Bone Marrow Versus Dental Pulp Stem Cells in Osteogenesis

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Introduction

Although the consideration of stem cells is currently approaching its hundredth year as one of the organizing principles of developmental biology, it demonstrates no sign of losing its youthful luster. A range of sources of stem cells have been identified that has the potential to self-renewal and capacity to form multiple lineages. Regardless of the discovery of existence of stem cells in various tissues and body fluids, bone marrow has been potentially considered as a persuasive and primeval source of stem cells for treating a wide horizon of disease [1, 2]. Although bone marrow-derived MSCs could be differentiated into mesodermal and non-mesodermal lineages [3–5], osteoblasts, responsible for osteogenesis, and hematopoietic cells, for hematopoiesis are closely associated with the bone marrow, suggesting a reciprocal relationship between the two [6]. Much of the work in MSCs found within the bone marrow stroma on its in vitro and in vivo applications involved in osteogenesis, adipogenesis, cartilage, and muscle formation including osteoblast, osteocytes, adipocytes, chondrocytes, myoblast, and myocytes are gaining importance due to its inherent bone formation capacity [7]. Hence, bone marrow resident stem cells made them the most primitive and promising source from ancient days for treating bone-related diseases. Nevertheless, it is unfortunate that these sources could have not been effective in treatment of all possible diseases due to various disadvantages of BM-MSCs; one of the main drawbacks is that osteogenic potential of bone marrow cells decreases with age [8], and hence, the search for alternate sources of adult stem cells is also underway. It has been demonstrated that stromal adipocytes in bone marrow cavity increases as age increases. In other words, adipocyte accumulation in the human bone marrow stroma correlates with trabecular bone loss with aging [9–12]. Thus, adipose stromal cells both isolated either from bone marrow or from adipose tissue itself has evolved as a contemporary source for bone regeneration [13, 14]. However, we predict that identifying a source that will be similar to the characteristics of bone marrow, possessing

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inherent bone-forming capacity might be more valuable in bone tissue engineering, repair, and regeneration.

One such source is stem/progenitor cells isolated from dental pulp tissue. Dental pulp tissue has the ability to regenerate dentin in response to dental disorders such as caries. Dentin regeneration is occurred by committed precursor cells called the odontoblasts. It is thus very likely that pulp tissue contains a loaded source of stem cells or progenitor cells which have the capacity to form odontoblasts. These stem/progenitor cells can also be used to induce differentiation resulting in various lineages, apart from dentin, such as osteocytes, adipocytes, chondrocytes, and neurons.

The concept of dental pulp stem cell banking has gained concentration in terms that the cells isolated from the deciduous teeth can be stored and utilized for future treatments since it is well known that dental pulp has stem cells that are multi-lineage [15, 16]. At this point, it is essential to identify the characteristics of deciduous and adult dental pulp mesenchymal stem cells and their differential potential especially across this specific lineage. Recently, stem cells have been isolated and expanded from pulp tissue of permanent teeth, deciduous teeth, periodontal ligament, and apical papilla from an immature tooth, and it has been reported that they generated dentin-like tissue. Subsequent regenerative procedures include the development of guided tissue regeneration (GTR) procedures for osteogenesis, chondrogenesis, neurogenesis, and also dentinogenesis. Gronthos et al. in his study reported that the deciduous tooth contained multipotent stem cells which were extremely proliferative and clonogenic capable of differentiating, into variety of cell types including neural cells, adipocytes and odontoblasts. After in vivo transplantation, they were able to induce bone, generate dentin, and stay alive in mouse brain along with expression of neural markers. Although it could serve a better tool in treating multitude of diseases, its role in osteogenesis and its therapeutic efficacy in bone-related diseases are promising due to its inherent bone formation capacity similar to that of bone marrow stem cells.

Therefore, the present study reviews the need, significance, and advantages of bone marrow stem cells in bone repair and regeneration in the former part. However, during crisis with the negative attributes of bone marrow cells, as mentioned, we demonstrate the dental pulp stem cells might be an ideal alternative source for in vivo bone regeneration capacity in latter half, due to its similarity of inherent bone-forming capacity as that of bone marrow.

Bone Marrow Stem Cells

Bone marrow has traditionally been seen to be composed of two main distinct lineages, the hematopoietic compartment, and the nonhematopoietic cells including the reticular, fat, and endothelial cells, fibroblasts, osteoblasts, and mesenchymal progenitors. Among the cells that compose the stromal tissue, the mesenchymal stem/progenitor cells represents the key component, which are able to differentiate into its progeny, such as osteocytes, chondrocytes, and adipocytes. The mesenchymal stem/progenitor cells have received, in the past years, plenty of attention from the scientific community for their ability to differentiate into different lineages. The first detailed functional description of the tri-lineage differentiation potential of BM-MSCs was provided by Pittenger and colleagues in 1999 [17]. They isolated populations of human BM-MSCs from the bone marrow taken from the iliac crests with a frequency ranging from 1 out of 10.000–100.000, analyzing their immunophenotype and the in vitro differentiation potential. They showed that some of the clones, which they obtained, were able to differentiate in osteoblast, chondroblasts, and adipocytes, pointing out a multipotent ability for these clones. Not every clone retained these abilities. Out of six colonies analyzed, all of them were able to differentiate in osteoblasts, five were able to differentiate in chondrocytes, and only two were able to differentiate in adipocytes, confirming that these populations were very heterogeneous and made

up of multipotent stem cells and progenitors already committed towards a specific cell lineage [17].

The most encouraging differentiation trait of BM-MSCs is osteoblastic differentiation in vitro and bone formation in vivo using beta glycerophosphate, dexamethasone, ascorbic acid, throughout the period of 2–3 weeks. Thus, development of osteoblast differentiation from human bone marrow in combination with biomimetic scaffolds provides the possibility of tissue engineering for bone and cartilage [18]. Osteoblasts are responsible not only for forming bone in the normal adult bone remodeling process but also for providing a specific niche microenvironment for hematopoietic stem cells (HSCs) governed by bone morphogenetic protein (BMP), parathyroid hormone (PTH), and Tie2/angiopoietin-1 signaling pathways [19]. These emerging evidences suggest a functional role of osteogenic cells for controlling HSC niches in vivo [2]. This demonstrates the efficacy of osteogenic potential of BM-MSCs.

Why BM-MSCs in Osteogenesis?

