Chapter

13

CRANIOFACIAL TISSUE RECONSTRUCTION WITH MESENCHYMAL STEM CELLS DERIVED FROM DENTAL TISSUE AND BONE MARROW

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13.1. INTRODUCTION

In craniofacial region, there are a variety of specified tissues including bones, teeth, muscles, cartilages, blood vessels and nerves. Bones and teeth, which are parts of the craniofacial hard tissues, work as a functional unit and provide structural support, protection, sensation and allow movement. Defect and dysfunction of bones and teeth may occur due to pathological factors such as congenital malformations, progressive diseases, trauma and infections; or due to treatment procedure such as surgery. Whole or partial loss of bone or tooth structures has an enormous impact on patient's physical and psychological life. It is important to restore function and preserve esthetics during a craniofacial reconstruction to ensure patient's regaining self-esteem and maintaining a good quality of life [1].

For craniofacial reconstruction, bone grafting has become a major treatment modality in past decades. Various bone grafting materials, including autograft, allograft, xenograft, and alloplastic graft, provide a wide range of options to clinicians. Autologous grafting has been used as a gold standard in craniofacial reconstruction for years with its superior osteogenic, osteoinductive and osteoconductive properties. However, its further application is limited as a result of the potential donor site morbidity and insufficient amount of bone graft [2]. Although bone allograft materials such as demineralized bone matrix have good osteoconductive and osteoinductive capabilities, these foreign materials may induce immune rejection from the host. Immune suppression may also be caused by treatment alternatives to prevent this rejection [3]. Alloplastic grafts, which are usually synthetic materials such as hydroxyapatite and calcium carbonate, have an inferior osteoinductive and osteoconductive capability, but they can be customized to fill defects of different shapes [4].

In the last two decades, tissue engineering and regenerative medicine have been developed and advanced. Dr. Langer first defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [5]. For regenerative medicine, the principles of tissue engineering are utilized to regenerate human tissues or organs in order to restore normal functions [6]. Modern concept of tissue engineering was developed in the late 1980s, when synthetic biodegradable materials were introduced as scaffolds and stem cells technology was advanced. Since then, the cell-based tissue engineering modality along with a combination of stem cells, biomaterials and growth factors has been widely studied and applied. In this method, cells and growth factors are delivered to the defect site *via* scaffolds. Scaffolds not only support cell attachment and proliferation, but also contain and release growth factors to promote cell differentiation towards a specific lineage for tissue regeneration.

Tissue engineering using stem cells and biomaterials has been applied to the reconstruction of craniofacial bone defects. This chapter highlights the recent progress of tissue engineering in reconstruction of craniofacial bone and tooth tissues by focusing on two types of mesenchymal stem cells(MSCs): bone marrow mesenchymal stem cells (BMMSCs) and dental stem cells (DSCs).

13.2. MESENCHYMAL STEM CELLS

MSCs are a heterogeneous cell population with self-renewal, clonogenic and multipotent characteristics existing in almost all tissues. They were first isolated from hematopoetic tissues in 1974 [7]. Since then MSCs have been successfully isolated from a variety of tissues such as bone marrow, skeletal muscle, dermis, peripheral blood, adipose tissue, umbilical cord blood, amniotic fluid, cornea and tooth [8,9].

13.2.1. Origin of MSCs

MSCs have complex ontogeny. The exact histological origin of MSCs is still not well-known, mainly because of the lack of a unique MSC marker. Three possible origins of MSCs have been proposed.

a. Epithelial to mesenchymal transition (EMT)

EMT is a cellular process in which epithelial cells lose their epithelial traits and obtain mesenchymal cell properties. During EMT, cell polarization occurs and junctional structure of the epithelial cells gets lost, followed by cytoskeleton reorganization and transition to spindle shape mesenchymal-like cells [10]. Accompanying the morphological changes during EMT, the expression of epithelial related genes is down-regulated while the expression of MSC related gene is up-regulated. After the completion of EMT, the cells express MSC markers and exhibit the capability of multilineage differentiation [11-13].

b. Adventitial cells

Human vascular adventitial fibroblasts in pulmonary arteries are found to contain mesenchymal stem progenitor cells. These cells express MSC marker genes and undergo osteogenesis and adipogenesis under appropriate culture conditions [14]. Recently, a cell population characterized by CD34+ CD31− CD146− CD45− was isolated from the *tunica adventitia*. These cells expressed MSC markers and had a similar multipotency to BMMSCs [15].

