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Concise Reviews: Characteristics and Potential Applications of Human Dental Tissue-Derived Mesenchymal Stem Cells

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ABSTRACT

Recently, numerous types of human dental tissue-derived mesenchymal stem cells (MSCs) have been isolated and characterized, including dental pulp stem cells, stem cells from exfoliated deciduous teeth, periodontal ligament stem cells, dental follicle progenitor cells, alveolar bone-derived MSCs, stem cells from apical papilla, tooth germ progenitor cells, and gingival MSCs. All these MSC-like cells exhibit self-renewal, multilineage differentiation potential, and immunomodulatory properties. Several studies have demonstrated the potential advantages of dental stem cell-based approaches for regenerative treatments and immunotherapies. This review outlines the properties of various dental MSC-like populations and the progress toward their use in regenerative therapy. Several dental stem cell banks worldwide are also introduced, with a view toward future clinical application. STEM CELLS *2015;33:627–638*

INTRODUCTION

Mesenchymal stem cells (MSCs) are spindle-shaped cells with the potential for clonogenic proliferation. MSCs were initially reported as fibroblast-like cells that could be isolated from bone marrow via their adherence to plastic in culture and subsequently confirmed as a population (the colony-forming unit-fibroblast) of bone-marrow-derived nonhematopoietic cells [1].

MSCs can differentiate into all mesodermal lineages, which prompted the investigation into the role of MSCs in mediating tissue regeneration [2]. The capacity for the differentiation of MSCs into mesodermal [3], ectodermal [4], and endodermal [5] cell lineages has since been fully characterized and forms the basis for most current work on bone marrow-derived MSCs (BMMSCs). In 2006, the International Society for Cellular Therapy (ISCT) [6] proposed the minimal characterization criteria for human MSCs, including their propensity for adherence to plastic when maintained under standard culture conditions and their ability to differentiate into osteoblasts, adipocytes, and chondroblasts in vitro. In addition, most ($\geq$95%) MSCs positively express CD105 (endoglin), CD73 (ecto-5′-nucleotidase), and CD90 (Thy1) while negatively expressing ($\leq$2%) CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR [6].

Since the discovery and characterization of BMMSCs, MSC-like populations from other tissues have been characterized based on the standard criteria established for BMMSCs [1–3, 6, 7]. In addition to bone marrow, MSC populations can be readily obtained from skeletal muscle [8] and a variety of other tissues, such as umbilical cord blood [9], synovium [10], the liver [11], adipose tissue [12], the lungs [13], amniotic fluid [14], tendons [15], placenta [16], skin [17], and breast milk [18].

The search for MSC-like cells in specific tissues led to the discovery of a distinctive population of MSCs from a variety of human dental tissues during previous decades. To date, eight unique populations of dental tissue-derived MSCs have been isolated and characterized. Postnatal dental pulp stem cells (DPSCs) were the first human dental MSCs to be identified from pulp tissue [19]. Gradually, other dental MSC-like populations, such as stem cells from human exfoliated deciduous teeth (SHED) [20], periodontal ligament stem cells (PDLSCs) [21], dental follicle progenitor cells (DFPCs) [22], alveolar bone-derived MSCs (ABMSCs) [23], stem cells from apical papilla (SCAP) [24], tooth germ progenitor cells (TGPCs) [25], and gingival MSCs (GMSCs) [26], were also reported (Fig. 1).

Preliminary data suggest that these dental tissue-derived MSCs not only display self-renewal and multidifferentiation potential but

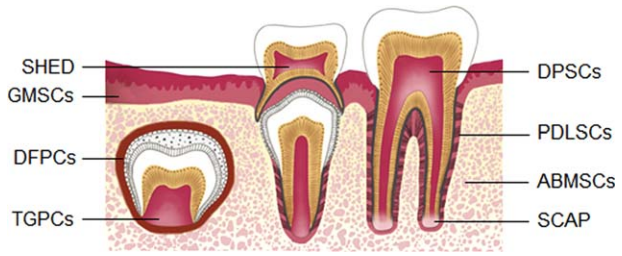

Figure 1. Schematic drawing illustrating sources of human dental tissue-derived MSCs. Abbreviations: ABMSCs, alveolar bone-derived mesenchymal stem cells; DFPCs, dental follicle progenitor cells; DPSCs, dental pulp stem cells; GMSCs, gingiva-derived MSCs; PDLSCs, periodontal ligament stem cells; SCAP, stem cells from the apical papilla; SHED, stem cells from exfoliated deciduous teeth; TGPCs, tooth germ progenitor cells.

also possess immunomodulatory functions and potent tissue regenerative properties (Fig. 2). A better understanding of the biological characteristics of dental MSCs is essential to investigate their potential for clinical application. Herein, we review many aspects of the current investigations into various dental MSC populations.

Dental MSCs

Dental Pulp Stem Cells

DPSCs were first isolated by enzymatic digestion from dental pulp tissues [19] and found to express several surface markers, such as CD73, CD90, and CD105, but not CD14, CD34, or CD45 (Table 1) [27]. These cells have a fast population doubling time, they possess immunosuppressive properties, and they are prone to forming a dentin-pulp-like complex [28].

In Vitro Multipotency. Aside from their odontogenic potential, DPSCs can develop into adipocytes and neural cells, which was confirmed by evaluating the expression of specific gene markers [27]. Recently, DPSCs exhibited the additional potential to differentiate into osteoblasts, chondrocytes, myocytes, cardiomyocytes, active neurons, melanocytes, and hepatocyte-like cells (HLCs) in vitro (Table 1) [29–34].

