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INVITED REVIEW

Bone marrow-derived mesenchymal stem cells for regenerative medicine in craniofacial region

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The craniofacial region contains many specified tissues including bone, cartilage, muscle, blood vessels and neurons. Defect or dysfunction of the craniofacial tissue after post-cancer ablative surgery, trauma, congenital malformations and progressive deforming skeletal diseases has a huge influence on the patient's life. Therefore, functional reconstruction of damaged tissues is highly expected. Bone marrow-derived mesenchymal stem cells (BMMSCs) are one of the most well characterized postnatal stem cell populations, and considered to be utilized for cell-based clinical therapies. Here, the current understanding and the potential applications in craniofacial tissue regeneration of BMMSCs are reviewed, and the current limitations and drawbacks are also discussed.

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Introduction

In recent years, stem cell research provides fundamental knowledge for translating stem cell-mediated tissue regeneration into clinical therapies. The craniofacial region contains many specified tissues including bone, connective tissue, fat, blood vessels, neural tissue and muscle. Reconstruction of craniofacial components is one of the most important and intricate objectives in stem cell-mediated regenerative medicine (Warren *et al*, 2003; Cowan *et al*, 2004; Warnke *et al*, 2004). In addition, craniofacial deformities have enormous psychosocial impacts for individuals, with the most common causes for craniofacial defects being post-cancer ablative surgery, trauma, congenital malformations, and progressive deforming skeletal diseases (Phillips *et al*, 1992; Jeffcoat, 1993; Nguyen and Sullivan, 1993). Autogenous graft, allogeneic graft, and various alloplastic materials, such as demineralized bone matrices, synthetic bone pastes, and semisynthetic scaffolds, which have been utilized to reconstruct craniofacial defects have all led to improved clinical outcomes of various degrees. However, these approaches showed inherent limitations, such as insufficient autogenous resources, donor site morbidity, contour irregularities, disease transmission, major histoincompatibility, graft-versus-host disease (GVHD), immunosuppression, unpredictable outcome for bone formation, and infection of foreign material (Jackson *et al*, 1986; Oklund *et al*, 1986; Sawin *et al*, 1998; Warren *et al*, 2003). To overcome these limitations, stem cell-based tissue regeneration offers a promising approach to providing an advanced and reliable therapeutic strategy for craniofacial tissue repair.

One potential avenue for developing a stem cell-based therapy is the use of embryonic stem (ES) cells, which are derived from the inner cell mass of the blastocyst and possess pluripotent differentiation capacity (Evans and Kaufman, 1981; Martin, 1981; Shamblott *et al*, 1998; Thomson *et al*, 1998). Although ES cells are considered as important cell resources in the future for many tissue repair/regeneration applications, the clinical use of ES cells is still limited because of ethical issues, uncontrolled differentiation of ES cells, and immune rejection problems between donor and recipient. Moreover, long-term clinical trials are necessary to exclude the possibility of chromosomal instability and tumorigenesis when ES cells are utilized *in vivo*.

Tissue-specific postnatal stem cells have been isolated from a variety of organs and tissues, including but not limited to, bone marrow (Castro-Malaspina *et al*, 1980; Civin *et al*, 1984; Baum *et al*, 1992; Craig *et al*, 1993; Prockop, 1997; Pittenger *et al*, 1999; Kondo *et al*, 2003), neural tissue (Flax *et al*, 1998; Johansson *et al*, 1999), muscle (Chen and Goldhamer, 2003; Huard *et al*, 2003), skin (Lavker and Sun, 2000; Janes *et al*, 2002), eye (Lavker and Sun, 2003), intestine (Marshman *et al*, 2002), and liver (Alison *et al*, 1997; Shafritz and

