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Chapter 6

Oral and Maxillofacial Tissue Engineering with Adipose-Derived Stem Cells

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1. Introduction

Oral and maxillofacial tissues are a complex array of bone, cartilage, soft tissue, nerves and vasculature. Damage to these structures, even when minimal, usually leads to noticeable deformities. Therefore, the repair of large segmental bone defects of the jaw or mandible due to trauma, inflammation, or tumor surgery remains a major clinical problem. For many years, simple autogenic, allogenic, or xenogenic bone grafts, or combinations thereof, have been the mainstay for tissue replacement [1]. However, when large bone defects are present, advanced approaches such as free tissue transfer with microvascular reanastomosis of vascularized flaps from distant sites including the fibula, iliac crest, scapula, and radius are needed to repair or regenerate a functionally complex tissue such as maxillofacial tissue [2, 3]. While these procedures have proven to be reliable and effective, they require extended hospitalization, and a secondary donor site with the associated morbidity and complications. As an alternative to current surgical techniques or approaches, developments in tissue engineering using the gene therapy and stem cell biology strive to utilize cells, biomaterial scaffolds and cell signaling factors to regenerate large oral and maxillofacial tissues defect with precise replication of normal body contours. A tissue engineering approach offers several potential benefits, including a decrease in donor site morbidity, a decrease in technical sensitivity of the repair, and the ability to closely mimic the in vivo microenvironment in an attempt to recapitulate normal craniofacial development [1].

Mesenchymal stem cells (MSCs) derived from bone marrow have been used experimentally for tissue engineering applications [4-6]. MSCs can differentiate into several different cell types, such as those that produce bone, cartilage, tendon, and other connective tissues, as well as muscle, adipose, and dermal cells [7-10]. MSCs can be expanded in culture while maintaining their multipotency.

The concept of prefabricated bone engineering with MSCs for large bone defects may play a pivotal role in future therapies. However, bone marrow-derived MSCs have been reported to require selective sera lots and growth factor supplements for culture expansion [11]. Furthermore, traditional bone marrow procurement, particularly in volumes larger than a few milliliters may be painful, frequently requiring general or spinal anesthesia [12-14].

Bone marrow tissue provides the most universal and attractive source of MSCs; however, other tissues such as periosteal [15], muscle [16], synovial membrane [17] and adipose [18-20] tissues also appear to possess MSCs. Particularly, adipose tissue is an important source of stem cells because subcutaneous adipose tissue is an abundant and accessible source of both uncultured stromal vascular fraction (SVF) cells and cultured homogeneous adipose-derived stem cells (ASCs) (21). ASCs obtained from lipoaspirates have multilineage potential and will differentiate into adipogenic, chondrogenic, myogenic, osteogenic, and neurogenic cells [19, 22, 23]. Thus, ASCs have great potential for clinical applications such as the repair of damaged tissues and angiogenic therapy. Injection of human ASCs was recently shown to improve neovascularization in an ischemic hindlimb mouse model and osteoid matrix formation in immunotolerant mice [24-26]. Further, ASCs have been shown to increase the functional capacity of damaged skeletal muscle in vivo [27]. Therefore, these reports suggest that ASCs may also have the potential for use in large bone tissue engineering techniques such as prefabrication. Recently, prefabricated bone engineered with ASCs was reported both with in vivo studies in rat and a clinical human case. Thus, the use of ASCs in maxillofacial tissue reconstruction should be viewed favorably and these novel approaches may have advantages for tissue reconstruction.

In this chapter, the current approaches and the biomaterials used for repair of large bone defects are presented, and the novel approach of prefabricated bone engineering with MSCs and ASCs is introduced.

2. Current therapy for large bone reconstruction

Bone tissue is composed of heterogeneous cell types embedded in a three-dimensional mineralized extracellular matrix. The scaffolds for repair of large bone defects, including autogenous bone grafts or biomaterials, must provide the necessary support for cells to proliferate while maintaining their potential to differentiate, and must possess an architecture suitable for matching the final shape of the newly formed bone [28].

2.1. Autologous bone reconstruction

The current standard of care for repair of critical large bone defects consists of autogenous bone grafting using bone from the rib or iliac crest of the patient. An autologous bone graft is still the ideal material for the repair of craniofacial defects; however, the availability of autologous bone is limited and harvesting can be associated with complications [29]. Vascularized and avascular autogenous bone has a greater osteogenic capacity than any other bone replacement material, as revascularization attracts mesenchymal

differentiation into osteogenic, chondrogenic and other cell types. Autogenous bone transplants possess an inherent biocompatibility and are therefore more easily incorporated without immunogenic responses [30]. However, the clinical use of autologous bone transplants is limited by considerable donor site morbidity, which increases with the amount of harvested bone. Bleeding, hematomas, infections, and chronic pain are common complications of autologous bone graft harvests [31, 32].