In Vivo Ectopic Formation Capacity. A dentin-pulp-like complex associated with vascularized pulp-like tissue and surrounded by a layer of odontoblast-like cells can be generated by expanded DPSCs ex vivo when transplanted into immunocompromised mice with hydroxyapatite/tricalcium phosphate (HA/TCP) as a carrier [19, 35]. Additionally, DPSCs can form mineralized nodules with a reparative dentin-like tissue on the surface of human dentin in vivo [35], and many other scaffold or carrier materials can be used in vivo to generate dentin-pulp-like structures, such as calcium phosphate scaffolds [36], polylactic acid [37], and hexafluoro-2-propanol silk [38]. Moreover, a bone-like tissue was formed in DPSC-transplanted samples in vivo with various scaffold or carrier materials [39].

Reports have shown that DPSCs can differentiate into adipocyte-like cells, endotheliocytes, and myofibers and enhance

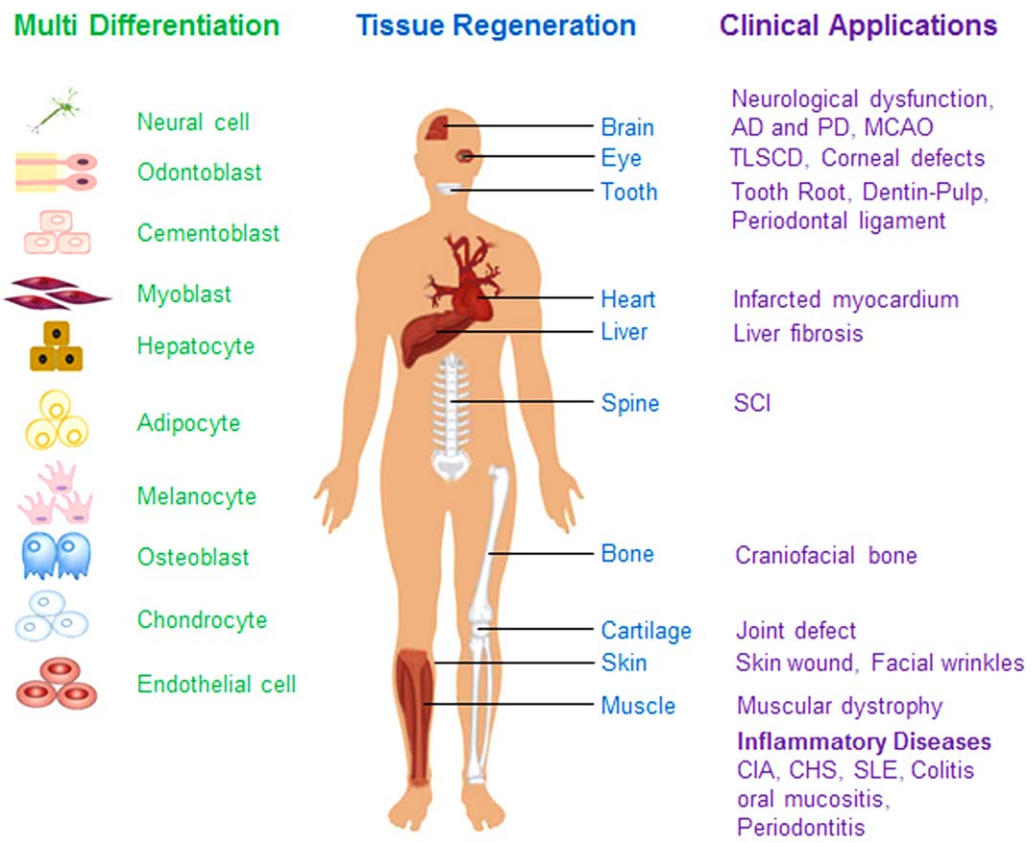

Figure 2. Multilineage differentiation capacity, tissue regeneration, and potential clinical applications of human dental tissue-derived MSCs. Abbreviations: AD, Alzheimer's dementia; CHS, contact hypersensitivity; CIA, collagen-induced arthritis; MCAO, middle cerebral artery occlusion; PD, Parkinson's disease; SCI, spinal cord injury; SLE, systemic lupus erythematosus; TLSCD, total limbal stem cell deficiency.

Table 1. Characteristics of human dental tissue-derived MSCs

Cell Type	Isolation	PD	CD antigen expression		In vitro Multipotency	In vivo Tissue Formation Capacity
			Positive	Negative		
DPSC	++++	>120	CD9, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146, CD166, CD271	CD14, CD19, CD24, CD31, CD34, CD45, CD117, CD133	Adipo, chondro, myo, osteo, neuro, odonto, cardiomyo, HLCs, melanocyte	Adipose, muscle, dentin-pulp, bone
SHED	+++	>140	CD13, CD29, CD44, CD56, CD73, CD90, CD105, CD146, CD166	CD11b, CD14, CD19, CD34, CD43, CD45	Adipo, chondro, myo, osteo, neuro, odonto, endothelial cell	Bone, dentin-pulp, microvessel
PDLSC	++++	ND	CD9, CD10, CD13, CD29, CD44, CD59, CD73, CD90, CD105, CD106, CD146, CD166	CD14, CD31, CD34, CD45	Adipo, chondro, osteo, neuro, cemento	Cementum, PDL
DFPC	++	ND	CD9, CD10, CD13, CD29, CD44, CD53, CD59, CD73, CD90, CD105, CD106, CD166, CD271	CD31, CD34, CD45, CD133	Adipo, chondro, osteo, neuro, cemento, HLCs	Alveolar bone, PDL, cementum
ABMSCs	++++	>30	CD13, CD29, CD44, CD71, CD73, CD90, CD105, CD146, CD166	CD11b, CD14, CD19, CD31, CD34, CD45,	Adipo, chondro, osteo	Bone
SCAP	+++	>70	CD13, CD24, CD29, CD44, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD146, CD166	CD14, CD18, CD34, CD45, CD117, CD150	Adipo, neuro, osteo, odonto, HLCs	Dentin-pulp
TGPC	+	ND	CD29, CD44, CD73, CD90, CD105, CD106, CD166	CD14, CD34, CD45, CD133	Adipo, osteo, chondro, odonto, neuro, hepato endothelial cell,	Bone
GMSC	++++	>20	CD29, CD44, CD73, CD90, CD105, CD106, CD146, CD166	CD34, CD45, CD117	Adipo, chondro, osteo, neuro, endoderm cell	Cartilage, bone, muscle

+: the ease and efficiency of isolation.
Abbreviations: adipo, adipocyte; cardiomyo, cardiomyocyte; cemento, cementoblast; chondro, chondrocyte; hepato, hepatocyte; HLCs, hepatocyte-like cells; myo, myoblast; ND, not determined; neuro, neuronal cell; PD, population doubling; PDL, periodontal ligament; odonto, odontoblast; osteo, osteoblast.

angiogenesis in vivo [30, 40]. Several studies also indicate that DPSCs survive in the central nervous system, express neuronal markers, and acquire neuronal morphology following transplantation into chicken embryos [32].