Bone tissue is capable of regeneration, yet the natural bone healing process is in some cases insufficient. This is because regeneration of damaged bone is related to four fundamental processes such as osteogenesis, osteoinduction, osteoconduction, and osteopromotion [20]. Excessive loss of bone due to trauma, tumor resection, nonhealing fractures, and so on are cases which lose natural regeneration process, thus requiring transplantation of large bone tissues or substitutes to restore the structural integrity [21]. The use of autologous and allogenic bone grafts was into clinical practice. Although autogenous, spongeous bone graft was considered the "golden standard" among tissue transplants supporting bone regeneration, but it does not suit ideal to clinical practice. Grafts were associated with donor site morbidity and the possible transmission of diseases [21]. Besides, lack of an adequate supply of autologous bone grafts and the unsuitability of allografts do exists. Attention of contemporary research is therefore directed to the finding of an optimum substitute for the standard bone grafts used. BMPs combined with the osteoconductive materials such as hydroxyapatite, tricalcium phosphate, and so on [22, 23] showed promising results. The application of BMPs has yielded positive results in supporting bone regeneration, yet their exclusively osteoinductive property presents a certain strategic limitation.

Thus, there has been some impetus to use MSCs to encourage repair and regenerate bones. Bone regeneration using transplantation of MSCs alone or combined with biomaterials has become the interesting areas of recent research due to its successful healing of the particular bone defect [24]. Despite the availability of stem cells from various adult tissues [25, 26], bone marrow is gaining consensus from ancient times, due to the fact that these stem cells are abundant in the bone marrow which is their suitable source [27]. When bone integrity is damaged (e.g. after fracture), under normal circumstances, MSCs from bone marrow play an important role in its healing. This is due to its inherent bone formation capacity. During fetal bone development, a part of MSC population in bone marrow remains unchanged and forms the source of undifferentiated stem cells [25]. Repair mechanism takes place by chemoattractive property of MSCs through release of cytokines from the damaged bone matrix. MSCs from periosteum and bone marrow are transferred to the damage site, where they continue to multiply and differentiate into its respective lineages to heal the repair. Some study explains a mechanism where bone regeneration occurs through the migration of distant MSCs from peripheral blood to the site of bone injury were they reinforce the healing potential of local MSCs [28]. Bone regeneration is analogous to embryonic development of the skeleton. It is provided by a sum of cellular, humoral, and mechanical factors involved in the formation of new bone in which MSCs play an important role. It is, thus, validated that MSCs from bone marrow serves an appropriate source of bone regeneration.

Applications of BM-MSCs in Bone Regeneration

Formation of new bones during repair is dependent on the quality of MSCs which is directly proportional to the source of osteogenic lines of cells capable of forming bone matter. In this sense, the strategy of using the MSCs that possess more osteogenic potential transplanted into the bone defect appears promising. Studies on murine model showed very promising results especially for bone repair and metabolic bone disorders [24]. Since their first use in 1951, MSCs have been successfully applied for bone regeneration. The subject of intensive research in the field of tissue engineering is the application of MSCs alone or in combination with suitable scaffolds in order to achieve bone tissue regeneration. For successful tissue engineering approaches, implantation of MSCs will require the use of growth and differentiation factors that will favor differentiation and maintenance of bone or chondrocyte phenotype together with an appropriate scaffold to provide a three-dimensional environment. Defining the optimal combination of stem cells, growth factors and scaffolds is thus essential to provide functional bone and cartilage [21]. This would be a contribution for clinical practice in patients with extensive bone defects (tumor resection, traumatic injuries with bone loss, complicated fractures) or in cases of decreased healing ability of bone tissue (older age, osteoporosis) or genetic diseases of the skeleton (osteogenesis imperfecta) [29].

A number of studies have been performed on the use of growth factors and biomaterials to improve tendon-to-bone healing. Besides, interest in using MSCs for tissue engineering has been validated in numerous preclinical models and is under evaluation in clinics. Several clinical trials are recruited for the therapeutic application of MSCs for cartilage defects, osteoporosis, bone fracture, or osteonecrosis. Methods have also been developed for the expansion of bone marrow osteoprogenitors, which indicates the possibility of using autologous human stromal progenitors in the regeneration of large bone defects [30]. Several researchers have described the purification and expansion of bone marrow cells from mice, rats, rabbits, dogs, and humans, and their repair and functional recovery of diaphyseal defects/segmental bone defects have also been reported with the use of osteoprogenitor cells grown on scaffolds of macroporous of hydroxyapatites or other carriers. Other applications of using MSCs as a vehicle for gene delivery approaches have also been demonstrated [31–34]. After successful BM-MSCs, 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 bone/marrow organ structure (craniofacial). Thus, the use of this cell-based tissue-engineering approach to treat patients with large bone defects is also underway [22], thereby leading to substantial improvement in our ability to repair large defects in long bones.

Apart from tissue-engineering-based approach, several clinical investigators from various parts of the world have reported on the safety and therapeutic effect of direct BM-MSCs administration in patients with osteoarthritis and other bone diseases [22, 35]. As an example, Nejadnik and colleagues [36, 37] compared the efficacy of first-generation autologous chondrocyte implantation with that of autologous BM-MSCs, and identified BM-MSCs for cartilage repair showed a better outcome. Besides, a number of studies on the direct use of MSCs to improve the repair of tendon defects have been carried [38, 39]. As an example, Lim et al. studied the role of MSCs at the tendon-bone junction during reconstruction of the ACL in the rabbit [40].

However, as compared to uncommitted BM-SCs, freshly isolated heterogenous bone marrow cell transplantation has not proven successful. This is because uncommitted BM-MSCs were identified to express many osteogenic markers such as CBFA 1/Runx2, osterix, osteopontin, parathyroid hormone receptor, and osteocalcin which are not expressed by freshly isolated BM-MSCs [41]. Interestingly, study reported that a subset of high-proliferating single colony-derived BM-MSCs clones (approximately 60 %) was capable of forming ectopic bone upon in vivo

transplantation into immunocompromised mice [42]. Ex vivo expanded BM-MSCs successful repair of bone defects has been achieved in both calvaria and long bone in various animal models [43–47].

Dental Stem Cells

The quest for MSC-like cells in different tissues has led to identification of a variety of stem cells in all organs and tissues in the body in the past decades. Dental-tissue-derived MSC-like populations are among many other stem cells residing in specialized tissues that have been isolated and characterized [48]. The first kind of stem cells was isolated from the human pulp tissue and termed "postnatal dental pulp stem cells" (DPSC) [49]. Later, four additional types of dental-MSC-like populations were recognized: stem cells from exfoliated deciduous teeth (SHED) [50], periodontal ligament stem cells (PDLSCs) [51], stem cells from apical papilla (SCAP) [52], and dental follicle precursor cells (DFPCs) [53].

