TISSUE ENGINEERING IN MAXILLOFACIAL BONE RECONSTRUCTION

David B. Kamadjaja[1], Purwati[1],Ferdiansyah[1], Fedik Abdul Rantam[1]

[1]Stem Cell Research and Development Center, Universitas Airlangga Surabaya, East Java, Indonesia

ABSTRACT

Maxillofacial bone defects due to tumor resection, trauma or infections should be reconstructed to maintain the bone continuity in order to preserve its masticatory, speech and esthetic functions. Autogenous bone graft have been the gold standard for mandibular defects reconstruction, however, it is associated with limitation in volume and availability as well as the donor site morbidities. Tissue engineering approach has been proved to be a good alternative to overcome the limitation of autogenous bone graft. Tissue engineering studies have been conducted combining various sources of mesenchymal stem cell, scaffolds, and or signaling molecules. The paper aims to provide information on the development of bone tissue engineering researches to reconstruct bone defects through results of numerous studies obtained in the English literature. As the conclusion, bone tissue engineering is a potential approach to reconstruct maxillofacial bone defects.

Keywords: scaffold,osteoconduction, mesenchymal stem cell, bone regeneration, bone integration.

Introduction

Surgical reconstruction of critical size mandibular defect has been a great challenge in oral and maxilla facial surgery. Gold standard for mandibular reconstruction is autogenous bone graft. However, autogenous bone grafthas limitation in shape, size, and its availability; furthermore, it has been attributed to donor site morbidity (Arrington *et al*., 1996; Heary *et al*., 2002). While the transfer of autologous tissue such as bone grafts or tissue free flaps are well-described, they are not without complications.To overcome these limitation, one can expect more on bone tissue engineering. Tissue engineering or tissue regeneration is a multidisciplinary approach to replace tissue loss as a result of traumatic defects, tumor resection or infection (Srisuwan *et al*., 2000). The prospect of using principles of tissue engineering to reconstruct defects in oral and maxillofacial defects continues to gain the attention of the reconstructive surgeon.(Susarla *et al*., 2011)Numerous studies in bone tissue engineering have attempted to search for effective combination of stem cells, scaffolds, and signaling molecules for reconstruction of bone defects in various animal models. A tissue engineering approach provides numerous prospective benefits, including a decline in donor site morbidity, a decrease in procedural sensitivity of the repair, and the capacity to intimately mimic the *in vivo* tissue environment

into recapitulate normal craniofacial development (Ward *et al*., 2010).The paper aims to provide information on the development of tissue engineering to reconstruct maxillofacial bone defects.

Principles of tissue engineering

Principles of tissue engineering are basically a triad of stem cells, signaling molecules, and scaffolds or extracellular matrix (Figure 1). (Rai *et al.*, 2015). A stem cell is defined as an unspecialized cell that can renew and maintain itself for a longer period of time with the potential to commit to a cell or tissue lineage with specialized functions. The use of stem cells, either embryonic or adult-derived (ADSCs), is a reality of regenerative medicine and dentistry. ADSCs are multipotent cells not derived from embryonic or primordial germ cell lineage, and they have the potential to differentiate into bone, muscle, cartilage, nerve, and vasculature under appropriate conditions (Conrad *et al*., 2005). These stem cells can differentiate into various cells like chondrocytes, osteoblasts, myoblasts, hematopoietic cells, and neural cells (Figure 2).

Signaling molecules: Various growth factors and cytokines are mixed to the ECM, like bone morphogenetic proteins (BMP), fibroblast growth factor-2 (FGF-2), interleukin-6, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor-β1, etc. This co-localization works as a storage pool of growth factors and may diminish growth factor degradation, protecting them from the local micro environment while facilitating the presentation of the growth factors to cell surface receptors (Schnaper *et al*., 1993; Roberts *et al*., 1988).