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Dabeva, 2002). It was reported that craniofacial tissues also contain postnatal stem cells such as bone marrow-derived mesenchymal stem cells (BMMSCs), dental pulp stem cells, periodontal ligament stem cells, and stem cells from human exfoliated deciduous teeth (SHED) (Gronthos *et al*, 2000, 2002; Miura *et al*, 2003; Seo *et al*, 2004; Matsubara *et al*, 2005; Akintoye *et al*, 2006). However, bone marrow is the only system thus far that provides stem cells for successful and routine treatment of hematopoietic diseases, cancer therapy, and GVHD (Mulder *et al*, 1989; Broun *et al*, 1997; Thomas, 1999; Le Blanc *et al*, 2004). Bone marrow contains at least two populations of postnatal stem cells; hematopoietic stem cells (HSCs), which can reconstitute all blood cell lineages (Lemischka *et al*, 1986; Osawa *et al*, 1996), and mesenchymal stem cells (MSCs), which are derived from bone marrow stromal tissues with the capacity for multipotent differentiation into cell types of mesodermal origin such as osteoblasts, chondrocytes, adipocytes, and muscle cells (Castro-Malaspina *et al*, 1980; Prockop, 1997; Pittenger *et al*, 1999). Both HSCs and MSCs have been used to treat a variety of human diseases and tissue defects (Korbling and Estrov, 2003). Transplantation of whole bone marrow cells or mobilized peripheral blood, which includes HSCs and MSCs, has been used to treat a variety of hematopoietic diseases and malignancies, following systemic infusion into patients. To date, there is no evidence to show that bone marrow-derived stem cells have given rise to any primary cancers or had any association with severe side effects since the first successful allogeneic marrow graft performed in a patient 38 years ago (Thomas, 1999). Taken together, BMMSCs have been considered of an effective and safe resource for stem cell-based clinical therapy. In this review, we discuss the potential of BMMSC-mediated tissue regeneration for repairing craniofacial tissues.

Biology of BMMSCs

The resource of MSCs

Mesenchymal stem cells have been isolated from different organs, including bone marrow, skeletal muscle, dermis, adipose tissue, muscle, dental pulp, and periodontal ligament (Gronthos *et al*, 2000; Lee *et al*, 2000; Halvorsen *et al*, 2001; Jankowski *et al*, 2001; Zuk *et al*, 2001; Gimble and Guilak, 2003; Miura *et al*, 2003; Seo *et al*, 2004). Interestingly, peripheral blood may also contain MSCs (Fernandez *et al*, 1997; Zvaifler *et al*, 2000; Kuznetsov *et al*, 2001). It was reported recently that human nonadherent osteocalcin-positive cells from peripheral blood possess an osteogenic potential *in vivo*, which supports the possibility of the existence of functional MSCs in circulating peripheral blood (Eghbali-Fatourechi *et al*, 2005). Additionally, the existence of MSCs in umbilical cord blood was also reported (Goodwin *et al*, 2001; Gang *et al*, 2004). These evidences suggest that MSCs can be obtained from a variety of tissues for MSC-mediated therapies although MSCs from bone marrow have been studied intensively. However, it is necessary to be cautious while selecting the stem cell resource for the therapies because of the fact that organ- or tissue-specific traits may affect the stem cell behavior (Phinney *et al*, 1999b; Gronthos *et al*, 2001; Shi *et al*, 2005; Akintoye *et al*, 2006). For instance, recent studies have suggested that BMMSCs derived from craniofacial bone may differ from those derived from long bone in terms of cell proliferation and differentiation (Matsubara *et al*, 2005; Akintoye *et al*, 2006). Human orofacial bone marrow mesenchymal cells showed an increased proliferation rate and bone-forming capacity *in vitro* and *in vivo* but did not support an organized hematopoietic marrow organ as seen in BMMSC transplants from long bone (Akintoye *et al*, 2006). Inherent nature of the specific organ/tissue may account, at least in part, for this discrepancy. Craniofacial skeletal tissues including maxilla, mandible, and teeth are thought to be derived from neural crest cells, whereas long bones are derived from the mesoderm (Chai *et al*, 2000). Moreover, the existence of orofacial-specific skeletal diseases such as cherubism (Ueki *et al*, 2001), hyperparathyroid jaw tumor syndrome (Simonds *et al*, 2002), and craniofacial fibrous dysplasia (Akintoye *et al*, 2003) supports the notion that craniofacial bones are different from long bones in their development, function, and disease progress. Furthermore, there are limitations to collecting sufficient bone marrow samples from the orofacial region to obtain adequate numbers of BMMSCs for therapeutic purposes, because of the limited size of the orofacial bones. Therefore, utilizing appropriate stem cell populations from the most favorable donor site might be critical to achieve the ideal or optimal outcomes in stem cell-based therapies.