c. Pericyte origin

In multiple human organs, MSCs also have a perivascular origin [8,16]. It is believed that stem cells reside in a specialized "stem cell niche" in perivascular areas, which was first discovered in bone marrow and later was confirmed in other tissues [17,18]. Pericytes are the contractile cells surrounding the endothelial cells in capillaries. Some surface markers of MSCs are expressed in

pericytes, including CD146 and PDGF-Rβ [19,20], and MSCs express some pericyte markers such as pericyte-associated antigen 3G5 [21], suggesting that pericytes have a potential link with MSCs. Pericytes have the ability of differentiation into osteoblasts, osteocytes, adipocytes, chondrocytes and nerve cells [22-26], indicating the potential perivascular origin of MSCs. Thus, two perivascular MSC progenitors may co-exist: adventitial cells around larger vessels and pericytes in capillaries. It should be noted that MSC-like cells can be as well derived from vascular tissues such as intervertebral discs. These cells not only express MSC markers CD146 and CD166, but also have the capability of multilineage differentiation [27-29].

13.2.2. Functions of MSCs

Although the exact mechanisms of MSCs are not fully clear yet, the functions of MSCs in tissue engineering can be concluded as the followings:

a. Homing effect

MSCs have a specific capability to migrate to injured or damaged tissue sites when they are introduced into the host body, which is defined as "homing efficiency" [30,31]. The migration is induced by a group of molecules called "homing-related molecules (HRMs)". HRMs have three major categories: (a) chemokines, such as chemokine ligand 12 (CCL-12)-chemokine receptor 4 (CXCR4), and chemokine ligand-2 (CCL-2)-chemokine receptor 2 (CCR2) [32-34]; (b) adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [35]; and (c) matrix metalloproteinases (MMPs) [36]. HRMs can be regulated by inflammatory cytokines. For example, tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) up-regulate the expression of ICAM-1 and VCAM-1, leading to the MSCs chemotaxis [37,38].

b. Trophic effects

After delivered to the injured site, MSCs can be stimulated by the local micro-environment to produce trophic factors, such as insulin-like growth factor (IGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and CCL-2. The trophic factors can promote cell proliferation, anti-apoptosis and enhance angiogenesis [39,40]. For example, the conditioned media from mesenchymal stem cells have the effect of enhancing bone regeneration, suggesting that paracrine factors secreted by stem cells can promote osteogenesis [41].

c. Plasticity and multilineage differentiation

MSCs have the capability to differentiate into mature and functional cells of the same or different germ layer of origin, which is described as “plasticity” [42]. BMMSCs have been extensively studied, and their multipotency are proved to differentiate into different types of cells in bone, cartilage, adipose tissue and skeletal muscles [43-47]. BMMSCs can also differentiate into cardiomyocytes

[48], neural cells [49], epithelial cells [50,51], endothelial cells [52] and hepatocytes [53]. For regenerative medicine, the plasticity of MSCs provides the fundamental cellular basis. Four mechanisms of plasticity have been proposed: (a) stem cells from embryonic stage are persistent during the development and maintained in post-natal tissues; (b) "true" post-natal stem cells, such as multiple adult progenitor cells, reside in tissues [54]; (c) MSCs undergo de-differentiation, trans-differentiation or re-differentiation [55-57]; and (d) fusion of donor and host cells trigger the exchange of genetic information, leading to the MSCs trans-differentiation [58,59]. The plasticity of MSCs can be enhanced by environmental changes, stimulated by exogenous growth factors, or regulated by genetic manipulation. For example, the hypoxic condition can enhance osteogenic differentiation of BMMSCs [60], and genetically engineered MSCs with osteogenic factors bone morphogenetic protein-2 (BMP-2) promote osteogenesis [61,62].

d. Immunomodulatory effect

Studies have reported the immunosuppressive effect of MSCs [63,64]. MSCs can be stimulated to produce immunosuppressive mediators E2 by the inflammatory stimuli including nitric oxide and prostaglandin, as well as cytokines IFN-y, TNF-α and IL-1 [65-67]. These stem cells regulate both of innate and adaptive immune systems. They are able to inhibit the maturation of monocytes [68], and also inhibit the proliferation of B cell and T cell [63,69]. Based on their immunosuppressive effect, MSCs have been suggested to be used to treat patients with immune diseases, such as systemic lupus erythematosus [70,71], rheumatoid arthritis [72] and multiple sclerosis [73]. However, higher level evidences are required to support the clinical use of MSCs as an immunosuppressive reagent, such as randomized and well controlled studies to understand their immunomodulatory effect further.

