure 3).

Bone is known as a mineralized tissue containing high amount of calcium phosphate. Therefore, not surprisingly, the initial bone substitute materials were ceramic materials. Although in the past bone substitute materials were designed to be bio-inert, later the paradigm shifted towards the design of bioactive materials that integrated with biological cytokines and cells and regenerated tissues (30). Ideally, biomaterials for bone tissue engineering applications should be both osteoinductive and osteoconductive with the capacity of osseointegration (31). Therefore, these materials should rather encourage the differentiation of progenitor cells to osteoblastic lineage, support bone growth, stimulate the in-growth of the surrounding bone and finally, be able to integrate into the neighboring bone tissue. Materials for bone scaffolding can be generally categorized into bioactive inorganic materials, polymers and composites.

Most ceramics used for the reconstruction of oral and maxillofacial region are either preformed blocks, granules of porous hydroxyapatite (HA), TCP or setting cement commonly called biphasic calcium phosphate (BCP). These brittle materials have limited tensile strength (32).

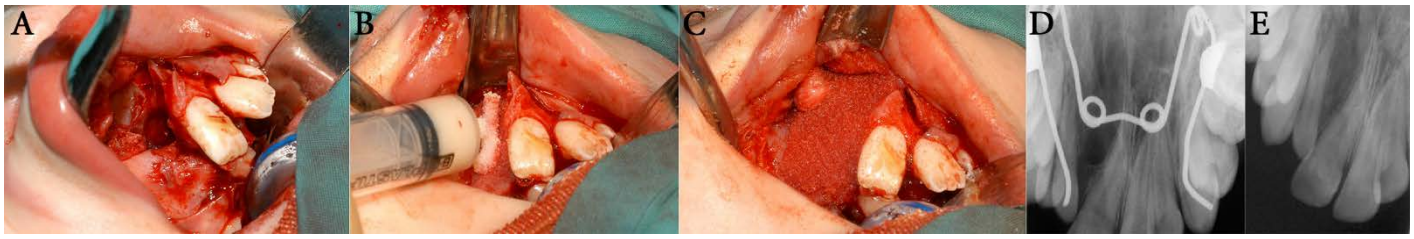

Figure 4. (A) Alveolar cleft; (B) Injection of rh-BMP-7 (Op-1) at the bony defect; (C) Adaptation of Op-1 to fill the defect before closure of the mucoperiosteal flap; (D) Immediate post-operative occlusal radiograph showing an alveolar cleft; (E) Three-month post-operative occlusal radiograph of the same case showing bone formation and eruption of the associated tooth

Bioactive glass is a combination of a silica-based material with a biocompatible material such as calcium phosphate, forming a bond between an implant and host tissue. This material has been used as a filler of bone cavities in the cranio-maxillofacial region ,i.e. frontal sinus obliteration. However, it is not suitable for the reconstruction of continuity defects of the jawbone due to the lack of mechanical properties (33).

Porous polyethylene has been used for the reconstruction of cranio-maxillofacial defects. This material is a dense porous polyethylene with a pore size of 100 to 250 μm. Bioresorbable plates and screws made of polylactic acid have been successfully used for paediatric craniofacial reconstructive procedures including the release of craniosynostoses and reconstruction of cranial defects (34).

Signalling for osteogenesis and bone remodelling

In bone tissue engineering, it is necessary to provide the physical and chemical environments required to induce bone remodelling. Bioactive molecules, mechanical loads and electromagnetic fields are famous stimuli for bone healing and remodelling since they trigger several molecular cascades in cells and the surrounding tissues.

Specifically, bioactive molecules including BMPs and transforming growth factor beta (TGF-β) are responsible for osteogenic differentiation and acceleration of extra-cellular matrix production and consequently tissue integration (35). The BMPs can attract mesenchymal progenitor cells and act as chemotactic mitogenic and differentiating agents to induce chondrogenic and osteogenic differentiation. This fact implies that BMPs are essential signaling molecules for intramembranous and endochondral bone formation. In maxillofacial reconstruction, BMP-2 and BMP-7 have been principally studied for clinical applications and use in combination with collagen/collagen composite scaffolds (36). To date, reconstruction of 4 to 8 cm mandibular defects and cleft alveolus as well as maxillary sinus augmentation have been successfully performed using BMP-2 or BMP-7 combined with collagen scaffolds (37). In 2001, US Food and Drug Administration (FDA) approved the application of BMPs for sinus augmentation and alveolar ridge augmentation associated with extraction sockets. The BMP-2 and 7 are now commercially available for clinical use. Recently, *Ayoub et al.*, demonstrated the successful clinical application of rhBMP-7 (Op-1) for reconstruction of alveolar cleft (Figure 4). This is the first application of this cytokine for the reconstruction of critical-size defects in the maxillofacial region (38).

It has been well demonstrated that bone cells respond to mechanical loads both *in vivo* and *in vitro*. In bone tissue engineering, it is promising to take advantage of such mechanical responses to stimulate matrix production and induce osteogenic differentiation (39). Also, cells can sense mechanical properties of the scaffold or surface and react. Surfaces with higher stiffness or Young's modulus can encourage mesenchymal stem cells to differentiate to bone lineage. It has been demonstrated that mechanical stress increases alkaline phosphatase activity, a marker of osteoblast differentiation, increases the expression of osteocalcin, which is an osteoblast-specific extracellular matrix (ECM) protein, induces runt-related transcription factor 2 (Runx2) activation and increases the expression of osterix in osteoblast-like MC3T3-E1 cells (40). Ultrasound is a method to generate mechanical stimulation for clinical application. Ultrasound treatments are successfully used for acceleration of osseointegration of metal biomaterials in osteoporotic patients. Moreover, these treatments have shown to diminish the healing period of fresh fractures of the extremities by up to 38%, and to heal delayed and non-unions by up to 90% and 83%, respectively (41). This implies that mechanical stimulation has a promising future in oral and maxillofacial surgery.