Immunomodulatory Properties. Previous reports have demonstrated that DPSCs can suppress T-cell proliferation and therefore might be suitable for preventing or treating T-cell alloreactivity associated with hematopoietic or solid-organ allogeneic transplantation [41]. Ex vivo-expanded DPSCs significantly inhibited the proliferation of peripheral blood mononuclear cells (PBMCs) via the expression of soluble factors partly induced by the secretion of interferon (IFN)-γ by activated PBMCs [42]. In another study, Toll-like receptors (TLRs), key molecules that bridge the innate and adaptive immune responses, were shown to trigger the immunosuppression of DPSCs by upregulating the expression of transforming growth factor (TGF)-β and interleukin (IL)-6 [43]. In addition, DPSCs could induce activated T-cell apoptosis in vitro and ameliorate inflammation-related tissue injuries in mice with colitis, which was associated with the expression of the Fas ligand (FasL). Knockdown of FasL expression reduced the immunoregulatory properties of DPSCs in the context of inducing T-cell apoptosis [44].

Stem Cells from Human Exfoliated Deciduous Teeth

The transition from deciduous teeth to adult permanent teeth is a unique and dynamic process in which the development and eruption of permanent teeth are coordinated with resorption of the deciduous teeth roots [45].

A distinct population of multipotent stem cells can be isolated from the remnant pulp of exfoliated deciduous teeth and expanded ex vivo, thereby unexpectedly providing a unique and accessible tissue source of MSCs [20]. SHED are distinct from DPSCs due to their higher proliferation rate, increased cell population doublings rate, and ability to form sphere-like cell cluster. SHED display surface markers that conform to the minimal criterion for MSCs proposed by ISCT with DPSCs (Table 1) [46, 47], and they also express the embryonic stem cell markers Oct4 and Nanog, the neural stem cell marker nestin, and the stage-specific embryonic antigens SSEA-3 and SSEA-4 [20].

In Vitro Multipotency. Similar to DPSCs, SHED exhibit a tendency for osteogenesis, odontogenesis, and adipogenesis under defined culture conditions. Furthermore, SHED express a variety of neural cell markers, form sphere-like clusters, and form multicytoplasmic processes when cultured under neurogenic conditions [20].

The myogenic and chondrogenic properties of SHED have been demonstrated [48]. Reports also support the observation that SHED can differentiate into endothelial cells when cultured on a dentin slice in vitro [49]. In hepatic differentiation medium, SHED were shown to produce specific hepatic proteins, and they acquired the morphological and functional characteristics of hepatocytes [50].

In Vivo Ectopic Formation Capacity. The neural developmental potential of SHED was studied by injecting SHED into the dentate gyrus of the hippocampus of immunocompromised

mice. The SHED could survive more than 10 days, and they continued to express neural markers, such as neurofilament M [20]. After in vivo transplantation into the intraperitoneal space of immunocompromised mice, SHED were shown to undergo dense engraftment in various tissues and organs, including the liver, spleen, and kidney, indicating their potent differentiation plasticity [48].

SHED can repair critically sized calvarial defects in immunocompromised mice through substantial bone formation [51]. Although SHED could not differentiate directly into osteoblasts, they did induce new bone formation by recruiting host osteogenic cells in vivo. These findings imply that deciduous teeth may not only provide guidance for the eruption of permanent teeth, as is generally assumed, but they may also be involved in inducing bone formation during the eruption of permanent teeth [20].

After transplantation into immunocompromised mice, ex vivo-expanded SHED yielded odontoblasts that were directly associated with a dentin-like structure, but they failed to reconstitute a dentin-pulp-like complex similar to that of in vivo DPSCs [20]. SHED seeded onto tooth slices/scaffolds were capable of differentiating into functional blood vessels that connected with the host vasculature and formed a dental pulp-like tissue and dentin after subcutaneous implantation into immunocompromised mice [52]. With a PEGylated fibrin carrier, SHED rendered a vascularized soft connective tissue similar to dental pulp after in vivo transplantation [53].

Immunomodulatory Properties. Systemic delivery of SHED resulted in significant engraftment in the muscles of dogs with golden retriever muscular dystrophy (GRMD), which is likely due to the immunomodulatory effect of SHED. Both the increase of cell engraftment with consecutive SHED transplantation and the absence of an immunological response in the GRMD dog model indicate important implications in designing future therapeutic trials [40]. In addition, SHED significantly inhibited T helper 17 (Th17) cell differentiation but increased the number of regulatory T cells (Tregs) in vitro. Furthermore, systemic infusion of SHED was able to effectively reverse systemic lupus erythematosus (SLE)-associated disorders, possibly because of their superior immunomodulatory effects that promote the recovery of the ratio between Tregs and Th17 cells. These data suggest that SHED may be an accessible and feasible source of MSCs for treating immune disorders, such as SLE [54].

Periodontal Ligament Stem Cells

The periodontal ligament (PDL) is a soft connective tissue embedded between the cementum and the alveolar bone socket. Early evidence showed that PDL not only plays an important role in supporting teeth, but it also contributes to tooth nutrition, homeostasis, and the regeneration of periodontal tissue [55].