Isolation of BMMSCs

Bone marrow-derived mesenchymal stem cells are a subpopulation of the total bone marrow mononuclear cells, which can be isolated from whole bone marrow aspirates or from isolated mononuclear cells following density-gradient Ficoll separation. (Castro-Malaspina *et al*, 1980; Simmons and Torok-Storb, 1991a,b; Falla *et al*, 1993; Waller *et al*, 1995; Prockop, 1997; Pittenger *et al*, 1999). BMMSCs adhere to tissue culture plastic and are identified based on their capacity to generate clonogenic cell aggregates when plated at low cell densities, initially termed colony-forming unit-fibroblasts (CFU-F) (Friedenstein *et al*, 1970; Friedenstein, 1976; Pittenger *et al*, 1999). However, these bone marrow-adherent cells consist of heterogeneous populations in which multipotent BMMSCs represent only a small fraction of the total CFU-F population (Castro-Malaspina *et al*, 1980; Falla *et al*, 1993; Waller *et al*, 1995; Kuznetsov *et al*, 1997; Pittenger *et al*, 1999; Gronthos *et al*, 2003). The establishment of methods to isolate a purified population of BMMSCs is essential for characterizing the multipotent BMMSCs. Although there is no ideal method to purify the homogeneous populations of BMMSCs from bone marrow, recent advances have identified cell-surface molecules expressed by immature noncommitted BMMSCs.

The monoclonal antibody, STRO-1 was identified by its reactivity with stromal cells in the adherent layers of long-term bone marrow cultures and CFU-F isolated

from human bone marrow (Simmons and Torok-Storb, 1991b; Gronthos *et al*, 1994). STRO-1, whose antigen has yet to be identified, does not react with hematopoietic committed progenitors, but binds to approximately 10% of bone marrow mononuclear cells. The STRO-1^+ fraction of bone marrow mononuclear cells showed a high clonogenic capacity when compared with unfractionated cells (Shi and Gronthos, 2003) and exhibited the ability to differentiate into multiple cell lineages including myelosupportive stromal cells, osteoblasts, adipocytes, and chondrocytes (Simmons and Torok-Storb, 1991a; Gronthos *et al*, 1994, 2003; Dennis *et al*, 2002). Furthermore, two other antigens, CD106 (VCAM-1) and CD146 (MUC18) have been reported as early progenitor markers for BMMSCs (Filshie *et al*, 1998; Gronthos *et al*, 2003; Shi and Gronthos, 2003). Their expression was found to be restricted to a minor fraction of STRO-1 high-expressing bone marrow mononuclear cells. CFU-F could be enriched up to approximately 5000-fold relative to their incidence by unfractionated cells on the basis of the use of STRO-1 combined with CD106 or CD146, (STRO-1^{bright}/$CD106^+$ or STRO-1^{bright}/$CD146^+$) (Gronthos *et al*, 2003; Shi and Gronthos, 2003). Other antigens including α-smooth muscle actin, mesenchymal associated surface molecules CD49a, CD73, CD90, and CD166, as well as endothelial progenitor related marker CD105 were also reported to be positive on BMMSCs following *ex vivo* expansion (Simmons and Torok-Storb, 1991b; Galmiche *et al*, 1993; Conget and Minguell, 1999; Pittenger *et al*, 1999). Some of these markers are known to be expressed by endothelial/perivascular cells. These observations and accumulating data suggest that mesenchymal stem/progenitor cells are closely related with vascular pericytes (Schor *et al*, 1995; Doherty *et al*, 1998; Shi and Gronthos, 2003). More recently, CD18 (β-2 integrin) has been identified as a new surface marker of BMMSC and shown to play a critical role in the osteogenic differentiation of BMMSCs (Miura *et al*, 2005b). The expression of CD18 is limited to the very early stem progenitors of BMMSCs, where it is rapidly lost as the cells undergo *ex vivo* expansion. Two-color fluorescence cell sorting using a combination of CD18 and STRO-1 resulted in the enrichment of CFU-F in the double-positive population (Miura *et al*, 2005b). These data lay the foundation for potential discovering of new cell-surface molecules expressed by BMMSCs to help the define discrete differentiation stages of stromal cell development. Moreover, this approach will help develop more effective strategies for isolating highly purified BMMSCs based on multiple antigen expression.