13.2.3. Isolation of MSCs

In order to isolate a population of MSCs, the first step is to separate cells from the tissue origins by either enzyme digestion or the explant culture [74]. Then the MSCs population can be isolated and identified from the entire cell population by the following methods: (a) selection based on physiological criteria including morphology, proliferation and differentiation properties; (b) immunohistochemical staining; (c) immunomagnetic bead selection; and (d) fluorescence-activated cell sorting (FACS).

Among these methods, FACS has become very popular, since it is sensitive, accurate and cost efficient. Immunohistochemistry method can help to confirm the location of MSCs in tissues. For example, immunohistochemical staining showed that a majority of STRO-1+ cells are found in the niche located in the perivascular and perineural sheath regions of the human dental pulp [75-79]; in the periodontal ligament, most cells with STRO-1+/CD146+/CD44+ are located in the perivascular regions [80].

13.3. BONE MARROW MESENCHYMAL STEM CELLS (BMMSCs)

Bone marrow contains heterogeneous populations of cells, in which only a small fraction are multipotent cells [7,43,45,81]. Two major distinct stem cell populations have been found in bone marrow: hematopoietic stem cells and mesenchymal stem cells. At present, BMMSC is one of the most well characterized postnatal stem cell populations that have been utilized for cell--based clinical therapies. BMMSCs express not only STRO-1, CD106 (VCAM-1), CD146 (MUC18), but also mesenchymal associated surface molecules CD49a, CD73, CD90, CD166, β-2 integrin (CD18), and endothelial progenitor related marker CD105 [45,82-86]. Cell flow cytometry is used to isolate different subpopulation of cells with desired stem cell characteristics with the help of single or a combination of these markers. Recently, a rare population of murine BMMSCs was discovered expressing high CD13 and stage-specific antigen I (SSEA-I). These cells, named as multipotent adult progenitor cells (MAPC), showed a very strong capacity of proliferation and differentiation towards cells originated from any of the three germ layers, indicating that MAPC had a great potential for cell-based stem cell therapy [54,87].

13.4. DENTAL STEM CELLS (DSCs)

Since the first isolation of human dental stem cells from dental pulp tissue in 2000 [75], at least five different types of mesenchymal stem cells have been isolated from tooth related tissues, including dental pulp stem cell (DPSC) [75], stem cells of human exfoliated deciduous teeth (SHED) [88], stem cells of the apical papilla (SCAP) [89], dental follicle progenitor cells (DFPC) [90] and stem cells from periodontal ligament (PDLSC) [91,92]. It has been reported that various types of DSCs exist in dental tissues such as dental pulp, periodontal ligament, apical papilla, tooth germ, dental follicle of mature and immature teeth, inflamed periapical [93,94]. Even induced bleeding during pulp regeneration procedure revealed a large amount of stem cells [95].

Similar to all other MSCs, DSCs reside in the perivascular and perineural sheath regions [76,77], and can be isolated by FACS based on the specific cell surface markers. The markers include but not limit to: positive markers such as STRO-1, CD13, CD44, CD24, CD29, CD73, CD90, CD105, CD106, CD146, Oct4, Nanog, β2 integrin and 3G5; and negative markers such as CD14, CD34, CD45 and HLA-DR [96]. In recent studies, a subpopulation of cells expressing c-kit+/CD34+/STRO-1+ was isolated from perivascular niche of their respective tissues. These cells are believed to more accurately represent multipotent stem cells population and are therefore recommended to be used as DSCs [83,86,96-99].

DSCs have shown stem cell properties including self-renewal and multilineage differentiation. For example, under specific culture conditions *in vitro*, DPSCs can undergo osteogenic / odontogenic, adipogenic, chondrogenic or neurogenic differentiation [75,88].

13.5. COMPARISON OF BMMSCs AND DSCs

Both BMMSC and DSC are considered as a subpopulation of mesenchymal stem cells. Similarities and differences have been found between them (Table 1).