Explant cultures or enzyme digestion treatment of the PDL released a population of PDLSCs, postnatal multipotent stem cells that could be readily expanded in vitro to generate a cementum/PDL-like complex. Additionally, PDLSCs show more population doublings in culture [21] and express STRO-1 and other MSC surface markers that are also present on DPSCs (Table 1). PDLSCs express a higher level of the tendon-specific marker scleraxis than do DPSCs, suggesting that PDLSCs might form a unique population of postnatal MSCs [21, 42].

In Vitro Multipotency. PDLSC populations express a heterogeneous assortment of makers associated with dentin, bone, smooth muscle, neural tissue, and formation of calcified nodules [56]. Similar to the other dental stem cells described above, PDLSCs have the ability to differentiate into osteogenic, adipogenic, and chondrogenic cells under defined culture conditions [57].

In Vivo Ectopic Formation Capacity. A typical cementum/PDL-like complex characterized by a layer of aligned cementum-like tissues and clearly associated PDL-like tissues can be regenerated after the transplantation of ex vivo-expanded PDLSCs into immunocompromised mice. The cementum/PDL-like structures have a completely distinct appearance compared with that of the typical dentin-pulp-like structures generated by DPSCs [21]. After transplantation into surgically created defects at the periodontal area of the mandibular molars in immunocompromised rats, a PDL-like tissue was regenerated, and PDLSCs were found to be closely associated with the alveolar bone, implying a potential functional role in periodontal tissue regeneration [21]. With HA/TCP as a carrier in the minipig model, transplanted PDLSCs generated a root/periodontal complex capable of supporting a porcelain crown, resulting in normal tooth function [24].

Immunomodulatory Properties. Previous studies have shown that activated human PBMCs induced PDLSCs to secrete soluble factors, including TGF-β, HGF, and IDO, that partly suppress PBMC proliferation [42]. PDLSCs were discovered to possess low immunogenicity and marked immunosuppressive activity via prostaglandin E2 (PGE2)-induced T-cell anergy [58]. Furthermore, a recent study reported that PDLSCs isolated from inflamed periodontium showed significantly diminished inhibitory effects on the proliferation index of T cells compared to those of healthy cells. In cocultures, stimulated PBMCs showed a significant decrease in the induction of Tregs, suppression of Th17 differentiation, and secretion of IL-10 and IL-17 in the presence of inflamed PDLSCs compared with healthy PDLSCs, demonstrating that inflamed PDLSCs had markedly dysfunctional immunomodulatory properties, which may explain the pathogenesis of periodontitis and facilitate the development of therapies for this condition [59].

Dental Follicle Stem Cells

The dental follicle is an ectomesenchymal tissue that surrounds the developing tooth germ prior to eruption. This tissue is thought to contain stem cells and lineage-committed progenitor cells for cementoblasts, periodontal ligament cells, and osteoblasts [22].

Progenitor cells have typically been isolated from the dental follicle of human third molars [22]. Similar to other dental stem cells, DFPCs have an extensive proliferative ability, express similar cell surface antigens (Table 1), and are capable of forming hard tissue both in vitro and in vivo [22, 46]. Moreover, they express the putative stem cell markers Notch-1 and Nestin and form the tissues of the periodontium, including alveolar bone, PDL, and cementum [22].

In Vitro Multipotency. Cultured DFPCs were demonstrated to exhibit osteogenic differentiation capacity under the appropriate

conditions. Long-term cultures of DFPCs with dexamethasone produced compact calcified nodules or appeared as membrane-like structures [22]. Cementoblast features were detected in cultured DFPCs stimulated by BMP-2/-7 and enamel matrix derivatives [60]. Moreover, DFPCs could differentiate into chondrocytes and adipocytes, as demonstrated by specific staining and the expression of specific markers [60]. The neural differentiation potential of DFPCs under in vitro conditions was therefore investigated [61]. Recently, DFPCs were reported to transdifferentiate into functional HLCs and acquire hepatocyte functions upon hepatogenic induction [34].

In Vivo Ectopic Formation Capacity. DFPCs combined with porous ceramic discs and transplanted into immunocompromised rats produced a cement/woven bone-like tissue with embedded cementocyte/osteocyte-like cells. However, no hard tissues formation, such as dentin, cementum, or bone, has been observed in the in vivo transplant [62]. Further studies are necessary to explore the potential for hard tissue regeneration.

Immunomodulatory Properties. Recent studies have shown that DFPCs produced TGF-β and suppressed the proliferation of PBMCs. Treatment with TLR3 and TLR4 agonists augmented the suppressive potential of DFPCs and potentiated TGF-β and IL-6 secretions [43, 63]. These properties of DFPCs are desirable for the treatment of diseases caused by chronic inflammation accompanied by tissue injury [43].

Alveolar Bone-Derived MSCs

Alveolar bone comprises the thickened ridge containing the tooth sockets in the bones that hold teeth, and it is embryonically derived from the dental follicle.

Recently, the successful isolation and culture of human ABMSCs (hABMSCs) were presented [23]. The isolated cells exhibit a spindle-shaped fibroblast-like morphology, plastic adherence, and colony formation. These cells express the surface markers CD73, CD90, CD105, and STRO-1 but do not express the hematopoietic markers CD14, CD34, and CD45 (Table 1) [23, 64, 65].

In Vitro Multipotency. Expanded ABMSCs can be differentiated into osteoblastic lineages, and they demonstrate high ALP expression [23]. Moreover, many studies have revealed that treatment of hABMSCs with the dichloromethane fraction of Dipsaci Radix [66], interferon-induced transmembrane protein 1 [67], nicotine [68], low-frequency pulsed electromagnetic fields [69], low-intensity pulsed ultrasound [70], low fluid dynamic shear stress [71], and orbital shear stress [72] could enhance osteogenesis in these cells. CS/HAp composite fabric may provide a good scaffold for ABMSC attachment, proliferation, migration, and differentiation for use in bone tissue engineering applications [73]. Additionally, ABMSCs showed chondrogenic and adipogenic differentiation potentials similar to those of other stem cell populations [65, 74].