Following *ex vivo* expansion in the presence of serum, BMMSCs show an altered gene expression profile to that described prior to culture (Gronthos *et al*, 2003; Shi and Gronthos, 2003). In particular, many genes are subsequently up-regulated in culture associated with committed osteoblast, adipocyte, and chondrocyte (Castro-Malaspina *et al*, 1980; Vilamitjana-Amedee *et al*, 1993; Rickard *et al*, 1996; Gronthos *et al*, 2003). Extensive *ex vivo* culture may also lead to a decreased capacity for osteogenic differentiation by BMMSCs *in vitro* and *in vivo* (Shi *et al*, 2002; Simonsen *et al*, 2002). These findings suggest that the 'stemness' of BMMSCs may not be maintained consistently under conventional *ex vivo* culture conditions. Therefore, in addition to isolating highly purified BMMSCs, it is necessary to establish optimal culture conditions, perhaps using serum-free medium (Gronthos and Simmons, 1995) for expanding BMMSCs to reach sufficient numbers of stem/progenitor cells for clinical therapies.

Plasticity of BMMSCs

Bone marrow-derived mesenchymal stem cells are capable of extensive self-renewal and are able to differentiate into multiple cell lineages including bone, cartilage, adipose tissue, hematopoiesis-supporting stroma, muscles, and tendon (Bruder *et al*, 1994; Prockop, 1997; Bianco and Robey, 2001). The most established differentiation trait of BMMSCs is to form bone and an organized bone-associated hematopoietic marrow when transplanted into immunocompromised animals (Ashton *et al*, 1980; Friedenstein *et al*, 1982; Bab *et al*, 1988; Goshima *et al*, 1991; Cassiede *et al*, 1996; Krebsbach *et al*, 1997). Recent reports indicated that adult bone marrow cells have a potential to differentiate into nonmesoderm-originated cell types, including neurons and glia, hepatocytes and bile duct epithelia, renal epithelia and podocytes, and gut mucosal cells and associated myofibroblasts (Poulsom *et al*, 2002). Moreover, existence of multipotent adult progenitor cell (MAPC) further supports the plasticity of BMMSCs (Jiang *et al*, 2002a,b). MAPCs, a rare population of murine BMMSCs, showed an unlimited proliferation capacity and differentiated into the cells originating from all three germ layers. In contrast, other reports identified that bone marrow cells are capable of fusing with differentiated hepatocytes, Purkinje neurons, and cardiomyocytes (Alvarez-Dolado *et al*, 2003; Vassilopoulos *et al*, 2003; Wang *et al*, 2003). At present, the plasticity of BMMSCs is inconclusive because transdifferentiation or cell fusion of bone marrow cells is based on utilizing the whole bone marrow cells, which include hematopoietic stem cells, mesenchymal stem cells and endothelial precursor cells known as angioblasts (Eglitis and Mezey, 1997; Shi *et al*, 1998; Asahara *et al*, 1999; Petersen *et al*, 1999; Brazelton *et al*, 2000; Mezey *et al*, 2000). Therefore, the mechanisms of transdifferentiation need to be clarified to elucidate if transdifferentiation is due to cell 'plasticity' or caused by stem cell fusion (Terada *et al*, 2002; Ying *et al*, 2002; Vassilopoulos and Russell, 2003; Alison *et al*, 2004).