2.2. Allogenic/Xenogenic bone reconstruction

Demineralized bone matrix (DMB) is the de-cellularized and organic component of bone, and is a commercially available osteoinductive and osteoconductive biomaterial. DMB represents a concentrated source of bone morphogenetic proteins (BMPs) and has been used in numerous animals systems since its initial description in 1965 [33]. The widespread use of DMB in humans still remains restricted since the immunologic properties of donor DMB are unknown [34].

With the disadvantages of host morbidity and the limits in suitable harvesting sites and material for autologous grafts, the use of xenografts might be considered for large bone reconstructions, although the histocompatibility issues between the human recipient and animal donor preclude the use of bone xenografts [34]. However, bovine-derived DMB is currently used in oral and maxillofacial surgery [35].

2.3. Synthetic scaffolds for bone reconstruction

A wide variety of synthetic (alloplastic) scaffolds such as ceramics and polymers are used clinically for bone grafting [30]. Ceramics are crystalline, inorganic, nonmetallic minerals that are held together by ionic bonds and usually densified by sintering [36]. Ceramics such as hydroxyapatite and β-tricalcium phosphate (TCP) are currently in use clinically for bone tissue regeneration of large bone defects.

Various synthetic polymer scaffolds exhibit different structural, mechanical and degradation properties that make then suitable for bone tissue engineering [36]. Blending polymers of different molecular weights can achieve both optimal degradation rates and mechanical properties [37]. Some synthetic polymer scaffolds such as polycaprolactone (PCL) scaffold, polylactic acid (PLLA), polyglycolic acid (PGA) and polylactic-co-glycolic acid (PLGA) materials have been approved by the FDA for craniofacial applications or as absorbable sutures and bone pins/screws [36].

2.4. Gene therapy for bone reconstruction

The use of exogenous cytokines and growth factors, which are essential for bone regeneration, promotes cell adhesion, proliferation, migration and osteogenic differentiation [28]. Growth factors such as BMPs, fibroblast growth factors (FGFs), insulin-like growth factors (IGF), vascular endothelial growth factors (VEGF) and platelet-derived growth factors (PDGF) have been used in bone regeneration [28, 36].

Recently the use of combinations of growth factors, such as BMP-2 and NEL-like molecule-1 (NELL-1), was tested in rapid distraction osteogenesis in a rabbit model. The combined

treatment produced significantly greater bone healing compared to single growth factor treatments after four weeks of treatment [38]. However, some reports have cautioned that the clinical use of BMPs and VEGF is in its infancy, and some risks may accompany their use. VEGF is commonly upregulated in various types of tumors to enhance their vascularization, and subcutaneous sarcomas were found in some rats administered recombinant human BMP-7 [39, 40], although no clinical relationship has been established between the use of these growth factors and tumor formation.

2.5. Prefabricated bone engineering for oral and maxillofacial tissue reconstruction

Prefabrication is an interesting area of oral and maxillofacial surgery and plastic and reconstructive surgery, because it represents a bridge between conventional reconstructive surgery and tissue engineering [41, 42]. The purpose of prefabrication is to build a tissue (muscle, bone, skin, or composite) with characteristics as similar as possible to those of the defect that is to be repaired [43]. Conventional osteomyocutaneous flaps do not always meet the requirements for repairing a composite defect. A prefabricated composite flap can be created according to the complex geometry of the defect. Prefabrication of multi-component flaps is a well established procedure in plastic and reconstructive surgery [41]. This concept is based on the revascularization phenomenon directly related to host tissue vascularity [44] and has significantly expanded the frontiers of reconstructive surgery.

Hirase et al. were the first to report the use of prefabricated myocutaneous and osteomyocutaneous tissue in a rat model [45]. Flap prefabrication using conventional bone grafts allows for generation of new types of flaps independent of the vascular anatomy of the bone transplant. However, the donor site morbidity after harvesting of bone for grafting is still a problem. Recently, biomaterials, osteogenic cells and osteoinductive growth factors have been used for generation of vascularized bone tissues in combination with a vascular axis or vascularized flaps. An inflammatory wound healing response as a reaction to the surgical implantation induces vascularization of the scaffolds [31]. Induction of axial vascularization protected the porous biomaterials from bacterial infection and transfer of this vascularized hard tissue as a free flap has been demonstrated [46]. Prefabricated vascularized bone grafts have been used in a clinical setting for mandibular reconstruction following thorough in vivo evaluation in a pig model [47-49]. In these studies, granules of xenogenic bone minerals soaked with recombinant Osteogenic protein-1 were implanted into the latissimus dorsi muscle and the neo-tissue was subsequently transferred to sites of mandibular defects using microsurgical techniques.