Scaffold: A scaffold is a permanently or temporarily placed three-dimensional porous and permeable natural or synthetic biomaterial that is biocompatible. It can be natural (Figure 2) or synthetic. It acts as a matrix and allows the attachment, migration, and differentiation of progenitor cells. Properties of scaffolds (such as biodegradability, porosity, stiffness, and strength) influence cell adhesion, migration, and proliferation (such as osteoconduction). The greatest challenges faced in tissue-engineered devices, regardless of tissue type, is promoting healing in three dimensions. Scaffolds have been made-up with a variety of innate and synthetic biomaterials, such as ceramics, metals, proteins, and polymers. An appropriate scaffold for tissue engineering will be one that is created with biology in mind. The functionof the scaffolds is providing structural support to cells, reservoir for growth factors and provide flexible, physical environment for remodeling (Patil *et al*., 2013).

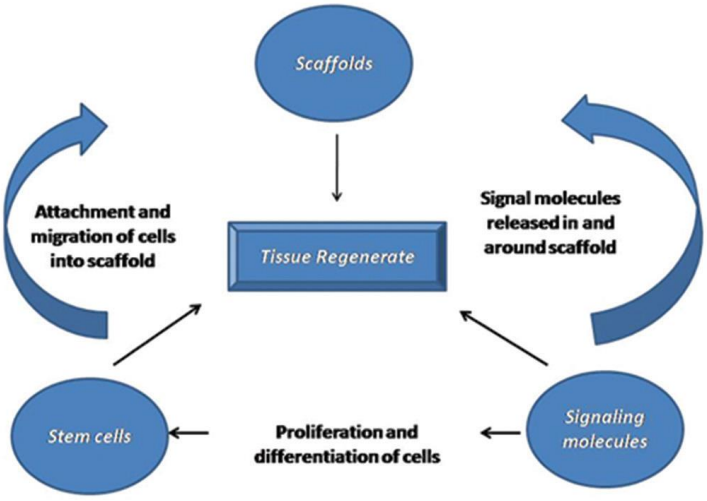

Figure 1: Triad of tissue engineering. Tissue engineering consists of three components which are scaffolds, stem cells, signaling molecules (Rai et al., 2015).

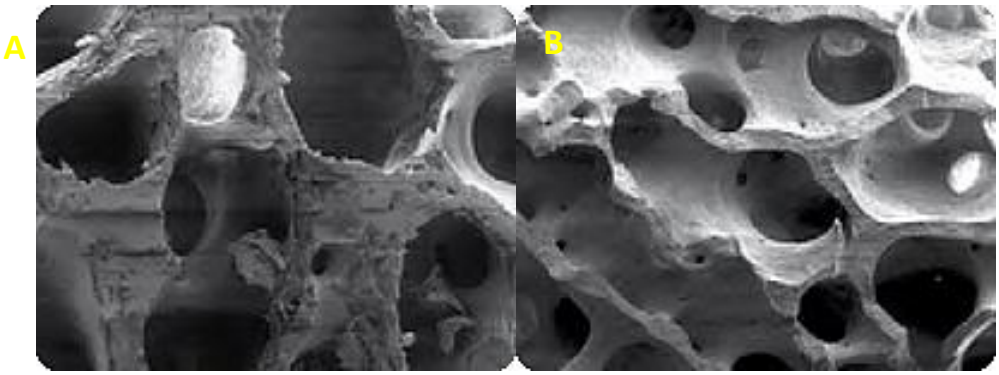

Figure 2.Microscopic structure of human *human* and*bovine cancellous bone*. Scanning electron microscope shows similarity in architecture and bony porosities between *human cancellous bone* (A) and *bovine cancellous bone* (B) (Fassina et al., 2010)