Postnatal Human Dental Pulp Stem Cells (DPSCs)

The postnatal human dental pulp stem cells (DPSCs) were first isolated by Gronthos and colleagues from pulp of permanent teeth and identified as clonogenic and rapidly proliferative stem cells [49]. Studies have demonstrated that multiple-colony-derived DPSCs can have a population doubling of more than 120, single-colony-derived strains of DPSCs proliferate 10–20 population doublings, and approximately two-thirds of the single-colony derived hDPSCs are able to form the same amount of dentin as multi-colony hDPSCs [54]. On comparison of these cells with BM-MSCs, the DPSCs were found to have an identical expression outline for a range of markers related to endothelium, smooth muscle, bone, and fibroblasts, as that for BM-MSCs [49]. The similarity between DPSCs and BM-MSCs was also confirmed by cDNA microarray profiling when DPSCs and BM-MSCs showed similar level of gene expression for more than 4000 known human genes. DPSCs expressed a high level of collagen type XVIII α-1, insulin-like growth factor-2 (IGF-2), discordin domain tyrosine kinase-2, NAD(P)H menadione oxidoreductase, homolog-2 of Drosophila large disk, and cyclin-dependent kinase-6, whereas the insulin-like growth factor binding protein-7 (IGFBP-7), and collagen type I α-2 genes are expressed in high levels in BM-MSCs. However, the functional roles of many of these genes in the development of dentin and bone can be an interesting concept for further study and research in future [55]. Since characterization studies revealed the mesenchymal stem-cell-like qualities of DPSCs such as self-renewal and multi-lineage differentiation potential, recently tri-lineage differentiation of DPSCs adipo-, osteo-, and chondro-differentiation became a common approach for identification of mesenchymal property of these cells in majority of published works [56]. Spontaneous differentiation of STRO-1+ DPSCs into odontoblasts, osteoblasts, and chondrocytes has been also observed in vitro [57].

DPSCs in Osteogenesis

In vitro expanded DPSCs are capable of differentiating into dentin/pulp-like tissue in vivo. In a study, in vitro expanded DPSCs were transplanted into immunocompromised mice with hydroxyapatite/tricalcium phosphate powder (HA/TCP). Six weeks after transplantation, dentin-like structures were observed lining the surface of the hydroxyapatite/tricalcium phosphate particles. Dentin matrix protein markers like bone sialoprotein, osteocalcin, and DSPP were found to be expressed in the DPSC transplants, and generated dentin was found to thicken over time [49]. DPSCs also demonstrated their capability in differentiation into dentin-like structure by seeding onto human dentin surfaces and implanting into immunocompromised mice [58]. It has been shown that DPSCs from inflamed pulps (DPSCs-IPs) has a decreased osteo-/dentinogenic potential when compared

with that of DPSCs normal pulps (DPSCs-NPs) [59]. Nevertheless, on transplantation of DPSCs-IPs into immunocompromised mice, pulp/dentin surfaces similar to that of DPSCs-NPs transplantation is formed [59]. Osteogenic differentiation potential of the human dental pulp cells was discovered when a subpopulation of these cells developed into bone-like tissue in vivo. The cells were termed as osteoblasts derived from human pulpar stem cells (ODHPSCs) [60].

In the field of orofacial and maxillofacial surgery, addition of mass to existing tissue is often essential to reconstruct largely damaged tissue. However, limited availability of autografts, and the inability of allografts to integrate with the surrounding tissue, confines their application [61, 62]. Under such scenarios, stem cell therapy and tissue-engineering technology, or its combination, have been found to be useful. Several studies have documented the in vitro and in vivo osteogenic potential of DPSCs [60–62]. Laino et al. demonstrated the formation of functional lamellar bone constructs in vivo from CD 44+/RunX 2+ (osteoblast precursor marker) differentiated DPSCs [63]. The potential of DPSCs to catalyze responses required to restore tooth structure and function following clinical procedures has been successfully utilized in regenerative endodontics [64]. Recent findings revealing the critical ability of DPSCs to vascularize engineered constructs has expanded its potential in hard tissue engineering [65–67].

Previous studies have also investigated the in vitro and in vivo behavior of DPSCs on 2-D and 3-D collagen, ceramic, and titanium scaffolds directed towards applications in orofacial tissue engineering [68–70]. Reports involving *3-D* porous HA/TCP showed bone-like hard tissue formation by STRO-1 selected DPSCs with distinct lamellae structure and bone marrow-like tissue [71]. In a clinical study, DPSCs were used in conjunction with a collagen sponge scaffold to repair alveolar bone defects caused due to wisdom tooth extraction [72]. Results from these investigations conclude that DPSCs in combination with a suitable scaffold system provide immense potential for the repair and regeneration of periodontal and maxillofacial tissues. However, the above-mentioned studies make use of porous scaffolds into which DPSCs are seeded for the purpose of reconstructing dental or bone tissue. Immobilization ensures sustainability and functionality of cells while avoiding physical stress and inflammatory responses caused at transplantation or delivery sites. Several types of biomaterials, synthetic polymers like polydimethylsiloxane (PDMS), polyethylene glycol (PEG), and natural materials like silk, collagen, and alginate have been adopted over the past decade to immobilize cells [73–75]. The material characteristics of alginate allow its mechanical strength, permeability, and degradability to be tailored to application requirements. The diffusibility of alginate under physiological conditions allows timely release of cells as well as replacement of the biomaterial with regenerated tissue. In addition, the hydrophilic nature of cross-linked alginate provides a framework similar to the extracellular matrix in which cells proliferate, differentiate, and form a functional tissue [76]. Recent studies have investigated the potential of periodontal ligament stem cells (PDLSCs) and gingival mesenchymal stem cells (GMSCs) encapsulated in oxidized alginate micro beads for applications in bone tissue engineering [77].

Results obtained from these studies investigating the morphology, growth, proliferation, immunophenotype, and genotype expression is crucial in determining the potential of utilizing immobilized DPSCs to deliver stem cells and to engineer functional native tissue constructs for oral and maxillofacial bone regeneration applications. Results from a study clearly emphasized the significance of immobilization of DPSCs in 3-D calcium alginate microspheres, leading to consistent cell survivability and functionality. In the same study, the improved osteogenic differentiation of immobilized DPSCs was evidenced by enhanced mineralization, protein secretion, and an upregulated osteo-related gene profile, and interestingly, it was also shown that immobilization triggered osteogenic differentiation of DPSCs without any use of conventional induction factors.

Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

Primary teeth also contain stem cells that referred as SHED [50]. Gronthos et al. in his study reported that the deciduous tooth contained multipotent stem cells which were extremely proliferative and clonogenic capable of differentiating into variety of cell types including neural cells, adipocytes, and odontoblasts. After in vivo transplantation, they were able to induce bone, generate dentin, and stay alive in mouse brain along with expression of neural markers. Although it could serve a better tool in treating multitude of diseases, its role in osteogenesis and its therapeutic efficacy in bone-related diseases are promising due to its similar characteristics inherent bone formation capacity similar to that of bone marrow stem cells.