3. Mesenchymal stem cells for oral and maxillofacial tissue reconstruction

3.1. Mesenchymal stem cells for bone engineering

The bone marrow is not only the site where hematopoiesis occurs in postnatal life, it is also a reservoir of pluripotent stem cells for mesenchymal tissues [50]. Plated at low densities, single precursor cells derived from bone marrow, and referred to as colony-forming units, give rise

to distinct and heterogeneous colonies. These colonies have been shown to undergo osteogenic, chondrogenic and adipogenic differentiation [51].

Chang and colleagues showed that MSCs can produce ectopic bone generation in a mouse model [52]. A suspension of osteogenically induced MSCs was added to 2% alginate, which was then gelled by mixing with calcium sulfate. The gel was injected subcutaneously on the dorsal side of the experimental animals. Histological examination of the implants revealed signs of endochondrosis with woven bone deposition. The equilibrium modulus of the newly formed bone increased with time up to 678 kPa at 30 weeks, as determined by biomechanical analysis. This value is approximately 1.62% of native bovine cancellous bone. In another study [53] of large mandibular bone defect repair, dog MSCs cultured with ß-TCP to generate osteogenic cells were co-implanted with a titanium plate into a 30 mm segmental mandible defect. Biomechanical tests showed a significant difference between the experimental group (with cells) and the control group (without cells), highlighting the importance of the MSCs in bone formation. Pedicled bone flaps based on collagen I scaffolds, bone marrow stromal cells and a PTFE membrane have been successfully generated using the carotid artery and jugular vein or the saphenous bundle as a vascular axis in a mouse model [54]. The osteogenetic stimulus was supplied by the injection of mouse MSCs cultured in osteogenic medium inside the space delimited by the PTFE membrane. After only 4 weeks islands of bone tissue were present inside the membrane.

3.2. Clinical trials for bone engineering with mesenchymal stem cells

There is some clinical experience with bone reconstruction using expanded MSCs combined with scaffolds. Constructs of expanded autologous MSCs in macroporous hydroxyapatite were used in three patients with large segmental bone defects [55, 56].

Warnke and Terheyden have developed a two stage procedure for mandible reconstruction in humans [57]. This study used prefabrication in the latissimus dorsi muscle with the aim of reconstructing a 70 mm defect in the mandible of a man who underwent a tumor resection years previously. The entire construction of the mandible was built using blocks of Bio-Oss® and MSCs that had been cultured in the presence of BMP-7. The Bio-Oss® and MSCs were placed in a titanium cage, and implanted into the latissimus dorsi of the patient and maintained in situ for 7 weeks. Subsequently, this unit, together with the vascular bundle that supplied it, was removed and re-implanted in the mandible defect by fixation with titanium plates and microvascular sutures connecting the vasculature to the external carotid artery and the cephalic vein.

4. Adipose-derived stem cells for oral and maxillofacial tissue engineering

4.1. Characterization of adipose-derived stem cells

There is a general consensus that SVF cells are a heterogeneous population, and no specific ranges for each subpopulation have been agreed upon formally [21]. In contrast, the Interna-

tional Society for Cell Therapy has provided guidelines for the definition of MSCs, as follows. (1) MSCs must be plastic-adherent when maintained in standard culture conditions. (2) MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface antigens. (3) MSCs must differentiate into osteoblasts, adipocytes and chondroblasts in vitro [58].

SVF cells include preadipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells (ECs), resident monocytes/macrophages, lymphocytes and ASCs [59]. Although the criteria to define SVF cells remain in contention, the heterogeneous SVF cell population includes putative ASCs (CD31-, CD34+/-, CD45-, CD90+, CD105-, CD146-), endothelial (progenitor) cells (CD31+, CD34+, CD45-, CD90+, CD105-, CD146+), vascular smooth muscle cells or pericytes (CD31-, CD34+/-, CD45-, CD90+, CD105-, CD146+), and hematopoietic cells (CD45+) in uncultured conditions [60]. Cultured ASCs show an extensive proliferative ability in an uncommitted state while retaining their multilineage differentiation potential. ASCs express the mesenchymal stem cell markers CD10, CD13, CD29, CD34, CD44, CD54, CD71, CD90, CD105, CD106, CD117, CD166 and STRO-1. They are negative for the hematopoietic lineage markers CD45, CD14, CD16, CD56, CD61, CD62E, CD104, and CD106 and for the EC markers CD31, CD144, and von Willebrand factor [20, 61, 62]. Morphologically, cultured ASCs are fibroblast-like and preserve their shape after expansion in vitro [20, 63]. The ASC specific surface markers CD29, CD90, and CD166 increase during culture [64]. In later passages, ASC cultures are homogeneous and exhibit fibroblastoid morphology. The composition of the subpopulations, therefore, may change during expansion [65, 66]. Therefore ASCs match the standard criteria for MSCs.