Immortalization/transformation of BMMSCs

Embryonic stem cells are capable of producing teratomas when injected into immunocompromised mice. However, any stem cell-based therapy will have to ascertain that their stem cell preparations retain a normal chromosomal complement after *in vivo* administration. As a result of their slower growth rate and absence of telomerase activity *in vitro*, MSCs are presumed to have a lower risk for tumor formation compared with embryonic stem cells (Rosenthal, 2003). However, it was recently reported that human adult

MSCs from adipose tissues and mouse BMMSCs underwent spontaneous transformation and formed osteosarcoma cells after long-term culture (Rubio *et al*, 2005). Conversely, rigorous assessment of human BMMSCs failed to demonstrate any potential for transformation under the culture conditions shown to induce spontaneous transformation of murine BMMSCs or human adipose-derived MSC (Miura *et al*, 2005a). The spontaneous immortalization of stem cells is, therefore, a critical issue in the context of human therapies, and warrants further investigations to elucidate potential mechanisms by which MSCs spontaneously transform into tumor cells.

Clinical application of BMMSCs for tissue regeneration in craniofacial region

The craniofacial region mainly consists of bone, cartilage, adipose tissue, muscle, nerve, and dental tissue. Many of these tissues have originated from either mesoderm-derived cells or ectoderm-derived neural crest cells during development. BMMSCs have been reported to differentiate, at least, into mesoderm-derived tissues, including bone, cartilage, adipose tissue, and muscle. Therefore, BMMSCs may be the ideal candidates for the regeneration of multiple tissue types in different craniofacial regions.

Bone tissue regeneration

When cultured in the presence of dexamethasone, inorganic phosphate and ascorbic acid, BMMSCs can differentiate into osteoblastic-like cells with the capacity to synthesize mineralized nodules (Gronthos *et al*, 1994; Pittenger *et al*, 1999). Under these conditions, uncommitted BMMSCs begin to express many osteogenic markers such as CBFA1/Runx2, osterix, osteopontin, parathyroid hormone receptor, and osteocalcin which are not expressed by freshly isolated BMMSCs (Gronthos *et al*, 2003). Interestingly, only a subset of high-proliferating single colony-derived BMMSC clones (approximately 60%) was capable of forming ectopic bone upon *in vivo* transplantation into immunocompromised mice (Kuznetsov *et al*, 1997; Gronthos *et al*, 2003). Despite the nature of heterogeneity in *ex vivo* expanded BMMSCs, successful repair of bone defects has been achieved in both calvarial and long bone in various animal models (Bruder *et al*, 1998; Krebsbach *et al*, 1998; Kon *et al*, 2000; Petite *et al*, 2000; Mankani *et al*, 2001). In addition, optimal outcomes have been achieved in studies by using autologous BMMSCs to treat human patients with different bone defects (Quarto *et al*, 2001; Warnke *et al*, 2004). For example, the study by Warnke *et al* (2004) designed a custom-made biomaterial scaffold that contained BMP-7 and autologous bone marrow in order to generate a functional mandible. Although the origin of the cells (presumed to be BMSSCs) responsible for the regeneration of the mandible was not defined, the patient developed an improved mastication function and was reported to be satisfied with the esthetic outcome.

Another important feature of *in vivo* osteogenesis of BMMSCs is the capacity of these cells to facilitate formation of organized hematopoietic marrow elements, which originate from recipient cells, when transplanted into immunocompromised mice with hydroxyapatite/tricalcium phosphate ceramic powder as a carrier (Ashton *et al*, 1980; Friedenstein *et al*, 1982; Bab *et al*, 1988; Goshima *et al*, 1991; Cassiede *et al*, 1996; Krebsbach *et al*, 1997). After successful BMMSC transplantation, donor cells actively form bone on the surface of the carrier vehicle and the recipient cells are induced to form hematopoietic marrow elements, leading to a bone/marrow organ-like structure. However, the mechanisms by which osteogenic differentiation of BMMSC influences the organization of recipient marrow components, are yet to be elucidated.