There are three main proposals taken in the branch of tissue engineering: Conduction, induction, and cell transplantation. The approach taken to regenerate a tissue relied upon numerous factors, together with the extent of the deficiency, the contribution of cells from adjoining areas, cell resettlement rate, and the available contiguous vasculature. When a pretty small amount of tissue is required, cell conduction and cell induction procedures are frequently used to uphold cell movement from host tissue keen on a scaffold. The alternate of little larger defects habitually requires the straight transplantation of cells. In conductive approach, like guided tissue regeneration, the innately derived or synthetic template simply acts as a submissive 3-D mechanical scaffoldto which cells can connect, propagate, transfer, and discriminate. The guided tissue regeneration approach is currently extensively used for the treatment of periodontal diseases. It is over and over again enviable, however, to manage the cell

colonization into the scaffold, discrimination of these cells, and consequent tissue fabrication. A potential tissue engineering method is an inductive approach in which bioactive scaffold signaling molecules are used to tempt cell movement and organized cellular behavior. A general inductive technique is the deliverance of soluble signaling molecules s like growth factors to the adjacent tissues. Gene therapy likewise is used to convey specific genetic information to host cells; once introduced, the host cells can then create definite growth factors to sway tissue development.When a huge tissue defect is there or the limited supply of appropriate cells in the tissue environment, cell transplantation is more suitable. This procedure typically includes a biopsy procedure from a donor source, separating and escalating the donor cells *in vitro*, and implanting the cells directly onto polymers characteristically made up of the physical forms of a fiber-based mesh, a sponge, or a hydrogel. The cells affix to the scaffold, propagate, and eventually form a tissue regenerate. This regenerated tissue is then set in into the individual in the tissue deficient area (Langer R., 1993). Steps involved in tissue regeneration (Figure 3): 1). Cell harvesting from body 2). Isolation, cultivation, and proliferation of cells into scaffold in presence of growth factors or signaling molecules (*in vitro*) 3). Implantation of the tissue regenerate.

Bone Regeneration with Tissue Engineering

Bone regeneration with tissue engineering approach requires combination of mesenchymal stem cell (MSC) and scaffold which serves as the porous matrix into which MSC attach, grow and differentiate into osteoblast. Mesenchymal stem cell can be isolated from various tissue either autologous or allogeneic such as umbilical cord, amniotic membrane or dental pulp. The collected tissue was enzymatically digested and cultured for expansionto obtain the desired amount. (Purwati *et al*., 2009) The cells was subsequently prepared into cell suspension into which the scaffold block is immersed and incubated for few days. The composite scaffold and cells are then implanted in bone defect and stabilized with fixation technique. (He *et al*., 2007; Eslamineja and Bagheri, 2009).

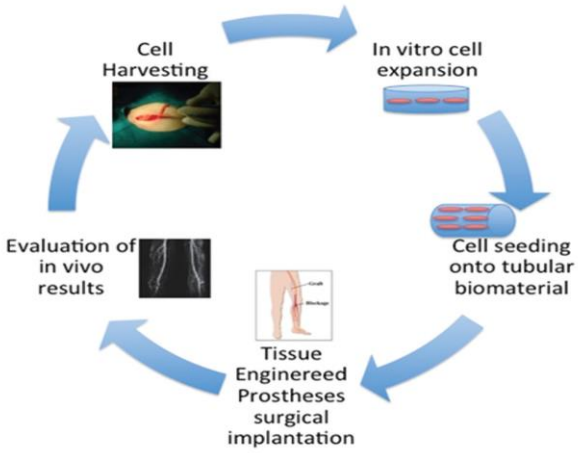

Figure 3. Steps of tissue regeneration and implantation.(Rai *et al*., 2015)

Bone healing in the defects after bone tissue engineering was believed to occur through two mechanisms. First, MSC growing in the porosities of the scaffold differentiate into osteprogenitor cells and osteoblast which subsequently produce bone matrix. Second, MSC attached on the *scaffold porosity* surfaces produce angiogenic, mitogenic and osteogenic growth factors leading to migration and differentiation of MSC into osteoblast. In such circumstances, bone forming will occur in scaffold construct at a higher magnitude compared to a mere scaffold. (Eslaminejad and Bagheri, 2009).

Clinical application of MSC-based bone tissue engineering has been reported. Segmental defects in human's long bone of 4-8 cm was reconstructed with composite graft made of HA ceramic construct seeded with autologous bone marrow-derived MSC. Following implantation of the composite graft it was demonstrated that good integration has occurred between composite graft construct and the host bone (Quarto *et al*., 2001).