13.5.1. Gene expression profile

A similar pattern of gene expression between BMMSCs and DPSCs can be showed by microarray analysis. Shi *et al.* compared the gene expression profiles between DPSCs and BMMSCs using microarray, and found that more than 4000 known genes had a similar expression level between these two types of cells [100]. On the other side, DPSCs expressed higher level of Col18a1, IGF-2, discoidin domain tyrosine kinase 2 (DDR2), NAD(P)H menadione oxidoreductase (NMOR1), cyclin-dependent kinase 6 (CDK6), homolog 2 of drosophial large disk (DLG2); while the expression of Col1a2 and IGF binding protein 7 (IGFBP-7), were higher in BMMSCs. Moreover, no gene was expressed solely in either of these two cell types [100]. In another study by Yamada *et al.*, over 12,000 genes were compared between osteogenic induced DPSCs and BMMSCs. The result showed that after osteoinduction, DPSCs expressed higher level of ALP, DMP1 and DSPP. Cluster analysis revealed the difference of gene expressions existing in gene groups that involved cell signaling, cell communication or cell metabolism [101]. Kim *et al.* performed a GeneChip analysis to compare the gene expression profiles between STRO-1+ BMMSCs and STRO-1+ DSCs. They identified that in BMMSCs, 379 genes were up-regulated and 133 genes were down-regulated; in DPSCs, 218 genes were up-regulated and 231 genes were down-regulated [102].

13.5.2. Proteomic profile

A study used a large scale of 2-dimensional electrophoresis and liquid chromatography to compare the differential proteomic expression profiles of DPSCs and BMMSCs derived from an individual donor. Eighteen proteins were found upregulated in DPSCs relative to BMMSCs, while no protein upregulation was observed in BMMSCs compared with DPSCs [103]. Wei *et al.* identified 23 proteins related to odontoblastic differentiation of dental pulp cells (DPCs) *in vitro*, including cytoskeleton proteins, nuclear proteins, cell membrane--bound molecules, proteins involved in matrix synthesis, and metabolic enzymes [104]. Aforementioned results suggest that differences exist in the characteristic growth and development capacity of DPSCs and BMMSCs, which

needs to be considered when the cells are used for particular tissues regeneration.

13.5.3. Colony-forming unit / cell proliferation

The percentage of colony-forming cells derived from dental pulp tissue was significantly higher than that derived from bone marrow [75,105]. DPSCs exhibit a higher proliferation rate and grow faster compared to BMMSCs in vitro [75,93,105]. Flow cytometry study showed that DSCs contained a higher portion of cells that in G2 / M phase and lower portion of cells that in G0/G1 phase, suggesting more DSCs are undergoing mitosis [93].

13.5.4. Multilineage differentiation

a. Neural differentiation

DPSCs and BMMSCs are both capable of undergoing neural differentiation under defined conditions, which is evidenced by cell morphology change from fibroblast-like to neuron-like appearance with multipolarity and elongated processes. The differentiated cells have an increased level of neural markers nestin, *β*III-tubulin and NF-200 [93]. Karaöz *et al.* found that human DPSCs expressed several specific transcripts and proteins of neural stem cells and demonstrated better neural and epithelial stem cell properties than BMMSCs [106].

b. Osteogenic / odontogenic differentiation

Both DPSCs and BMMSCs are able to differentiate and form the alizarin-red positive tissues [93]. Batouli *et al.* implanted DPSC and BMMSC transplants respectively into mice, and found that BMMSCs tended to form more bone / marrow structures, whereas DPSC transplants more tended to form dentin / pulp complex. The same study also found that FGF and MMP-9 were highly expressed in the connective tissue compartment of BMMSC transplants; while dentin sialoprotein (DSP) was highly expressed in DPSC transplants [107]. In a study by Yu *et al.*, DPSCs or BMMSCs were cultured in renal capsule of rat for 14 days, and DPSCs were found to present more striking odontogenic capability than BMMSCs [108]. Zhang *et al.* seeded rat and human BMMSCs and DPSCs respectively on HA / TCP and cultured *in vitro*, or implanted them into nude mice *in vivo*. They found that *in vitro* both types of cells showed abundant cell growth and mineralization of extracellular matrix were noted, but *in vivo* BMMSCs displayed more potent capability to form mature bone-like structures than DPSCs [109].

c. Adipogenic differentiation

BMMSCs and DSCs both have capability to differentiate into oil-red O positive cells [93], although Zhang *et al.* observed that DPSCs and SCAPs had weaker capability of adipogenesis in comparison with BMMSCs [89,110].

d. Chondrogenic differentiation

Although BMMSCs and DPSCs are both able to differentiate into chondrogenic lineage [111-114], the chondrogenic potential of DPSCs appears weaker than BMMSCs [80,110].

e. Muscular differentiation

Both of BMMSCs and DPSCs can differentiate into muscular lineage and have the potential in craniofacial and cardiac repair and regeneration [115-119].