Cartilage tissue regeneration

Craniofacial tissue contains several areas, which consist of cartilage, such as nose, ear, and temporomandibular joint (TMJ). Surgery, congenital deformity, trauma, or some types of TMJ disorders may lead to a loss or destruction of the cartilage matrix. It is anticipated that cartilage regeneration may offer an alternative approach to the treatment of these disorders. BMMSCs can differentiate to chondrocytes when cultured under a three-dimensional serum-free setting in the presence of transforming growth factor (TGF)-βs (Pittenger *et al*, 1999), which was confirmed by the expression of type II collagen and aggrecan (Pittenger *et al*, 1999; Gronthos *et al*, 2003). When ovine BMMSCs were seeded onto biodegradable scaffolds and subsequently implanted into fetal tracheas, they showed a significant chondrogenic differentiation (Fuchs *et al*, 2003). Moreover, improved cartilage repair in patellar groove defects was observed following implantation of rabbit BMMSC (Wakitani *et al*, 1994; Im *et al*, 2001). At the molecular and cellular levels, recent studies have demonstrated that the Wnt/β-catenin pathway may play a crucial role in regulating chondrogenesis of BMMSCs (Day *et al*, 2005; Hill *et al*, 2005). Ectopic canonical Wnt signaling leads to enhanced ossification and suppression of chondrocyte formation, while genetic inactivation of beta-catenin causes ectopic formation of chondrocytes. While the requirement of high-quality cell preparations, growth factors, and ideal scaffolds poses many challenges for cell-based therapies, BMMSCs have shown a great therapeutic potential to repair cartilage defects (Magne *et al*, 2005; Raghunath *et al*, 2005).

Adipose tissue regeneration

Adipose tissue in different craniofacial regions may have considerable impact on the appearance of facial features. BMMSCs can differentiate to adipocytes when cultured with the inductive medium, which contains hydrocortisone, indomethacin, insulin, and isobutylmethylxanthine (Pittenger *et al*, 1999; Gimble and Guilak, 2003). The adipocytes derived from BMMSCs show cytoplasmic lipid vacuoles positively stained with oil red red O. This differentiation can be genetically

confirmed by the expression of the fat-associated markers, peroxisone proliferator-activated receptor (PPAR)γ2 and leptin (Gronthos *et al*, 2003). On the other hand, adipose-derived adult mesenchymal cells have also been demonstrated as a population of multipotent stem cells, which can differentiate into adipogenic, osteogenic, chondrogenic, myogenic, and neuronal strains. BMMSCs, however, can be an alternative resource to regenerate adipose tissue for cosmetic purpose and for tissue repair in craniofacial reconstructive surgery.

Muscular tissue regeneration

It has been shown that demethylation compounds such as 5-azacytidine or amphotericin B can induce myogenic differentiation of BMMSCs *in vitro* (Wakitani *et al*, 1995; Makino *et al*, 1999; Phinney *et al*, 1999a). Many reports have demonstrated that implantation of cultured bone marrow mononuclear cells by intracoronary injection improves left ventricular function (Assmus *et al*, 2002; Strauer *et al*, 2002; Wollert *et al*, 2004) although the question of transdifferentiation of bone marrow-derived cells into cardiomyocytes has been raised (Balsam *et al*, 2004; Murry *et al*, 2004). Recently, several clinical trials have reported an improvement of myocardial function by autologous BMMSC transplantation after acute myocardial infarction (Chen *et al*, 2004; Price *et al*, 2006). Collectively, these studies demonstrate a potential therapeutic use of BMSSC for regeneration of cardiac and perhaps skeletal muscles, particularly for patients who have undergone a radical surgery or trauma, including patients who have problems in mastification.