In Vivo Ectopic Formation Capacity. hABMSCs induced significant new bone formation following subcutaneous transplantation into immunodeficient mice, and cuboidal osteoblasts and osteocytes were observed lining the surface along the margin of newly formed bone [23, 64, 65]. These data support the feasibility of using hABMSCs as a source of stem cells to treat bone defects.

Immunomodulatory Properties. Further studies are needed to verify the immunomodulatory potential of these cells and compare them with other stem cell populations.

Stem Cells from the Apical Papilla

The apical papilla is the soft tissue found at the apices of developing permanent teeth [24, 75]. In developing teeth, root formation begins with the apical proliferation of epithelial cells from the cervical loop. The dental papilla contributes to tooth formation and is eventually converted into pulp tissue, and an apical cell-rich zone lies between the apical papilla and the pulp [75].

A unique population of MSCs referred to as SCAP was discovered in the apical papilla of human immature permanent teeth [24, 75]. SCAP show a higher proliferation rate and mineralization potential than DPSCs, and they express typical MSC markers, including STRO-1, CD73, CD90, and CD105 (Table 1) [24, 76]. Similarly to DFPCs, SCAP represent a population of cells from a developing tissue and might thus exhibit greater plasticity than other dental stem cells (DSCs).

In Vitro Multipotency. Cultured SCAP can undergo adipogenic and odontogenic/osteoblastic differentiation following induction in vitro, analogous to the patterns exhibited by DPSCs and SHED [24]. Interestingly, ex vivo-expanded SCAP show positive staining for several neural markers without neurogenic stimulation [77]. After stimulation, additional neural markers are also expressed by SCAP, including neuronal nuclear antigen, neurofilament M, and neuron-specific enolase [75]. Additionally, SCAP demonstrated the capacity to differentiate into hepatocyte-like cells in vitro [34].

In Vivo Ectopic Formation Capacity. When ex vivo-expanded SCAP were transplanted into immunocompromised mice with an appropriate carrier matrix, a typical dentin-pulp-like complex was regenerated [24].

SCAP appear to be the source of the primary odontoblasts responsible for the formation of root dentin. In minipigs, transplanted SCAP and PDLSCs generated a bio-root periodontal complex capable of supporting a porcelain crown, resulting in functional tooth regeneration [24]. Human SCAP-mediated tissue regeneration may offer a promising cell-based therapy for root regeneration.

Furthermore, SCAP can generate cement/woven bone-like tissue with embedded cementocyte/osteocyte-like cells in vivo. However, whether the material is dentin, cementum, or bone could not be identified [62].

Immunomodulatory Properties. SCAP possess low immunogenicity and can inhibit T-cell proliferation in vitro through an apoptosis-independent mechanism. SCAP can also suppress the one-way mixed lymphocyte reaction in a dose-dependent manner. Certain soluble factors may be involved in SCAP-mediated immune suppression, but the exact mechanisms require further study [78]. In addition, cryopreservation did not affect the immune properties of SCAP [76].

Tooth Germ Progenitor Cells

TGPCs are novel stem cell population that were identified in the dental mesenchyme of the third molar tooth germ during the late bell stage [25]. TGPCs can be expanded and maintained for nearly 60 population doublings, during which they retain their spindle-shaped morphology and high proliferation rate. TGPCs express the MSC-associated markers STRO-1 and CDs (Table 1) and demonstrate a tendency for pluripotency-associated gene expression (*nanog, oct4, sox2, klf4*, and *c-myc*), indicating a mesenchymal phenotype [25, 79, 80].

In Vitro Multipotency. TGPCs show a similar multilineage differentiation capacity to that of other dental MSCs, including the ability to differentiate into adipocytes, osteoblasts/odontoblasts, chondrocytes, and neurons [25, 79–82].

Hepatic-induced TGPCs change morphologically from bipolar-spindle and fibroblast-like to polygonal and epithelial-like. These cells are strongly positive for the liver-specific albumin gene. In addition, the immature hepatocyte marker alpha-fetoprotein (AFP) and the specific biliary epithelial cell marker CK19 were expressed more strongly during the culture period. These results indicated that TGPCs can differentiate into cells with the morphological, phenotypic, and functional characteristics of hepatocytes in vitro [25]. TGPCs form tube-like structures when incubated on Matrigel, which might indicate a possible contribution to vascularization [79].

In Vivo Ectopic Formation Capacity. TGPCs or TGPCs transfected with Venus were subcutaneously implanted with HA into immunocompromised rats. The HA/TGPC implants showed new bone formation in the presence of osteocytes in the newly formed bone matrix and a cuboid-shaped active osteoblast lining on the matrix surface. The implants with Venus-positive TGPCs were located within the mineralized matrix, where osteoblasts and osteocytes are typically found [25].

Cultured TGPCs show engraftment when they are transplanted via the portal vein into the liver of carbon tetrachloride (CCl_4)-treated rats. The transplantation of hepatic induction-treated TGPCs was effective in suppressing liver inflammation and fibrosis and reduced both the increase in bilirubin and the suppression of albumin. These findings suggest that the multipotent TGPCs are a candidate for cell-based therapy to treat liver diseases [25].

Immunomodulatory Properties. There is little information on the immunomodulatory properties of TGPCs. Due to their multipotency for regeneration, further research focusing on the immunomodulation characteristics of TGPCs remains urgently needed.

Gingiva-Derived MSCs

The gingiva is a unique oral tissue overlaying the alveolar ridges and retromolar region that is recognized as a biological mucosal barrier and a distinct component of the oral mucosal immunity. Furthermore, this tissue can often be obtained as a discarded biological sample [83].