Table 1. Comparison between BMMSCs and DSCs

	BMMSCs	DSCs	Reference
General properties			
CFU	Lover (2.4–3.1 colonies / 10^4 cells plated)	Higher (22–70 colonies / 10^4 cellsplated)	[75]
Proliferation rate	Lower (46 % BrdUrd-positive cells ± 1.96 SEM)	Higher (72 % BrdUrd-positive cells ± 3.48 SEM)	[75,93]
Cells in G0 / G1 phase	Higher	Lower	[93]
Cells in G2 / M phase	Lower	Higher	[93]
Surface marker			
CD44	+	+	[93]
CD29	–	+	[120]
CD13	+	+	[103]
CD90	+	+	[93]
CD105	+	+	[93]
CD106	+	–	[114]
CD14	–	–	[93,103]
CD34	–	–	[103]
CD45	–	–	[103]
STRO-1	+	+	[120]
Gene expression profile			
Nanog	+	+	
Oct3 / 4	+	+	[93,122]

	BMMSCs	DSCs	Reference
Sox2	+	+	
Col1a2	++	+	[103]
Insulin-like growth factor binding protein 7	++	+	[100]
Col 18a1	+	++	[99,102]
IGF-2	+	++	[100]
Discoidin domain tyrosine kinase 2	+	++	[100]
NADP	+	++	[100]
Cyclin-dependent kinase 6	+	++	[100]
Homolog 2 of drosophial large disk	+	++	[100]
vimentin	+	+	[123]
nestin	+	+	[123]
Proteomic expression			
Upregulation	0 protein upregulated relative to DPSC	18 proteins upregulated relative to BMMSC	[103]
Osteo/ Odontogenic			
DMP1	+	++	[103]
ALP	+	++	[103]
BSP	+	+	[114]
OCN	+	+	[123]
Col1	+	+	[114]
Col3	+	+	[114]
Runx2	+	+	[123]
DSPP	–	++	[103]
Chondrogenic	++	+	[80,109]
Adipogenic	++	+	[38,109]
Neurogenic			
Nestin	+	+	[93]

	BMMSCs	DSCs	Reference
NF-200	+	+	[93]
Cytokine secretion			
VEGF	+	++	[106]
TGF-β1	+	++	[106]

+: Expression tested positive; ++: expression tested positive and strong; −: expression tested negative

As a summary, several different gene and protein signatures have been exhibited in MSCs from bone marrow and dental tissue, which makes the stem cells competent to give rise to a specific lineage [120]. When applying these mesenchymal stem cells for craniofacial reconstruction, it is very important to recognize: (a) the differentiation capacity of cells may change along with cell passages. *In vivo* transplantation study showed that rat DPSCs at passage 1 could develop into dentin, bone and cartilage structures respectively, while DPSCs at passage 9 could only generate bone tissues [121]; (b) craniofacial bones are derived from neural crest cells, while long bones are derived from mesoderm [122]. The MSCs derived from bone marrows of these two origins may show different osteogenic potency [123,124]; (c) DSCs generally appear to be more committed to the odontogenic rather than the osteogenic lineage [79,88,125,126]; (d) DSCs from different tissue origins have difference regarding their potential in dental tissue regeneration. for example, PDLSCs are more potent to regenerate cementum / PDL-like structure, while DPSCs are more potent to regenerate dentin-pulp complex [114]; (e) The osteogenic potential of BMMSCs varies among different donors [127]; (f) Currently there is no unique master mesenchymal stem cell that is appropriate to regenerate all target tissues to treat various diseases.

13.6. REGENERATION OF CRANIOFACIAL TISSUE USING MSCs

Tissue engineering technology makes it possible to locally deliver MSCs and growth factors. MSCs and specific growth factors are first loaded into the biomaterial scaffold. The whole system is then delivered into the required sites [128,129]. This technology has been extensively applied to craniofacial tissues regeneration at both of the bench and clinical levels.