Bone tissue engineering for reconstruction of mandibular defects

Few experimental studies on mandibular defects reconstruction using bone tissue engineering strategy have been existing in English literature since more than a decade ago. A study (Li and Li, 2005) investigated the effect of tissue engineering bone composed of bone marrow-derivedosteoblasts and demineralized bone in repairing mandible defect. Bonemarrow-derived osteoblasts of 20 rabbits were cultured and seeded into scaffold of allogeneic demineralized boneto construct tissue engineering bone graft *in vitro*, which was used to repair the 10×5-mm bone defect made in the same rabbit mandible edge. Implant of demineralized bone alone was as the control. Rabbits were killed according to the schedule: five after 2 weeks, five after 4 weeks, five after 8 weeks, five after 12 weeks, and the implants

were harvested for gross, radiographic, and histological observation. The result showed new bone formation at the margin region of defect and osteogenesis at the center were observed in the implant of tissue engineering bone, and the bone formation pattern included osteogenesis, osteoconduction, and osteoinduction. In the implant ofdemineralized bone alone, the major bone formation pattern was 'creeping substitute'.The conclusion of this study was that tissue engineering bone graft constructed by autogenous bone marrow-derived osteoblasts and allogeneic demineralizedbone was better than demineralized bone alone in bone formation capability, which might be an ideal graft for bone defect repair.

In another study, a 30mm long mandibularsegmental defect was repaired by engineered bone graft using osteogenically induced autologouse BMSCs seeded on porous β-tricalciumphosphate (β-TCP, n = 5). The repair of defects was compared with those treated with β-TCP alone (n = 6) or with autologous mandibular segment (n = 4). In the BMSCs/β-TCP group, new bone formation was observed from 4 weeks post-operation, and bonyun in was achieved after 32 weeks, which was detected by radiographic and histological examination. In contrast, minimal boneformation with almost fibrous connection was observed in the group treated with β-TCP alone (Figure 5). More importantly, the engineered bonewith BMSCs/b-TCP achieved a satisfactory biomechanical property in terms of bending load strength, bending displacement, bendingstress and Young's modulus at 32 weeks post-operation, which was very close to those of contralateral edentulous mandible andautograft bone ($p<0.05$). Based on these results, we conclude that engineered bone from osteogenically induced BMSCs andbiodegradable b-TCP can well repair the critical-sized segmental mandibular defects in canines (Yuan *et al*., 2007).