Isolation of high-quality human postnatal stem cells from accessible resources is usually a priority in the field of stem cell research. As every child loses milk teeth, the obtaining of SHED from them becomes a simple and convenient when compared with other sources of stem cells like as BM-MSCs; hence, this property has given a considerable advantage to SHED among other type of stem cells. In comparison to BM-MSCs, SHED are found to have a higher cell proliferation rate and show a higher number of single colony clusters (CFU-F) [78]. They are also able to proliferate more than 140 population doublings, which is significantly higher than BM-MSCs and DPSCs [50]. SHED are CD34-, CD45-, STRO-1+, SSEA4+, CD73+, CD105+, CD146+, and CD166+. These cells show significant higher levels of STRO-1 and CD146 and lower levels of CD105 [78]. Immature DPSCs (IDPSCs), stem cells isolated from deciduous teeth, have embryonic stem cell markers like Oct4, Nanog, stage-specific embryonic antigens (SSEA-3, SSEA-4), and tumor recognition antigens (TRA-1-60 and TRA-1-81) [79]. Heterogeneous population of SHED has molecular similarity with neural crest cells and stem cells in vitro.

SHED in Osteogenesis

SHED, on osteogenic induction medium, form alizarin red positive nodules, and various bone markers like CBFA1, ALP, MEPE, and bone sialoprotein get upregulated, indicating calcium accumulation and the ability of SHED to differentiate into odontoblastic lineage in vitro [50]. One-month-old culture of SHED-derived osteoblasts secreted extracellular mineralized matrix which went on to develop into 3D woven bone samples in vitro. These cells were positive for alkaline phosphates (ALP), alizarin red, and calcium and to specific antibodies [80]. Myogenic and chondrogenic potentials of SHED have also been demonstrated [79].

In vivo odontoblastic differentiation potential of SHED was demonstrated by transplanting the ex vivo expanded SHED into immunocompromised mice, where these cells developed into human-specific Alu-positive odontoblasts directly associated with a dentin-like structure, while the regenerated dentin being immune reactive to dentin sialophosphoprotein (DSPP), a dentin-specific antibody [70]. However, in vivo complete dentin pulp-like complex regeneration of SHED is not possible [50]. Although SHED are not able to differentiate directly into osteoblasts, but they found to be capable of inducing recipient murine cells to osteocytes, when transplanted into immunocompromised mice [50]. One-fourth of the single-colony-derived SHED clones exhibited the ability to generate ectopic dentin-like tissue equivalent to that generated by multi-colony-derived SHED, while all the single-colony-derived SHED clones were capable of inducing bone formation in immunocompromised mice. Therapeutic potential of SHED was discovered when SHED were found to be able to repair bone defects. In vivo transplantation of SHED-derived bone samples into immune-suppressed rats gave rise to lamellar bone containing entrapped osteocytes [80]. Another study revealed that in the process of SHED-mediated osteogenesis, the hematopoietic marrow elements often found in bone marrow

mesenchymal stem cell-generated bone were absent, while mesenchymal stem cell markers like CC9/MUC18/CD146, with an array of growth factor receptors such as transforming growth factor receptors I and II, fibroblast growth factor receptors I and III, and vascular endothelial growth factor receptor I were co-expressed, implying their comprehensive differentiation potential [81]. In vivo transplantation of SHED into immunocompromised mice demonstrated dense engraftment of these cells in various tissues and organs like the liver, spleen, and kidney; hence, the relative ease of recovery and the expression profiles of various markers justify further investigation of SHED for treatment of diseases [79].

Bony defects in the craniomaxillofacial skeleton remain a major and challenging health concern. Maxillofacial surgeons have been trying for centuries to restore functionality and aesthetic appearance applying different strategy including cell-based and protein-based therapies as new strategies without entirely satisfactory results. Nowadays SHED has been proved as a potential source of stem cells to be used in plastic surgery, particularly among craniofacial anomalies. The results of an investigation in the field of stem cell therapy which has been conducted to assess potential of SHED in reconstruction of large-sized cranial bone defects in non-immune-suppressed rats were shown that these stem cells with collagen membrane are able to induce new bone formation at the site of defects without stimulation of the allogenic graft rejection by recipient organism [82].

The curative efficacy of SHED in orofacial bone defects has also been proved when isolated stem cells from miniature pig deciduous teeth, engrafted into pre-generated critical-size bone defects in swine mandible models. Results of this study indicated that stem cells from miniature pig deciduous teeth are able to engraft and regenerate bone to repair critical-size mandibular defects [83]. Recently scientists have suggested that the tissue-engineered bone complex with nano-hydroxyapatite/collagen/poly (L-lactide) nHAC/PLA, recombinant human bone morphogenetic protein 2 (rhBMP-2), and autologous DPSCs might be a better alternative to autologous bone for the clinical reconstruction of periodontal bone defects. In this connection, the capacity of a tissue-engineered bone complex of rhBMP-2 mediated DPSCs and nHAC/PLA to reconstruct critical-size alveolar bone defects in rabbit was evaluated. Findings of this study indicated that nHAC/PLA is an acceptable scaffold for autologous DPSC seeding, proliferation, and differentiation and rhBMP-2 promotes osteogenic capability of DPSCs as a potential cell source for periodontal bone regeneration [84].

DPSCs and Osteogenesis: Our Short Experience

The abovementioned studies make use of porous scaffolds into which DPSCs are seeded for the purpose of reconstructing dental or bone tissue. As such, approaches involving DPSC immobilization aimed at stem cell delivery and hard tissue engineering need to be investigated. With regard to these discussed literatures, we investigated and examined the impact of immobilization on viability and osteogenic differentiation of DPSCs. Morphological analyses correlate with current literature that DPSCs assume spherical shape when immobilized due to matrix tension [77, 85–87]. It can also be inferred from our results that parameters governing immobilization like the concentration of alginate used, the choice of the cross-linker, its molar concentration, and the cell density (2×10^6 cells/mL of alginate) did not hinder the viability of cells (Fig. 1). Initial decrease in the viability of immobilized DPSCs could be attributed to cell shock observed due to the change in the microenvironment (*2-D to 3-D*). Immobilization causes mechanical stress on the cytoskeleton thereby influencing cell behavior. When exposed to sufficiently high stress, it is possible that cells undergo programmed death [88, 89].