Recently, GMSCs, a new population of stem cells isolated from human gingiva, were shown to exhibit clonogenicity, self-renewal, and multipotent differentiation capacity, and these cells possess both stem cell-like and immunomodulatory properties [26]. GMSCs express CDs and display positive signals for Oct4, Sox2, Nanog, Nestin, SSEA-4, and Stro-1 (Table 1) [26, 84, 85].

In Vitro Multipotency. Some studies have confirmed that GMSCs have multipotent mesenchymal precursor cell properties after differentiating into multiple mesenchymal-derived cell types, such as adipocytes, chondrocytes, and osteoblasts, as determined by the increased expression of specific markers [26, 84, 85].

The capacity of GMSCs to differentiate into a putative definitive endoderm (DE) lineage was further confirmed through demonstration of the expression of the DE markers Sox17, Foxα2, and CRCX4. When cultured on fibronectin-coated slides in endothelial cell growth medium, GMSCs expressed the endothelial cell marker CD31 [26]. Under neural differentiation conditions, GMSCs are positive for glial fibrillary acidic protein, neurofilament 160/200 (NF-M), MAP2, nestin, and βIII-tubulin [26]. When subjected to a glial differentiation regimen, GMSCs induce neuritogenesis and support survival of PC12 cells in serum-free medium [86].

In Vivo Ectopic Formation Capacity. Expanded GMSCs were transplanted with HA/TCP or fibrin gel as a carrier into immunocompromised mice. GMSCs consistently regenerated connective tissue-like transplants that exhibited the histological features of the collagenous connective tissue phenotype, including the presence of fibroblast-like cells and collagen fibers. GMSCs have a potent in vivo self-renewal ability, as confirmed by serial transplantation in the same model [26, 84].

Ex vivo-expanded GMSCs were seeded onto HA/TCP grafts, incubated in osteogenic medium, mixed with collagen gel, and transplanted subcutaneously into the dorsal surface of immunocompromised mice. High expression levels of osteocalcin, OPN, and Col I were observed, indicating the potential of GMSCs for in vivo bone regeneration [26]. Newly formed bone with a well-mineralized trabecular structure located at the inner site was also demonstrated for GMSCs transplanted into the mandible and calvarial defect model [26, 85].

Surprisingly, dexamethasone-treated GMSCs implanted subcutaneously into SCID mice revealed the formation of bilineage (mesodermal and ectodermal) mixed tumors that included fetal fat, striated muscle, cartilage, bone, epithelial tissue, and neural tissue. This finding implies that GMSCs are capable of giving rise to tissues in vivo that develop from cranial neural crest cells during embryogenesis [86].

Immunomodulatory Properties. GMSCs are capable of eliciting a potent inhibitory effect on T-cell proliferation in response to mitogen stimulation. Mechanistically, GMSCs exert their anti-inflammatory effect partly via the IFN-γ-induced expression of IDO, IL-10, cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase, which are known immunosuppressive factors. Cell-based therapy using a systemic infusion of GMSCs significantly ameliorated the severity of inflammation-related colonic injuries in experimental colitis by suppressing inflammatory cell infiltration and inflammatory

cytokine/mediator secretion and increasing Treg accumulation and IL-10 expression at local intestinal sites [26]. In addition, GMSCs can elicit M2 polarization of macrophages characterized by an increased expression of the mannose receptor (MR; CD206) and the secretory cytokines IL-10 and IL-6 and decreased induction of Th 17 cell expansion, which might contribute to a marked acceleration of wound healing [87]. Therapeutic administration of GMSCs dramatically alleviated both the sensitization and elicitation of contact hypersensitivity (CHS) by modulating the function of multiple types of innate and adaptive immune cells through the COXs/PGE2 pathway [88]. Furthermore, systemic infusion of GMSCs showed distinct immune tolerance in a murine skin allograft model via upregulation of putative systemic Tregs [84]. Functionally, three-dimensional (3D) spheroid GMSCs could mitigate chemotherapy-induced oral mucositis in the murine model by expressing increased levels of reactive oxygen species, hypoxia-inducible factor (HIF)-1 and HIF-2α, and manganese superoxide dismutase, which correlated with improved resistance to oxidative stress-induced apoptosis [89]. Most recently, the promising therapeutic effect of GMSCs was demonstrated in an experimental collagen-induced arthritis (CIA) model. The role of GMSCs in controlling the development and severity of CIA mostly depended on CD39/CD73 signals and partially depended on the induction of CD4+CD39+FoxP3+ Treg cells [90]. Taken together, GMSCs can function as an immunomodulatory and anti-inflammatory component of the immune system in vivo and represent a promising and easily accessible cell source for MSC-based therapies to treat inflammatory and allergic diseases.

Progress In Dental MSC–based Therapy

Stem cell-based therapy for regenerative treatment is considered a promising treatment modality for future therapy. Dental tissues-derived MSCs are currently an excellent candidate for the regeneration of teeth and other organs, and their progress in preclinical studies and possible applications are outlined here.

Bone Regeneration

Dental tissue-derived MSCs have been used to engineer bone for orofacial bone regeneration.

In vitro, DPSCs can produce a living autologous fibrous bone (LAB) tissue, which forms a lamellar bone with osteocytes after transplantation into immunocompromised rats [29]. DPSCs produced bone-like structures rather than dentin following transplantation in vivo with HA/TCP, poly(lactic-co-glycolic acid) (PLGA), HA, or nano-fiber hydrogel as a carrier [39, 91].

SHED can repair critical-sized calvarial defects with robust bone formation when implanted in vivo [51]. As these cells are derived from neural crest cells, SHED may share a similar tissue origin with mandibular bone cells and therefore might be a suitable resource for the regeneration of alveolar and orofacial bone defects. Although it is unclear whether transplanted cells directly differentiate into osteoblasts to create new bone, human DFPCs clearly support new bone formation after transplantation into a surgically created, full-thickness, critically sized partial defect in immunodeficient rats [92].