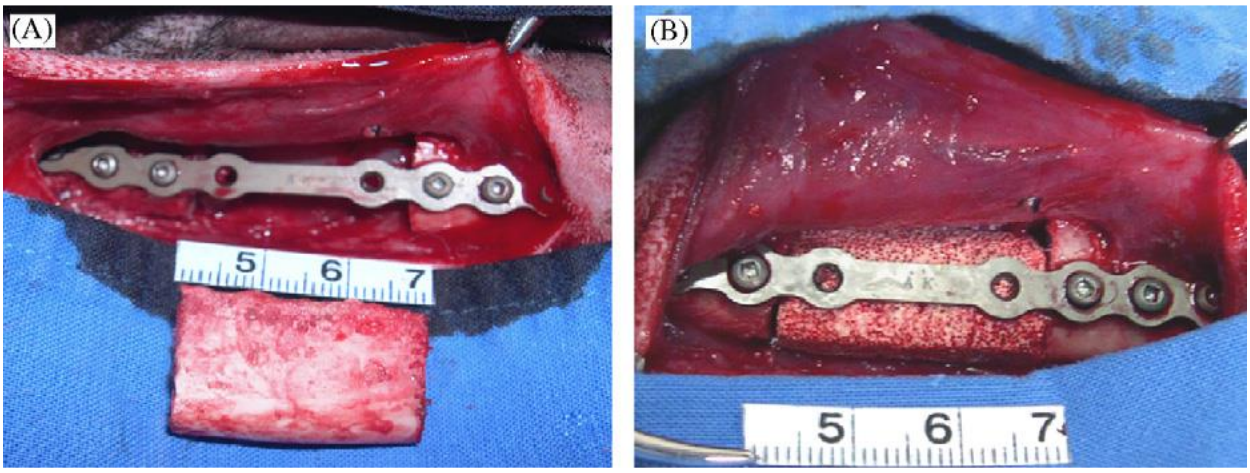

Figure 4. Implantation of cell-seeded scaffolds in mandibular segmental defect. Gross view of b-TCP cuboids of 30mm long, 15mm high and 10mm thick, with a tunnel of 3mm in diameter through each cuboid (top). Surgical procedure (bottom). (A) After adjustment of the titanium plate, an osteoperiosteal segmental mandibular defect of 30mm length was made at right side. (B) The defect was filled with BMSCs/b-TCP construct (Yuan *et al*., 2007)

Another similar study was conducted toproduce three-dimensional (3D) autologous tissue engineer deconstructs that combine auto genous cultivated bone marrow stromal cells with beta-tricalciumphosphate to reconstruct segmental mandibledefects without donor site morbidity. Bonemarrow stromal cells were isolated from a dog'scaput femoris. After differentiation and proliferationin vitro, the cells were seeded into a 3D beta-tricalciumphosphate scaffold. The constructs wereincubated under osteogenic culture conditions for 5days. Segmental defects of 30 mm length werecreated unilaterally in the mandibles of the animals.Reconstruction was performed using the construct inthree dogs and the scaffold only in three dogs asa control group.