The viability and proliferation of immobilized DPSCs were also compared to DPSCs grown in *2-D*. Results show that cells proliferated at a higher rate in *2-D* and reached saturation on day

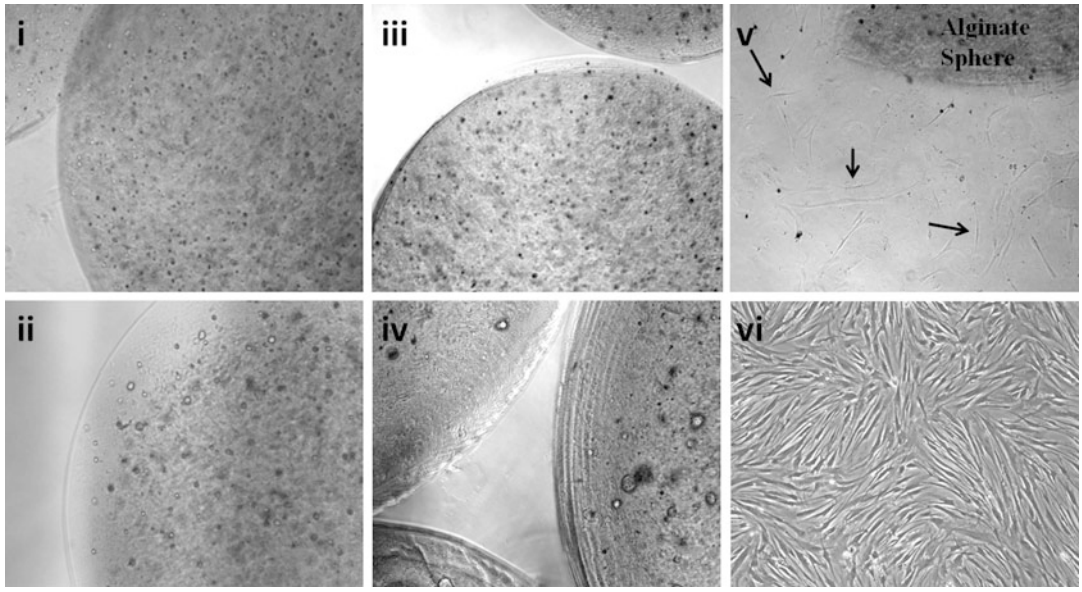

Fig. 1 Micrographs of DPSCs immobilized in alginate microspheres and supplied with conventional MSC medium (control spheres) on days 1 (i) and 10 (ii) supplied with osteogenic induction medium – induced spheres – on days 1 (iii) and 10 (iv) (*arrows* point towards cell aggregates). Immobilized DPSCs adhering to the culture dish upon release (v) (*arrows* point towards DPSC attaining fibroblast-like morphology). DPSCs in passage 3 in 2-D culture on reaching confluency (vi)

4 upon occupation of available surface area in the culture dish. Observably, the percentage viability of these cells reduced after day 4. Immobilized cells interact with the matrix, respond to mechanical cues, and undergo controlled proliferation. It can be concluded that while an immobilization matrix provides a more natural environment for the cells to grow in, it does not provide a platform for the expansion of cells. Immunophenotype analysis of the immobilized cells in *control spheres* on day 10 of culture revealed the expression of cell surface marker CD 73 and CD 90. This confirms that the stem cell characteristics of DPSC had not changed due to immobilization. Similarly, DPSCs in *induced spheres* marked for osteocalcin, a late marker of osteoblastic differentiation, showed maximum expression in aggregations of cells. Minimal osteocalcin expression was also observed in control spheres although the cells did not form aggregates. Calcium quantification analysis showed high calcium content in *induced spheres* as compared to DPSCs differentiated in *2-D*. Osteocalcin expression and the presence of calcium indicate that the alginate matrix provides optimal support for DPSCs to form aggregates, secrete bone-related proteins, and calcify the matrix when differentiation is induced. Alizarin red staining showed significant mineralization in *induced spheres* on days 14 and 21. Data concerning Alizarin red staining of matrix mineralization in immobilization systems are rare. However, reports of the use of alizarin red staining to support osteo-differentiation data have emerged lately [90]. As alginate hydrogels are capable of supplying nutrients to cells, the stain can also be diffused to interact with any mineralization. However, several wash steps need to be incorporated to remove unspecific binding of the stain. In this study, we found that results from Alizarin red staining corroborates with immunocytochemical and calcium quantification data indicating Alizarin red staining as yet another technique for determining mineralization.

It is likely that a *3-D* immobilization matrix, as against 2D, caused similar responses in DPSCs thereby elevating the production of the matrix proteins. Surprisingly, the expression of osteo-specific genes were also elevated in DPSC *control*

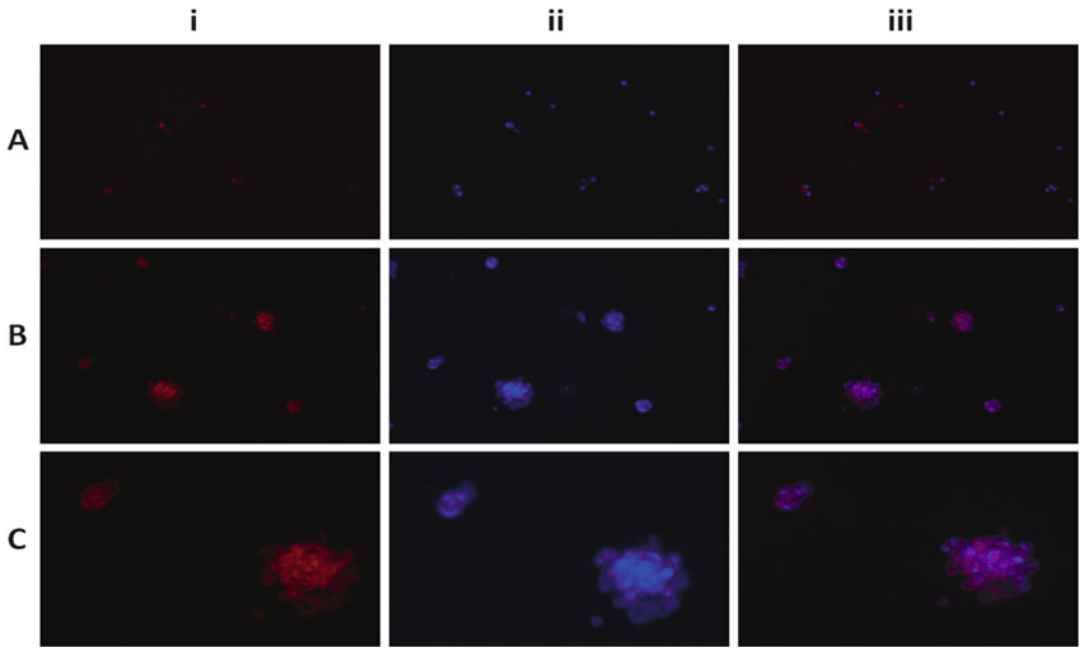

Fig. 2 Immunocytochemical evaluation of osteogenesis of immobilized DPSCs using osteoblastic specific marker, osteocalcin on day 21. (A in 10x) Individual DPSCs in control spheres (without any supplementation of induction medium) expressing osteocalcin. (B in 10x) Osteocalcin expression in cell aggregates in differentiation-induced spheres. (C in 20x) Osteocalcin expression can be observed clearly in cell aggregates in an induced sphere. PE-conjugated osteocalcin is expressed in (i), DAPI used to counterstain the nuclei of the cells is expressed in (ii), and the composite of the images is presented as (iii)

spheres supplemented with conventional MSC media suggesting correlation of the ectodermal origin of DPSCs with osteogenesis (Fig. 2). Stem cells from the human bone marrow (BM-MSCs), without supplementation of induction factors, have been shown to express osteogenic associated markers like OCN, osteopontin (BSP1), and ALP when grown on the surface of unmodified alginate [91]. DPSCs, like BM-MSCs, are equally capable of differentiating into osteoblasts by responding to specific environmental signals. As such, the presence of the markers could be due to (a) the innate quality of DPSCs being naturally prone to differentiate along the osteo-lineage owing to the source from which they are obtained and (b) the 3-D environment created by the alginate scaffold that allows cell-cell interaction imitating the physiological environment.