13.6.1. Regeneration of craniofacial bone tissues with BMMSCs

BMMSC is one of the most well characterized postnatal stem cells and have been used for cell based clinical therapies to regenerate hard tissues in craniofacial region.

13.6.1.1. Calvarial bone defect repair

The critical sized calvarial defect model is a well-established model to study the calvarial bone regeneration. A range of biomaterials have been used for calvarial bone defect regeneration, including silk fibroin, poly(lactic-*co*-glycolic acid) (PLGA), poly(vinylidene chloride), hydrogel, and minimally-invasive delivery of tissue-engineered bone (TEB). An injectable biopolymer scaffold seeded with BMMSCs and BMP-2 was used to repair rat calvarial defect, and significant regeneration of bone tissues was found at 4 weeks after implantation [130]. Lima *et al.* immobilized rat BMMSCs in the alginate beads before delivering them to bone defect area, and the results showed in accelerated bone regeneration [131]. Jiang *et al.* also reported that platelet-rich plasma scaffold loaded with BMMSCs promoted bone regeneration within 8 weeks in a rabbit model [132]. In a porcine model, a collagen scaffold cultured with BMMSCs led to the mineralization of newly formed bone tissue within 90 days after implantation [133]. BMMSCs can effectively support neovascularization mediated by endothelial cells, and promote angiogenesis for bone regeneration [134]. Furthermore, BMMSCs can be genetically modified to enhance their differentiation towards a specific lineage. Zou *et al.* reported that the transduced BMMSCs with angiogenic factor hypoxia-inducible factor-1α (HIF-1α) significantly improved blood vessel formation in calvarial defects [135].

13.6.1.2. Maxillary bone reconstruction

Maxillary sinus floor elevation is a common procedure for maxillary reconstruction. In a recent randomized controlled study, a bilateral sinus floor augmentation procedure was performed on 12 patients. The MSCs were seeded on BioOss® particles and then delivered to the defect sites. These cells were derived from either posterior iliac ridge or mandibular ridge in the retromolar areas. Both groups gained a sufficient volume of new bone formation, which enabled the reliable implants placement [136].

13.6.1.3. Mandibular bone reconstruction

Mandible reconstruction by tissue engineering has been reported on various animal models. In a study, BMMSCs were loaded on hydroxyapatite / tricalcium phosphate (PCL / TCP) to repair the mandibular defect in dogs, and significant bone formation was noted on day 21 [137]. To achieve vertical bone augmentation, Khojasteh *et al.* also loaded BMMSCs on PCL / TCP before implanting the system into mandible, and significant bone formation was observed in 8 weeks [138]. In another study, BMMSCs were loaded into PLGA scaffold and cultured for 10 days before being implanted into a mandibular defect site in minipig. The defect site was filled with bone-like tissues in 6 weeks [139]. Recently a clinical case of mandible reconstruction has been reported by Warnke *et al.*, in which a titanium mesh transplant was fabricated

by CT scanning and computer-aided design. The mesh was loaded with bone marrow and supplemented with BMP-7, followed by implantation into *latissimus dorsi* muscle for 7 weeks. It was then transplanted as a free bone--muscle flap to repair mandibular bone defect [140,141]. Hernández-Alfaro *et al.* reported another clinical case, in which bone marrow aspirate from the iliac crest was used to treat patient with mandibular bone defect. Bone marrow aspirate was supplemented with BMP-7 and seeded on a bovine xenograft blocks. The results showed adequate bone formation and recovery of esthetics and function [142].

13.6.1.4. Alveolar bone reconstruction

In secondary alveoloplasty, mesenchymal stem cells from a posterior iliac bone can be mounted on a biphasic scaffold combined with platelet derived growth factor (PDGF). In a recent study, the triads were placed into the alveolar cleft defects in patients. About 51.3 % of bone re-growth was observed in 3 months after the surgery [143].

13.6.2. Regeneration of craniofacial bone tissues with DSCs

DPCs provide an alternative MSC source to reconstruct craniofacial bone tissue. Otaki *et al.* reported that by mixing dental pulp cells with HA / TCP and subcutaneously implanting them to the immunocompromised mice, bone tissue was regenerated [144]. Yamada *et al.* demonstrated that stem cells from deciduous teeth mixed with platelet-rich plasma scaffold have the ability to form new bone to repair a significant osseous defect [145]. Tour *et al.* used periodontal ligament stem cells seeded on an extracellular matrix modified HA scaffold to repair calvarial critical-sized defect, resulting in a significant improvement of calvarial bone reparation [146]. In a clinical study, DPSCs were seeded on a collagen sponge scaffold to repair alveolar ridge defect, and optimal bone regeneration was observed one year after the grafting, indicating the combination of DPSCs with collagen sponge biocomplex completely repair human mandible bone defects [147].