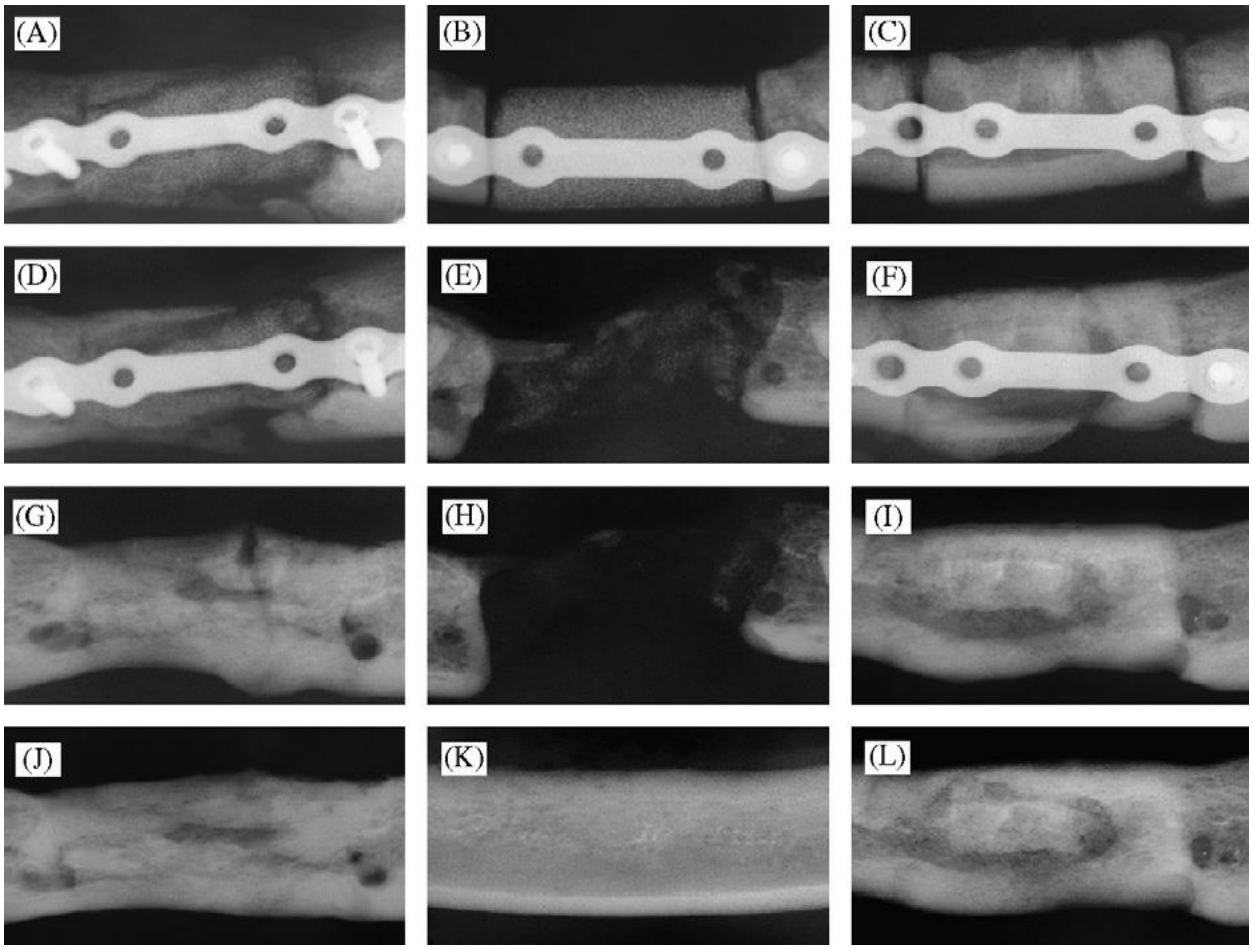

Figure 5. Radiographs of treated defects taken at different time points post-operation. In the BMSCs/b-TCP group, little calluses are formed at 4 weeks (A),and more calluses are observed at 12 weeks (D). At 26 weeks, the radiopacity is highly increased and bony-union is achieved (G). The radiopacity is closeto that of contralateral normal mandible (K) with smoothly remodeled bone contour at 32 weeks (J). In the b-TCP group, no callus is observed at neither 4weeks (B) nor 12 weeks (data not shown). Radiolucency and minimal callus formation at the cutting ends are observed at 26 weeks (E), while nearlycomplete radiolucency is observed at 32 weeks (H). In the autograft group, some callus formation is observed at the junction sites at 4 weeks (C), and theradiopacity decreased at 12 weeks (F). At 26 weeks, bony-union is achieved with some bone pieces in the middle portion of the graft (I). High density ofnew bone with insular bone pieces are coexisting in the graft after 32 weeks (L).(Yuan et al., 2007)

The specimens were retrieved 3months later, and the reconstructed areas were processed for gross observation, radiographic examination, 3D computed tomographic (CT) imaging, biomechanical evaluations, and histologic observation. The construct implanted group (n = 3) showedan average height of the reconstructed area of 18.54 mm and the control group 9.16 mm (*P* G 0.05). Higher radiodensity was present in the construct group than in the control group, as shown by radiograph. 3D CT imaging showed nearly two-thirds absorption of the reconstructed area in the control group. The biomechanical examination of the construct and control groups showed a compression strength of 102.77 N and 42.90 N andstress of 3.504 N/mm2 and 1.930 N/mm2, which demonstrates significant difference. Histologic micrographs showed new

bone formation in the scaffolds in central sections of the defects in theconstruct group 3 months later, with osteoblastseams, osteoclastic resorption, and cartilage formation.The construct of morphologic, 3D beta-tricalciumphosphate scaffold seeded, autologousbone marrow stromal cells ensure bone formationand vascularization throughout the procedure ofmandible segmental defect reconstruction, closely resembling how tissue engineering would be use to reconstruct a segmental mandible defect in theclinical setting (Yue *et al*., 2007).