4.2. Differentiation potential of osteogenic cells in vitro and in vivo

Numerous studies have presented results that clearly show that ASCs can differentiate into osteoblasts [20, 59, 63, 67, 68]. ASCs exhibit a time-dependent expression of genes and proteins associated with the osteoblast phenotype, including ALP, Type I Collagen, OPN, ON, RUNX2, BMP-2, BMP-4 and BMP receptors I and II [20, 67, 69, 70]. Additionally, between 2 and 4 weeks of culture, mineralization of the extracellular matrix begins and proceeds via the activity of ALP, an enzyme that hydrolyzes phosphate esters making available inorganic phosphate to form hydroxyapatite [19, 20, 71].

Furthermore, recent reports have shown that ASCs co-cultured with ECs exhibit enhanced osteogenesis [72, 73]. ASCs exhibited increased secretion of alkaline phosphatase and osteocalcin, and an overall increase in osteogenesis in the co-cultured situation compare with other experimental groups. These interactions may be important to regenerate bone in large bone defects since angiogenesis plays a key role in regeneration of large amounts of tissue.

4.3. Fabricated bone engineering with adipose-derived stem cells

To make a functional prefabricated bone, three elements are required: scaffolds to provide a three-dimensional support, growth factors to stimulate neovascularization, and MSCs to give an osteoinductive stimulus. Okuda et al. have reported prefabrication of tissue engineered bone grafts using ASCs in a rat model [74]. ASCs and porous β-TCP as scaffold material were

implanted into the superficial inferior epigastric artery flap. After prefabrication for eight weeks, the prefabricated flaps were elevated and the pedicles were clamped for 4 h; prefabricated tissue was harvested two weeks later. The osteogenic capacity of the prefabricated graft was not significantly different from non-prefabricated grafts examined after two weeks in a rat model. Furthermore, an analysis of angiogenesis suggested that the prefabricated model possessed significantly greater capillary density than the non-prefabricated model.

Recently, repair of a large bony defect using ASCs was clinically reported [75-77] (Table. 1). Mesima°ki and colleagues published a clinical case report of prefabricated bone tissue engineering [77]. The large bony defect was reconstructed with a microvascular flap using autologous ASCs, β-TCP and BMP-2, 36 months after a hemimaxillectomy due to a large keratocyst. After expansion of ASCs and cultivation with β-TCP and BMP-2 in vitro, a titanium cage filled with ASCs and β-TCP was inserted through a vertical incision into a pouch prepared in the patient's left rectus abdominis muscle. The rectus abdominis free flap was raised. Before severing the vascular supply to the muscle, the muscle pouch was carefully opened and the titanium cage was opened. After severing the vessels, the flap was placed in the maxillary defect; the inferior epigastric artery was anastomosed end-to-end to the facial artery and the vein end-to-end to the facial vein.

Clinical reports/trials with ASCs	Design	Results	Ref
Widespread calvarial defect	Autologous SVFs with fibrin glue	Success, follow-up: 3 months after operation	[75]
Large calvarial defect	Implant autologous cultured ASCs with β-TCP	No complications, follow-up: 3 months after operations	[76]
Maxillary reconstruction	Fabricated bone tissue using autologous cultured ASCs with β-TCP and BMP-2	Success, follow-up: 8 months after operations	[77]
Large osseous defect	Autologous ASCs with different scaffolds	Recruiting	NCT01218945 *
Avascular Necrosis of the Femoral Head	Autologous adipose tissue derived MSCs transplantation	Phase 1, 2	NCT01532076 *

Abbreviations: SVF; Stromal Vascular Fraction, ASCs; Adipose-derived Stem Cells, β-TCP; Beta-tricalcium phosphate, MSCs; mesenchymal stem cells, BMP; bone morphogenetic protein

(*Identifier on Clinical trials website: *http://clinicaltrials.gov/ct2/results?term=adipose+derived+cells+bone).

Table 1. Clinical reports/trials for large bony defect using adipose-derived stem cells

5. Future perspective

In the past decade, basic research characterizing ASCs shows that these cells have the potential to regenerate tissue defects such as large bone defects, and clinical studies have examined the potential use of ASCs to reconstruct oral and maxillofacial tissue. Although clinical studies have only just begun, the use of ASCs in the clinical setting is extremely promising because ASCs are a readily available, multipotent, and abundant cell type with the capability to undergo robust osteogenesis. However, further studies, including research to determine the mechanism of osteogenic differentiation and studies to evaluate the safety of ASC usage, will be necessary to realize the potential of ASCs in clinical regenerative medicine of the future.

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