Subcutaneous implantation of SCAP combined with HA scaffolds into immunocompromised rats formed bone-like mineralized tissues [91]. Furthermore, when SCAP were seeded onto synthetic scaffolds and then transplanted into immunodeficient mice, a continuous layer of dentin-like tissue was deposited onto the canal dentinal wall [93]. These findings provide evidence that SCAP can be used as an approachable stem cell source for osteo/odontogenic differentiation and bone formation. GMSCs can repair mandibular wounds and calvarial defects in vivo with local implantation, implying that GMSCs could be a novel source of stem cell-based therapy during bone reconstruction [85]. A clinical application of DPSCs for human bone defects has been reported. DPSCs obtained from the mandibular third molars demonstrated the capacity to completely restore human mandible bone defects when they were transplanted with a collagen sponge scaffold [94].

Tooth Root Regeneration

Several clinical cases have demonstrated the role of apical papilla in root formation. A bio-root periodontal complex constructed with SCAP and PDLSCs was able to support an artificial porcelain crown to provide normal tooth function in a swine model, suggesting the feasibility of using a combination of autologous SCAP/PDLSCs in conjunction with artificial dental crowns for functional tooth regeneration [24]. After root canal treatment, root-tip formation continued, and the surviving SCAP appeared to produce odontoblasts responsible for complete root formation in several cases of apexogenesis in infected immature teeth with periradicular periodontitis or abscess, supporting a pivotal role for the apical papilla in root formation [95].

In addition, treated dentin matrix (TDM) was used as a natural biological scaffold for tooth root reconstruction in an animal model. TDM was able to induce and support DFPCs to develop root-like tissues with dentin-pulp-like tissues and cementum-periodontal complexes, implying successful tooth root regeneration [96]. Therefore, DFPCs could be used for the treatment of root or tooth defect or loss in the future.

Dentin-Pulp Regeneration

The use of DPSCs, SHED, and SCAP for dentin-pulp tissue regeneration has been investigated.

A study using human tooth root fragments with an empty root canal space indicated the regeneration of vascularized dentin-pulp-like tissue when the canal was filled with a poly-D,L-lactide/glycolide (PLG) scaffold seeded with DPSCs [93]. When DPSCs treated with preameloblast culture medium were transplanted into immunocompromised mice, they generated pulp-like structures lined with odontoblast-like cells [97]. These studies have primarily demonstrated the potential of complete pulp regeneration under experimental conditions.

A pulp-like tissue with well-established vascularity was induced and a continuous layer of dentin-like tissue was deposited onto the canal dentinal wall when SCAP were inserted into tooth fragments and then transplanted into immunodeficient mice [93, 95]. These data provide evidence that SCAP can be used for tissue engineering/regeneration.

Xenogeneic transplants containing HA/TCP with SHED generated donor-derived dentin-pulp-like tissues with distinct odontoblast layers lining the mineralized dentin-matrix [56]. SHED seeded onto biodegradable scaffolds prepared within human tooth slices successfully differentiated into odontoblast-like cells on the dentin surface with a cytoplasmic process

extending into a dentinal tubule, suggesting that SHED constitute a viable source of cells for dental pulp tissue engineering [49]. SHED seeded onto polylactic acid scaffolds with the addition of BMP-2 and TGF-β1 produced pulp tissue constructs, suggesting that future regenerative endodontic treatment may involve the cleaning and shaping of root canals followed by the implantation of vital dental pulp tissue constructs created in the laboratory [98]. The establishment of reproducible and safe methods for regenerating dentin-pulp complexes to achieve the desired pulp tissue regeneration is expected.

Periodontal Regeneration

Stem cell-based regenerative periodontal therapy has gained attention since the isolation of MSCs from various tissues.

The bone morphogenetic protein BMP-2 has been used to promote for the differentiation of DFPCs into cementoblasts and odontoblasts to reestablish the integrity of the PDL [99]. Due to their periodontal ligament derivation and their capacity to differentiate into osteoblasts, cementoblasts, and fibroblasts, PDLSCs may be the first candidate cellular source for PDL regeneration. It has been demonstrated that expanded PDLSCs are capable of regenerating a typical cementum/periodontal ligament-like structure with HA/TCP as a carrier [21]. Extensive research exploring the potential use of PDLSCs to treat periodontal diseases in various larger animal models is ongoing. Several pilot studies have demonstrated that transplantation of PDL cell sheets was able to regenerate periodontal tissue in experimental defect models in rats, dogs, and swine [58]. In addition to animal models, a retrospective pilot study in humans has also demonstrated the therapeutic benefit of autologous periodontal ligament progenitor cells (PDLPs) when implanted with bone grafting material into intrabony defects in patients. All periodontal defects were reconstructed, and the clinical and experimental evidence support the potential efficacy and safety of using autologous PDL cells in the treatment of human periodontitis [100]. Furthermore, one human clinical study has demonstrated the development of new tissue consistent with PDL on the surface of dental implants. This proof-of-principal investigation shows great potential and may provide efficient methods for enhancing the outcome of implant treatment using PDLSCs [101].

An optimal protocol for the extraction, expansion, and characterization of human PDL cells has been demonstrated, and the safety and efficacy of the PDL sheet for clinical trials have been validated [102]. One clinical trial using PDL sheet technology is currently under way to develop a government-approved periodontal cell transplantation therapy [103].

Neural Tissue Regeneration

Transplanted DPSCs can survive [32] and may induce neuroplasticity [104] in the central nervous system. Injection of DPSCs into the right dorsolateral striatum of experimental animals subjected to middle cerebral artery occlusion (MCAO) induced a significant recovery from neurological dysfunction [105]. SHED could be induced to form neural-like spheres in a medium optimized for neural stem cells in vitro. Additionally, transplantation of SHED spheres into parkinsonian rats partially improved the apomorphine-evoked rotation of behavioral disorders, suggesting that SHED may be a promising source for the treatment of neurodegenerative diseases [106]. In a recent study, neuronally predifferentiated DPSCs were injected into the cerebrospinal fluid of rats with induced cortical lesions, these cells integrated into the host brain and exhibited neuronal properties, suggesting that they may serve as useful sources of neuro- and gliogenesis in vivo [107].