13.6.3. Regeneration of dental tissue with BMMSCs

13.6.3.1. Regeneration of periodontium

Kawaguchi *et al.* demonstrated that new cementum, alveolar bone and periodontal ligament in class III periodontal defects in dogs could be regenerated by transplantations of *ex vivo* expanded autologous BMMSCs. A 20 % increase in new cementum length and bone area was revealed by morphometric analysis [148]. In a subsequent study, autologous BMMSCs with a collagen scaffold were transplanted to treat patients with periodontal osseous defects. The results showed a significantly improved bone formation [149]. Zhou *et al.* reported engraftments of BMMSCs were differentiated into

periodontal specific cells when BMMSCs were delivered to the injured dental tissue site, suggesting that BMMSCs may communicate with surrounding dental tissues to become tissue-specific mesenchymal progenitor cells, and participate into the regeneration process [150].

13.6.3.2. Regeneration of whole tooth

Ohazama *et al.* reported using cultured stem cells could induce a significant progress toward the creation of tissue-engineered embryonic tooth *primordia*. Various mixtures of non-dental-derived mesenchymal cells, such as embryonic stem cells, neural stem cells, and adult bone marrow cells, with embryonic oral epithelium cells were detected and transplanted into the renal capsules of adult mice. All mixtures led to the development of a tooth structure and bone. Interestingly, the fact was noted that the host tissues made no contribution to the donor tissue. Moreover, transfer of embryonic tooth *primordia* into the adult jaw resulted in the development of tooth structures, indicating that an embryonic *primordium* can develop in its adult environment [151].

Although it is believed that the ultimate goal of tooth regeneration is to develop a whole bioengineered tooth that is fully functional, at the current stage, growing a complete bioengineered tooth from a single kind of cells still remains very challenging [152].

13.6.4. Regeneration of dental tissues with DSCs

In periodontal field, guided bone regeneration (GBR) and guided tissue regeneration (GTR) are surgical procedures that utilize a barrier membrane to prevent the periodontal epithelium ingrowth, and to maintain the space for bone and tissue regeneration [153]. Various resorbable or nonresorbable membranes along with bone graft materials have been used for these procedures [154-162]. Additional growth factors can be added to scaffold to promote their regeneration capability [163]. In endodontic field, the routine root canal treatment for a necrotic tooth is very challenging, especially in the cases of immature permanent teeth with open apex. As a new treatment modality, regenerative endodontics is able to create and deliver tissues to replace diseased and missing pulp. The clinical procedure of regenerative endodontics was first described by Dr. Nygaard-Ostby [164] and further developed by Dr. Martin Trope [165]. Since then techniques and materials have been advanced to promote continued root formation and apical closure [166]. The principles of regenerative endodontics are to initiate bleeding into the root canal through mechanical irritation of the apex, thus producing a blood clot to form a scaffold. Stem cells from apical papilla were meanwhile introduced into the root canal by bleeding [95]. Numerous clinical case reports have shown the successful regain of root length and width [167,168]. Advancements have been made in regenerative endodontics *via* the tissue engineering technology. Huang *et al.* observed that DPSCs implantation in a mechanically enlarged root

canal led to the formation of dentin-pulp complex [169]. Recently, several novel scaffolds have been designed for pulp regeneration, such as the self-assembling peptide amphiphile nanofibers [170], and the electrospun nanocomposite composed of polydioxanone and halloysite nanotubes [171]. These scaffold materials have greatly promoted the tissue regeneration.

13.7. FUTURE PERSPECTIVE

In craniofacial hard tissue reconstruction, the future advancement of tissue engineering will continuously be centralized on three essential components: mesenchymal stem cells, scaffolds and growth factors. Before animal studies and preclinical models further contribute to the clinical practice of regeneration, it is necessary to address several major challenges.