Results from this study clearly exhibited the significance of immobilization of DPSCs in 3-D calcium alginate microspheres leading to consistent cell survivability and functionality. The improved osteogenic differentiation of immobilized DPSCs was evidenced by enhanced mineralization, protein secretion, and an upregulated osteo-related gene profile. Interestingly, it was also shown that immobilization triggered osteogenic differentiation of DPSCs without any use of conventional induction factors. Collectively, our results demonstrate the potential of immobilized DPSCs to be utilized in stem cell delivery and hard tissue regeneration.

Why DPSCs Are Better than BM-MSCs at Instances

Irrespective of its prehistoric source, bone marrow-derived stem cells were not promising in attempting curative therapies for all diseases.

It became acknowledged from the advancement occurred in bone marrow stem cells by understanding the basic biology and molecular pathways. The first and foremost of the disadvantages put forward is the frequency of lesser number of nucleated cells obtained from large quantity of sample [92]. The second important disadvantage of BM-MSCs is that the proliferation and differentiation capacity of MSC decline with age, reducing their therapeutic potential [92, 93]. Additionally, low frequency of mesenchymal stem cell and the heterogeneity of mononuclear cells with granulocyte interface might create a threat for cell migration and engraftment [94, 95].

In concert with the decreased osteogenic potential of bone marrow cells with age, adipocytes accumulate in the bone marrow stroma. In neonates, adipocytes are barely present in the bone marrow stroma, but the number and size of stromal adipocytes increase with aging, and more than 90 % of the bone marrow cavity is occupied by adipocytes in the aged bone [96]. Interestingly, adipocyte accumulation in the human bone marrow stroma correlates with trabecular bone loss with aging [96–98]. Mice with premature aging (SAMP 6 strain) also show decreased bone formation and increased number of adipocytes in the bone marrow stroma. In hypokinetic rats, bone loss resulting from a decreased osteoblast number [99] is associated with increased adipocyte number and size in the bone marrow cavity. This inverse correlation between the two differentiation processes suggests the disadvantage of bone marrow.

Conclusion

This volume, thus, demands alternative valuable source of stem cell similar to that of bone marrow without compromising its quality. This opens the interesting possibility of promoting dental pulp stem cells. Discovery and advances in dental pulp stem cell biology and behavior have blazed new hopes and promises in the field of regenerative medicine. Although dental pulp stem cells are easily accessible and very good resource of MSCs, [100] conflicting results, possibly due to donor-associated variability, reduce its potential applicability [101], thereby, demanding further tremendous amount of work in order to complement the recent advances in bone tissue engineering. Some important hurdles need to be addressed include multi-differentiation potential, bioscaffolds, and inductive factors that implants and integrates into the surrounding environment for the reconstruction of functional complex organ systems.

References

1. Kumar AA, Kumar SR, Narayanan R, Arul K, Baskaran M (2009) Autologous bone marrow derived mononuclear cell therapy for spinal cord injury: a phase I/II clinical safety and primary efficacy data. Exp Clin Transplant 7:241–248
2. Miura Y, Gao Z, Miura M, Seo B-M, Sonoyama W, Chen W, Gronthos S, Zhang L, Shi S (2006) Mesenchymal stem cell-organized bone marrow elements: an alternative hematopoietic progenitor resource. Stem Cells 24:2428–2436
3. Hattan N, Kawaguchi H, Ando K, Kuwabara E, Fujita J, Murata M, Suematsu M, Mori H, Fukuda K (2005) Purified cardiomyocytes from bone marrow mesenchymal stem cells produce stable intracardiac grafts in mice. Cardiovasc Res 65:334–344
4. Mimeault M, Batra SK (2006) Concise review: recent advances on the significance of stem cells in tissue regeneration and cancer therapies. Stem Cells 24:2319–2345
5. Oh S-H, Muzzonigro TM, Bae S-H, LaPlante JM, Hatch HM, Petersen BE (2004) Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. Lab Invest 84:607–617
6. Wilson A, Trumpp A (2006) Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol 6:93–106
7. Kopp H-G, Avecilla ST, Hooper AT, Rafii S (2005) The bone marrow vascular niche: home of HSC differentiation and mobilization. Physiology 20:349–356
8. Rebelatto CK, Aguiar AM, Moreto MP, Senegaglia AC, Hansen P, Barchiki F, Oliveira J, Martins J, Kuligovski C, Mansur F, Christofis A, Amaral VF, Brofman PS, Goldenberg S, Nakao LS, Correa A (2008) Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med 233:901–913
9. Meunier P, Aaron J, Edouard C, Vignon G (1971) Osteoporosis and the replacement of cell

populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. Clin Orthop 80:147–154
10. Verma S, Rajaratnam JH, Denton J, Hoyland JA, Byers RJ (2002) Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis. J Clin Pathol 55:693–698
11. Burkhardt R, Kettner G, Bohm W, Schmidmeier M, Schlag R, Frisch B, Mallmann B, Eisenmenger W, Gilg T (1987) Changes in trabecular bone, hematopoiesis and bone marrow vessels in aplastic anemia, primary osteoporosis, and old age: a comparative histomorphometric study. Bone 8:157–164
12. Jilka RL, Weinstein RS, Takahashi K, Parfitt AM, Manolagas SC (1996) Linkage of decreased bone mass with impaired osteoblastogenesis in a murine model of accelerated senescence. J Clin Invest 97:1732–1740
13. Rodriguez AM, Elabd C, Amri E-Z, Ailhaud G, Dani C (2005) The human adipose tissue is a source of multipotent stem cells. Biochimie 87:125–128
14. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295
15. Kanafi MM, Pal R, Gupta PK (2013) Phenotypic and functional comparison of optimum culture conditions for upscaling of dental pulp stem cells. Cell Biol Int 37(2):126–136
16. Kanafi M, Ramesh A, Gupta P, Bhonde R (2014) Dental pulp stem cells immobilized in alginate microspheres for applications in bone tissue engineering. Int Endod J 47(7):687–697
17. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
18. Kamran Kaveh, Rashid Ibrahim, Md. Zuki Abu Bakar, Tengku Azmi Ibrahim (2011) Mesenchymal stem cells, osteogenic lineage and bone tissue engineering: a review. J Anim Vet Adv 10: 2317–2330
19. Arai F, Ohneda O, Miyamoto T, Zhang XQ, Suda T (2002) Mesenchymal stem cells in perichondrium express activated leukocyte cell adhesion molecule and participate in bone marrow formation. J Exp Med 195:1549–1563
20. Albrektsson T, Johansson C (2001) Osteoinduction, osteoconduction and osteointegration. Eur Spine J 10:S96–S101
21. Patel DM, Shah J, Srivastava AS (2013). Therapeutic potential of mesenchymal stem cells in regenerative medicine. Stem Cells Int:496218
22. Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M (2001) Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 344(5):385–386
23. Marcacci M, Kon E, Moukhachev V, Lavroukov A, Kutepov S, Quarto R, Mastrogiacomo M, Cancedda R (2003) Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng 13(5): 947–955
24. Griffin M, Iqbal SA, Bayat A (2011) Exploring the application of mesenchymal stem cells in bone repair and regeneration. J Bone Joint Surg Br 93(4):427–434
25. Stocum DL (2000) Stem cells in regenerative biology and medicine. Wound Repair Regen 9(6): 429–442
26. Fuchs E, Segre JA (2000) Stem cells: a new lease on life. Cell 100:143–155
27. Li M, Ikehara S (2013) Bone-marrow-derived mesenchymal stem cells for organ repair. Stem Cells Int:132642
28. Devine MJ, Mierisch CM, Jang E, Anderson PC, Balian G (2002) Transplanted bone marrow cells localize to fracture callus in a mouse model. J Orthop Res 20:1232–1239
29. Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WWK, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, Brenner MK (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med 5:309–313
30. Ahdjoudj S, Lasmoles F, Holy X, Zerath E, Marie PJ (2002) Transforming growth factor beta2 inhibits adipocyte differentiation induced by skeletal unloading in rat bone marrow stroma. J Bone Miner Res 17:668–677
31. Goshima J, Goldberg VM, Caplan AI (1991) Osteogenic potential of culture-expanded rat marrow cells as assayed in vivo with porous calcium phosphate ceramic. Biomaterials 12:253–258
32. Gundle R, Joyner CJ, Triffitt JT (1995) Human bone tissue formation in diffusion chamber culture in vivo by bone-derived cells and marrow stromal fibroblastic cells. Bone 16:597–601
33. Bruder SP, Kurth AA, Shea M et al (1998) Bone regeneration by implantation of purified culture-expanded human mesenchymal stem cells. J Orthop Res 16:155–162
34. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP (1997) Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. J Cell Biochem 64:295–312
35. Warnke PH, Springer IN, Wiltfang J et al (2004) Growth and transplantation of a custom vascularised bone graft in a man. Lancet 364:766–770
36. Nejadnik H, Hui JH, Feng Choong EP, Tai B-C, Lee EH (2010) Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. Am J Sports Med 38:1110–1116
37. Gupta PK, Das AK, Chullikana A, Majumdar AS (2012) Mesenchymal stem cells for cartilage repair in osteoarthritis. Stem Cell Res Ther 3(4):25

38. Young RG, Butler DL, Weber W et al (1998) Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res 16:406–413
39. Awad HA, Boivin GP, Dressler MR et al (2003) Repair of patellar tendon injuries using a cell-collagen composite. J Orthop Res 21:420–431
40. Lim JK, Hui J, Li L et al (2004) Enhancement of tendon graft osteointegration using mesenchymal stem cells in a rabbit model of anterior cruciate ligament reconstruction. Arthroscopy 20:899–910
41. Gronthos S, Zannettino ACW, Hay SJ, Shi S, Graves SE, Kortesidis A, Simmons PJ (2003) Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. J Cell Sci 116:1827–1835
42. Kuznetsov SA, Friedenstein AJ, Robey PG (1997) Factors required for bone marrow stromal fibroblast colony formation in vitro. Br J Haematol 97:561–570
43. Bruder SP, Horowitz MC, Mosca JD, Haynesworth SE (1997) Monoclonal antibodies reactive with human osteogenic cell surface antigens. Bone 21:225–235
44. Krebsbach PH, Mankani MH, Satomura K, Kuznetsov SA, Robey PG (1998) Repair of craniotomy defects using bone marrow stromal cells. Transplantation 66:1272–1278
45. Kon E, Muraglia A, Corsi A et al (2000) Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. J Biomed Mater Res 49:328–337
46. Petite H, Viateau V, Bensaid W et al (2000) Tissue-engineered bone regeneration. Nat Biotechnol 18:959–963
47. Mankani MH, Krebsbach PH, Satomura K, Kuznetsov SA, Hoyt R, Robey PG (2001) Pedicled bone flap formation using transplanted bone marrow stromal cells. Arch Surg 136:263–270
48. Huang GT-J, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806
49. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630
50. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG et al (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812
51. Seo B-M, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J et al (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155
52. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S et al (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34(2):166–171
53. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C et al (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165
54. Suchanek J, Soukup T, Visek B, Ivancakova R, Kucerova L, Mokry J (2009) Dental pulp stem cells and their characterization. Biomed Pap Med Fac Univ Palacký Olomouc Czech 153(1):31–35
55. Yamada Y, Fujimoto A, Ito A, Yoshimi R, Ueda M (2006) Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy. Biomaterials 27(20):3766–3781
56. Koyama N, Okubo Y, Nakao K, Bessho K (2009) Evaluation of pluripotency in human dental pulp cells. J Oral Maxillofac Surg 67(3):501–506
57. Yu J, He H, Tang C, Zhang G, Li Y, Wang R et al (2010) Differentiation potential of STRO-1+ dental pulp stem cells changes during cell passaging. BMC Cell Biol 11:32
58. Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S et al (2003) Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 82(12):976–981
59. Alongi DJ, Yamaza T, Song Y, Fouad AF, Romberg EE, Shi S et al (2010) Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regen Med 5(4):617–631
60. Carinci F, Papaccio G, Laino G, Palmieri A, Brunelli G, D'Aquino R et al (2008) Comparison between genetic portraits of osteoblasts derived from primary cultures and osteoblasts obtained from human pulpar stem cells. J Craniofac Surg 19(3):616–625, discussion 626–627
61. Liu H-C EL-L, Wang D-S, Su F, Wu X, Shi Z-P et al (2011) Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly(L-lactide). Tissue Eng Part A 17(19-20):2417–2433
62. Mori G, Centonze M, Brunetti G, Ballini A, Oranger A, Mori C et al (2010) Osteogenic properties of human dental pulp stem cells. J Biol Regul Homeost Agents 24(2):167–175
63. Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F et al (2005) A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). J Bone Miner Res 20(8):1394–1402
64. Huang GT-J (2011) Dental pulp and dentin tissue engineering and regeneration: advancement and challenge. Front Biosci (Elite Ed) 3:788–800
65. Marchionni C, Bonsi L, Alviano F, Lanzoni G, Di Tullio A, Costa R et al (2009) Angiogenic potential of human dental pulp stromal (stem) cells. Int J Immunopathol Pharmacol 22(3):699–706
66. Hazaimeh-NAL, Beattie J, Duggal M, Yang XB (2012). Angiogenic potential of human dental pulp