Another study was designed to compare mesenchymal stem cell (MSC)-based alveolar bone regeneration inbiphasic bone substitutes and natural bone mineral in a canine full-thickness alveolar defect model. MSCs were isolated from bone marrow aspirates and culture expanded through 3 successive sub cultures. The bone differentiation potential of third passage cells was evaluated and confirmed in vitro before cellswere used in the transplantation experiment. Undifferentiated cells were then incubated with 3 x 3 x 3 mm hydroxyapatite/β-tricalcium phosphate (HA/TCP) matrices (Kasios, Lanauguet, France) and 1- to 2-mm Bio-Osspongiosa (Geistlich Biomaterials, Osteohealth, Switzerland), which is a natural bovine bone mineral (NBM). Kasios/cell, Kasios alone, Bio-Oss/cell, and Bio-Oss alone were implanted in masseter muscle and 4 cylindrical (10-mmdiameter) through-and-through bilateral mandibular body defects in 4 mongrel dogs. Histomorphometric analysis wasperformed 6 weeks after insertion of the scaffold loaded with MSCs**.** The result of H&E staining of the decalcified scaffold and scanning electron microscopy demonstrated large MSC coverageof the HA/TCP and Bio-Oss. Cell-loaded Kasios matrices showed the greatest amount of the bone regeneration amongthe groups in both the muscle (29.11%) and the bone specimens (65.78%). Cell-free biphasic scaffold revealed 44.9%bone fill in bone defects and 23.55% in muscle specimen, and Bio-Oss alone matrices had the least amount of newbone formation: 36.84% and 24.16% in bone and muscle specimens respectively. Kasios loaded with MSCs demonstrated more bone regeneration than Bio-Oss/cell but there was no significant statistical difference (P >0.05). From this study it was concluded that new biphasic synthetic bone substitutes may offer better conditions for bone regeneration than traditional bone substitute in combination with MSCs. They remained in the defect and contributed to bone regeneration (Jafarian *et al*., 2008).

Transplants of culture-expanded bone marrow stromal cells (BMSCs) combined with hydroxyapatite/tricalciumphosphate (HA/TCP) scaffolds successfully form cortico-cancellous bone to reconstruct the dog cranio facial skeleton. Yet, these transplants' long-term stability in large animal models has not been evaluated. This study's purpose was the evaluation of long-term BMSC transplant stability when used to augment the mandible. Here, autologous BMSC–HA/TCP transplants

were introduced ontothe unilateral dog mandible as on lay grafts, while contralateral control mandibles received HA/TCP onlays alone. QuantitativeCT (qCT) scans were obtained both early and late after transplantation. Transplants were harvested up to 19months later for histologic and mechanical analyses. In all dogs, BMSC transplants formed significantly greater amounts of bone over their control counterparts. The new bone formed an extensive union with the underlying mandible. BMSC transplants retained the majority of their initial volume, while control (HA/TCP only) transplants were nearly completely resorbed. By qCT, the extent of newly formed bone could bedetermined non-invasively. In summary, HA/TCP particles alone undergo a high degree of resorption, while autologous cultured BMSC–HA/TCP transplants provide long-term bony augmentation of the mandible (Kusnetzov *et al*., 2008)

One other similar studycompared the bone healing mechanism and osteogenic capacity between bovine bone mineralloaded with hAMSC and autogenous bone graft (ABG) in the reconstruction of critical size mandibular bone defect of 45 New Zealand white rabbits. Specimens from sacrificed rabbit were collected for histology and immune histo chemistry staining. The result showed that expressions of VEGF, BMP2 and Runx2 as well as the amount of angiogenesis were higher in ABG compared with BBM-hAMSC group in the first and second weeks of healing. The result of twelfth week of healing showed that expressions of Runx2 and osteocalcin as well as the thickness of collagen type-I fibres were significantly higher in BBM-hAMSC compared to ABG group, while there was no statistically difference in trabecular area and bone incorporation between BBMh-AMSCand ABG group. This study concluded that early healing activities were higher in autogenous bone graft than in BBM-hAMSC, while osteogenic activities in the late stage of healing were higher in BBM-hAMSC compared to autogenous bone graft. It was also concluded that the osteogenic capacity of BBM-hAMSC was comparable to autogenous bone graft in the reconstruction of critical size defect in the mandible (Kamadjaja *et al*., 2015).

CONCLUSION

Bone tissue engineering is a potential strategy for reconstruction of maxillofacial defects. The future challenge envisaged for the successful reconstruction with tissue engineeirng is the fabrication of highly osteoconductive 3D scaffold and the optimum cell seeding technique using proper bioreactor before implantation of the composite construct in maxillofacial bone defects.

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