13.7.1. Source of stem cells

Clinical application of allogeneic MSCs is limited by the potential immunosuppresive effect from the host. Even the MSCs with low immunogenic profile *in vitro* can still induce immune response when they are transplanted *in vivo* [172]. Autologous stem cells from the same patient are ideal to avoid immune rejection. The concept of "stem cell banking" has been raised, and it will potentially provide an essential infrastructure for cell-based tissue regeneration [173]. However, the effectiveness and economical aspects of stem cell banking, especially dental stem cell banking, need further evaluation. Recently researches on the induced pluripotent stem cells (iPS cells) from somatic [174,175] and dental tissues have advanced, and therefore provide an alternative source of MSCs [176].

13.7.2. Microbial control

Microbial control in oral cavity will remain as a major challenge for the dental tissues regeneration since microorganism invasion and biofilm formation are the fundamental etiologies of oral diseases, especially endodontic and periodontal diseases. A higher level of disinfection may be required in regenerative endodontics. A variety of antibacterial irrigants have been used in clinic, including sodium hypochlorite, chlorhexidine, ethylene diamine tetraacetic acid and iodine potassium iodide. As an alternative, mixture of multiple antibiotics has also been introduced to the clinical procedure of dental pulp regeneration [163]. It should be noted that the success of tissue engineering requires the survival and proliferation of stem cells. Therefore, the goal of microbial control should be to maximize the antimicrobial effect but meanwhile to minimize the harmful effects on stem cells [177].

13.7.3. Biomaterial scaffold

In tissue engineering, biomaterial based scaffolds play a critical role. Scaffolds are used to seed cells and act as extracellular matrix. The ultimate fate of stem cells is determined by the properties of scaffolds. Recently, the dynamic reciprocity between the MSCs and scaffolds has received increased attention. The reciprocity is not only referred to the chemical or biological characteristics of scaffolds, but also linked to its mechanical characteristics [178-181]. Scaffolds are used to integrate and release growth factors to guide stem cells differentiation. During stem cells differentiation, specific growth factors may be required only during a specific period of time and at a specific location. Therefore, obtaining the temporal and spatial control of growth factors release is still a challenge for designing a new scaffold in tissue engineering.

13.7.4. Regulation of stem cell differentiation

Currently BMMSCs are the most widely used stem cells in craniofacial reconstruction [30,182,183], yet DSCs will greatly contribute to this field due to their origination from the same region and more commitment to the odontogenic lineage [79,88,125,126]. However, the detailed molecular signaling mechanisms of DSCs differentiation are still not fully clear. During stem cell differentiation towards a specific cell lineage, it is of great importance to understand the sequential activation or inactivation of signal cascades in order to help and promote tissue regeneration [184].

13.7.5. Risk of using stem cells

Stem cell laden scaffolds are not totally risk free but hold potential for malignancy. Currently most researchers focus on how to regenerate tissue. The next challenges will be how to control the process of regeneration, how to command the over-regeneration, and how to prevent the malignant transformation of cells. The genetic stability of these cells must be maintained during their differentiation.

13.8. CONCLUSION

Tissue engineering technology has been applied to the reconstruction of craniofacial tissue. The reconstruction of craniofacial tissue with MSCs has provided an alternative treatment modality, and various preclinical studies and case reports have proved its high potential of success. Stem cell-based tissue engineering in craniofacial reconstruction is promising, and it may finally replace autologous bone grafting to improve the life quality of patients. However, several challenges are still remaining. Successful craniofacial tissue regeneration depends on the appropriate selection of the source of stem cells. Dental tissue regeneration especially tooth regeneration needs the interaction

between epithelial and mesenchymal stem cells. It is challenging to control and guide the differentiation of MSCs towards the target craniofacial tissue, meanwhile to prevent their transformation during the differentiation.

The optimal combination of stem cells, growth factor and biomaterial scaffold is the current focus in order to achieve more advanced tissue regeneration. In the field of craniofacial tissue regeneration, researchers who are utilizing tissue engineering technology still face two major challenges: reestablishment of effective vascularization and functionality regaining of the bioengineered constructs. Various strategies have been proposed, including growth factor delivery, three dimensional culturing systems and biomimetic scaffold design. The spatial and temporal control of growth factors release from biomaterial scaffold is also a hot area of research. Furthermore, the translation of the cell-based technology from *in vitro* to *in vivo* is still a challenge. After transferring MSCs from *in vitro* to *in vivo* microenvironment, the interaction of MSCs and the microenvironment they reside, and the fate of stem cells *in vivo* need to be further studied. Safety precautions and regulations should also be considered before clinical application.

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