Although electrical stimulation (ES) is effective in rehabilitation of nerve and muscle tissues, it has been successfully applied clinically to stimulate osteogenesis in bone defects for more than 40 years (42). Several common modes of

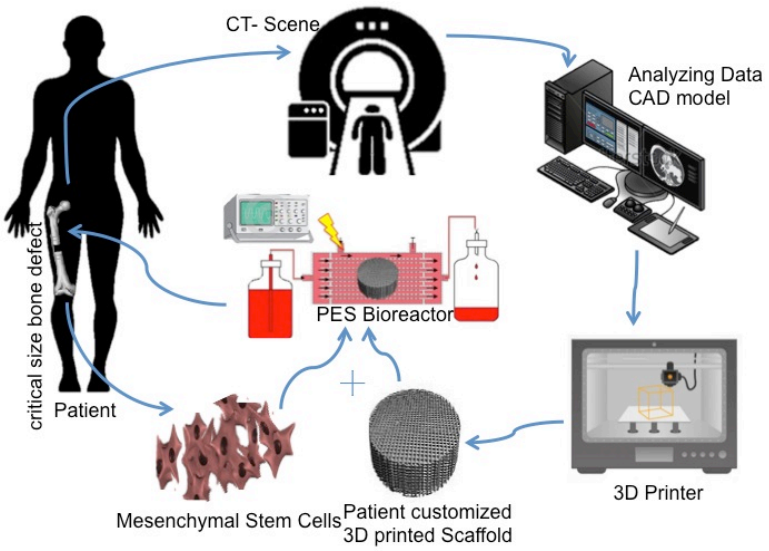

Figure 5. The future of bone bioengineering

electrical stimulation, such as pulsed electromagnetic field (PEMF), capacitive coupling (CC) and direct current (DC), have been used both experimentally and clinically to stimulate bone healing. Due to the piezoelectric properties of bone tissue, (43) rising the amount and speed of osteogenic differentiation by means of ES could be expected. Recent researches show that electrical stimulation can influence the expression of osteogenic marker genes in MSCs and greatly enhance alkaline phosphatase expression (44, 45). An *in vivo* study demonstrated that, electrical stimulation during mandibular lengthening accelerates the formation of new bone in rabbit model (46). Electrically stimulated periosteum grafting to a 12-year-old female patient who had a segmental mandibulectomy was successfully applied for mandibular reconstruction (47). These efforts suggest that electrical stimulation could be a promising tool for bone tissue engineering, particularly in the maxillofacial region.

Bioreactors in bone tissue engineering

Bioreactors are described as devices in which biological and biochemical processes are established under tightly controlled environment. Bioreactors enable the development of 3D tissues by providing biochemical (growth factors, proteins, etc.) and physical (*e.g.* oxygen level, mechanical stress, electrical stimulation, etc.) regulatory signals to cells and encouraging them to proliferate, differentiate and/or produce extracellular matrix (48). Originally, the goal behind developing most bioreactors was to test biomaterials by mimicking *in vivo* conditions; however, some of them were later invented with the goal of 3D *ex vivo* tissue development. Bioreactors have come a long way from simple cell culture dishes to complex co-culture, perfusion, electro-/magnetic-/mechanical-stimulation systems. Recently developed bioreactor systems, invented to improve cell survival in scaled-up scaffolds (in terms of size and number), reach a new step of complexity in tissue engineering techniques (49).

Bioreactors have been widely researched for bone tissue engineering applications. Particularly, the most important outcomes of the usage of bioreactors are enhanced mineralized matrix formation and osteoblastic differentiation (50). From a technical point of view, bioreactors can be classified into two main types: rotating wall vessels/spinner flasks and perfusion systems. These systems can be equipped with additional stimulation systems, which can provide magnetic, electrical and/or mechanical stimulations.

In the context of bone tissue engineering, it is essential to note that bone tissue requires control of mechanical conditions. Spinner flask as a simple bioreactor system is designed to mix the culture medium by means of a stirrer, while scaffolds are secured from the top. Spinner flasks are often used in the culture of cells for bone tissue engineering. They have been shown to increase expression of osteoblastic markers in comparison with static culture and rotating vessel bioreactors. However, spinner flasks and rotating vessel bioreactors are not able to efficiently perfuse media into a 3D scaffold. Bioreactors, in which pump systems perfuse media directly through a scaffold, are known as perfusion bioreactors. These bioreactors not only circulate culture medium and control nutrition, pH and temperature conditions, but also

cause the exposure of cells to fluid shear stress and provide mechanical stimulation, which is evidenced to be effective in osteogenic differentiation (51). Recently, presence of electric fields in bioreactors has attracted considerable attention. It has been shown that electrical fields have distinct effects on osteoblast lineage as they increase mineral formation (52). In this respect, the application of electrical fields in bioreactors seems to be a promising methodology in bone tissue engineering.

Future outlook

Tissue bioengineering is emerging to address a broad spectrum of clinical needs. However, it is still in its infancy. The sophistication and the range of human tissue that can be generated will increase dramatically in the future. There is a great deal of excitement in the clinical and academic circles to develop a reliable bone substitute with satisfactory mechanical properties for maxillofacial reconstruction capable to promote osteoinduction, osteoconduction and osteogenesis at the surgical site. Because of the pluripotency of stem cells they continue to be a major area of development. Technical industrial advances, on the other hand, are underway to improve the quality of tissue engineered products and their safety. This multidisciplinary approach for bone bioengineering will have a significant impact on the quality of service delivered to patients (Figure 5).

Conflict of Interest: 'None declared'.

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