Other tissue regeneration

Bone marrow-derived mesenchymal stem cells have been reported to differentiate to other tissues (Korbling and Estrov, 2003) of non-mesodermal origin by their capacity to regenerate neural cells (Eglitis and Mezey, 1997; Azizi *et al*, 1998; Kopen *et al*, 1999; Woodbury *et al*, 2000) and recovery of injured spinal cord (Hofstetter *et al*, 2002). Although all these studies encourage further investigations into the therapeutic potential of BMMSCs, many questions still remain to be answered, such as the cell origin of regenerated tissues, mechanisms of transdifferentiation, donor variation of growth/differentiation potential, and long-term effects of cell transplantation.

Delivery of BMMSCs

There are two important issues for efficiency of stem cell-mediated tissue regeneration. First, there is a requirement to deliver sufficient numbers of functional cells to the desired site. Local transplantation of BMMSCs is a practical method for tissue regeneration in craniofacial regions as the treatment area is relatively easy to access. The efficiency of delivery of BMMSCs injected systemically into the desired site is still unpredictable and often limited. However, BMMSCs have been reported to engraft and differentiate site-specifically in multiple organs after systemic injection (Liechty *et al*, 2000) and migrate into an injured site (Wang *et al*, 2002a,b). There are some suggestions that genetic manipulation of BMMSCs may improve the capacity of these cells to home into specific sites of tissue damage in the future. The genetic manipulation of BMSSCs may also improve the maintenance of 'stemness' and/or induce the lineage-specific commitment prior to implantation. Studies of enforced human telomerase expressing BMMSCs have been reported to extend their lifespan and enhance their osteogenic potential *in vitro* and *in vivo* (Shi *et al*, 2002; Simonsen *et al*, 2002). In addition, BMP-transfected BMMSCs have been successfully used to treat large segmental femoral and calvarial bone defects in animal models (Lieberman *et al*, 1998; Gysin *et al*, 2002). The drawback of genetically manipulated BMMSCs is that this approach is difficult to translate directly into any clinical application because of the possibility that the components used in genetic manipulation (i.e. vector) can be negative factors for a normal human body. Secondly, any local transplantation of BMSSC will also need to be performed in combination with suitable biocompatible scaffolds which will be critical in determining the survival rate and differentiation potential of the cells (Raghunath *et al*, 2005). Therefore, it is necessary to develop tissue-specific scaffolds to facilitate optimal tissue regeneration and repair of stem cells *in vivo*.

Future direction

Many recent reports suggest that BMMSCs have a promising potential to be utilized for regenerative medicine including craniofacial regions. However, it is clear that there are several critical questions which have to be addressed in order to develop effective treatments. First, BMMSCs are a heterogeneous population of cells which contain cells at different stages of development. Although many molecules have been reported as cell-surface markers specific for BMMSCs, it is still difficult to isolate highly purified BMMSCs. Secondly, it is critical to understand how to maintain the 'stemness' of BMMSCs following *ex vivo* expansion, and then how to direct tissue-specific regeneration in the presence of complex interactions with recipient tissues. In order to understand the stem cell properties of BMMSCs in depth, identification of the local microenvironment or stem cell 'niche' is essential for finding the markers to define BMMSCs at early stages of development and to assess their functions. Thirdly, tissue regeneration in craniofacial regions demands more predictable outcomes because craniofacial tissues consist of many relatively small and complicated components that have a huge impact on an individual's psychosocial condition. To enhance or control the differentiation of stem cells, proper application methods such as delivering methods, genetic manipulation, combinations of stem cells with growth factors and suitable biomaterials have to be further improved. Although there are many challenges ahead of us in terms of utilizing stem cells

for tissue regeneration, BMMSCs are one of the most promising postnatal stem cell populations for tissue repairing and regeneration for a wide range of organs, including craniofacial tissues.

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