stem cells for skeletal tissue engineering. J Bone Joint Surg:94-B (SUPP XXXVI 43)
67. Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD et al (2008) Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells Dayt Ohio 26(3):638–645
68. Zhang W, Walboomers XF, van Kuppevelt TH, Daamen WF, Bian Z, Jansen JA (2006) The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials 27(33):5658–5668
69. Prescott RS, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J et al (2008) In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J Endod 34(4):421–426
70. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S et al (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 34(8):962–969
71. Yang X, Walboomers XF, van den Beucken JJJP, Bian Z, Fan M, Jansen JA (2009) Hard tissue formation of STRO-1-selected rat dental pulp stem cells in vivo. Tissue Eng Part A 15(2):367–375
72. D' Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A et al (2009) Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 18:75–83
73. Bünger CM, Tiefenbach B, Jahnke A, Gerlach C, Freier T, Schmitz KP et al (2005) Deletion of the tissue response against alginate-pll capsules by temporary release of co-encapsulated steroids. Biomaterials 26(15):2353–2360
74. Kasoju N, Bora U (2012) Silk fibroin based biomimetic artificial extracellular matrix for hepatic tissue engineering applications. Biomed Mater Bristol Engl 7(4):045004
75. Munoz-Pinto DJ, Jimenez-Vergara AC, Hou Y, Hayenga HN, Rivas A, Grunlan M et al (2012) Osteogenic potential of poly (ethylene glycol)-poly(dimethylsiloxane) hybrid hydrogels. Tissue Eng Part A 18(15–16):1710–1719
76. Drury JL, Mooney DJ (2003) Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24(24):4337–4351
77. Moshaverinia A, Chen C, Akiyama K, Ansari S, Xu X, Chee WW et al (2012) Alginate hydrogel as a promising scaffold for dental-derived stem cells: an in vitro study. J Mater Sci Mater Med 23(12): 3041–3051
78. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S et al (2010) Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther 1(1):5
79. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV et al (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184(3–4):105–116
80. Laino G, Graziano A, d' Aquino R, Pirozzi G, Lanza V, Valiante S et al (2006) An approachable human adult stem cell source for hard-tissue engineering. J Cell Physiol 206(3):693–701
81. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K et al (2008) SHED repair critical-size calvarial defects in mice. Oral Dis 14(5):428–434
82. De Mendonça CA, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD et al (2008) Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. J Craniofac Surg 19(1):204–210
83. Zheng Y, Liu Y, Zhang CM, Zhang HY, Li WH, Shi S et al (2009) Stem cells from deciduous tooth repair mandibular defect in swine. J Dent Res 88(3): 249–254
84. Liu H-C, L-L E, Wang D-S, Su F, Wu X, Shi Z-P et al (2011) Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly (L-lactide). Tissue Eng Part A 17(19–20):2417–2433
85. Aquino D', Riccardo ADR, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G (2009) Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 18:75–83
86. Markusen JF, Mason C, Hull DA, Town MA, Tabor AB, Clements M et al (2006) Behavior of adult human mesenchymal stem cells entrapped in alginate-GRGDY beads. Tissue Eng 12(4):821–830
87. Nuttelman CR, Tripodi MC, Anseth KS (2004) In vitro osteogenic differentiation of human mesenchymal stem cells photoencapsulated in PEG hydrogels. J Biomed Mater Res A 68(4):773–782
88. Fletcher DA, Mullins RD (2010) Cell mechanics and the cytoskeleton. Nature 463(7280):485–492
89. Discher DE, Mooney DJ, Zandstra PW (2009) Growth factors, matrices, and forces combine and control stem cells. Science (New York, NY) 324(5935):1673–1677
90. Penolazzi L, Tavanti E, Vecchiatini R, Lambertini E, Vesce F, Gambari R et al (2010) Encapsulation of mesenchymal stem cells from Wharton's jelly in alginate microbeads. Tissue Eng Part C Methods 16(1):141–155
91. Wang L, Shelton RM, Cooper PR, Lawson M, Triffitt JT, Barralet JE (2003) Evaluation of sodium alginate for bone marrow cell tissue engineering. Biomaterials 24(20):3475–3481
92. Rebelatto CK, Aguiar AM, Moreto MP, Senegaglia AC, Hansen P, Barchiki F, Oliveira J, Martins J, Kuligovski C, Mansur F, Christofis A, Amaral VF, Brofman PS, Goldenberg S, Nakao LS, Correa A (2008) Dissimilar differentiation of mesenchymal

stem cells from bone marrow, umbilical cord blood, and adipose tissue. Exp Biol Med 233:901–913
93. Stolzing A, Jones E, McGonagle D, Scutt A (2008) Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 129:163–173
94. Kuethe F, Richartz BM, Kasper C, Sayer HG, Hoeffken K, Werner GS, Figulla HR (2005) Autologous intracoronary mononuclear bone marrow cell transplantation in chronic ischemic cardiomyopathy in humans. Int J Cardiol 100: 485–491
95. Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, Blake J, Schwager C, Eckstein V, Ansorge W, Ho AD (2005) Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 33: 1402–1416
96. Rozman C, Feliu E, Berga L, Reverter JC, Climent C, Ferran MJ (1989) Age-related variations of fat tissue fraction in normal human bone marrow depend both on size and number of adipocytes: a stereological study. Exp Hematol 17:34–37
97. Charbord P, Tavian M, Humeau L, Peault B (1996) Early ontogeny of the human marrow from long bones: an immunohistochemical study of hematopoiesis and its microenvironment. Blood 87:4109–4119
98. Kirkland JL, Tchkonia T, Pirtskhalava T, Han J, Karagiannides I (2002) Adipogenesis and aging: does aging make fat go MAD? Exp Gerontol 37:757–767
99. Machwate M, Zerath E, Holy X, Hott M, Modrowski D, Malouvier A, Marie PJ (1993) Skeletal unloading in rat decreases proliferation of rat bone and marrow-derived osteoblastic cells. Am J Physiol 26:E790–E799
100. Alge DL, Zhou D, Adams LL, Wyss BK, Shadday MD, Woods EJ, Gabriel Chu TM, Scott Goebel W (2010) Donor-matched comparison of dental pulp stem cells and bone marrow-derived mesenchymal stem cells in a rat model. J Tissue Eng Regen Med 4(1):73–81
101. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S (2005) The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 8:191–199