Recently, three independent groups reported that pulp stem cells showed neuroregenerative activity in rodent spinal cord injury (SCI) models. Human dental pulp cells (HDPCs) transplanted into a mouse model of compressive spinal cord injury showed higher levels of trophic-factor expression in the tissue, better tissue organization, and the presence of many axons or oligodendrocytes and neurons with synapses, indicating that HDPCs may be feasible candidates for therapeutic intervention after SCI and during central nervous system disorders in humans [108]. Functional recovery was promoted when undifferentiated or neural-induced SHED were transplanted into a rat spinal cord contusion injury model, suggesting that engrafted SHED or their derivatives could be suitable candidates for the treatment of SCI and other neurodegenerative diseases [109]. Transplantation of human SHED into the completely transected adult rat spinal cord significantly improved the recovery of hind limb locomotor functions. Thus, engrafted SHED may provide therapeutic benefits for treating SCI through both cell-autonomous and paracrine neuroregenerative activities [110]. A recent study indicated that hTGPCs use angiogenic antioxidative and antiapoptotic mechanisms to exert neuroprotective effects on in vitro models of Alzheimer's dementia and Parkinson's disease, which might provide insight into the therapeutic potential of tooth germ-derived MSCs as a cellular treatment for neurological disorders [111]. These reports suggest that dental stem cells offer valuable therapeutic potential in the central nervous system.

Regeneration of Nondental Tissues

Subcutaneous transplantation of human PDLSCs can lead to the formation of substantial amounts of collagen fibers and improve facial wrinkles in mice [112].

The induction of smooth and skeletal muscle cells from human SHED in vivo has been reported [40, 48]. Systemic application of SHED to animals suffering from muscular dystrophy improves the clinical symptoms [40].

DPSCs can repair infarcted myocardium associated with an increase in the number of vessels and a reduction in the infarct size, probably due to their ability to secrete proangiogenic and antiapoptotic factors. Therefore, this study suggests that DPSCs could provide a novel alternative cell population for the treatment of ischemic diseases [113].

When TGPCs were transplanted into CCl_4-treated liver-injured rats, they prevented the progression of liver fibrosis and contributed to the restoration of liver function. These findings suggest that TGPCs are a candidate for cell-based therapy to treat liver diseases and offer unprecedented opportunities for developing therapies to facilitate tissue repair and regeneration [25].

Vascular endothelial growth factor (VEGF)-induced DPSCs maintain endothelial cell-like features when cultured in a 3D fibrin mesh, displaying focal organization into capillary-like structures [114]. DPSCs were reported to show an increase in blood flow with a high density of capillary formation during experimentally induced mouse ischemia, suggesting their potential for promoting angiogenesis/vasculogenesis [115].

SHED are capable of reconstructing the eye surface following the induction of unilateral total limbal stem cell deficiency

in rabbits, suggesting that SHED might be used as a potential alternative source of cells for corneal reconstruction [116]. Transplantation of a tissue-engineered cell sheet composed of human SHED into rabbits with experimentally induced corneal defects resulted in successful reconstruction of the corneal epithelium [117].

Transplantation of DPSCs ameliorated ischemic tissue injury in the rat brain and accelerated functional recovery after MCAO, indicating that DPSCs could be a potential candidate for the treatment of stroke [118]. SHED-conditioned medium promoted the migration and differentiation of endogenous NPCs, induced vasculogenesis, and ameliorated ischemic brain injury after intranasal administration to sprague-dawley (SD) rats subjected to permanent MCAO [119].

In a murine excisional full-thickness skin wound model, systemic infusion of GMSCs significantly enhanced the repair process, as indicated by more rapid re-epithelialization and increased angiogenesis, providing evidence that GMSCs are a promising cell source for stem cell-based therapies of inflammatory diseases and skin wounds [84]. Systemic infusion of GMSCs also dramatically suppressed CHS via PGE(2)-dependent mechanisms before the sensitization and challenge phase [88]. In addition, systemic infusion of GMSCs mitigated chemotherapy-induced oral mucositis in murine models, as demonstrated by the reversal of body weight loss and the stimulation of regeneration in the disrupted epithelial lining [89]. Infusion of GMSCs into mice with CIA significantly reduced arthritis severity, decreased histopathology scores, and downregulated the production of inflammatory cytokines (IFN-γ and IL-17A), suggesting that GMSCs provide a promising approach for the treatment of autoimmune diseases [90].

Dental Stem Cell Banking

Dental stem cells appear to be a promising source for the treatment of various ailments already discussed herein. Personalized medicine is strongly believed to be the most promising avenue for treating challenging diseases and injuries throughout life; however, the use of an individual's own dental stem cells during a time of therapeutic necessity has serious limitations because it requires the extraction of remaining teeth. Thus, the ability to harvest and safely store stem cells from deciduous teeth and extracted permanent teeth is important to provide the greatest future benefit for life. Individuals have various opportunities at each stage of their life to bank these valuable cells, and it is best to recover stem cells from young and healthy individuals when the cells are strong and proliferative. Once stem-cell-containing tissues, such as pulp, apical papilla, periodontal ligament, follicle, gingiva, or the tooth itself, have been obtained from the patient, they can be cryopreserved for many years to retain their regenerative potential for use in future regenerative therapies.

Tooth banking is not currently a popular practice, but the trend is catching up mainly in developed countries. Current licensed tooth banks include the following [120]:

Advanced Center for Tissue Engineering, Japan (http://www.acte-group.com/)
Teeth Bank, Co., Japan (http://www.teethbank.jp/)
BioEDEN (http://www.bioeden.com/)
StemSave (http://www.stemsave.com/)
Store-A-Tooth (http://www.store-a-tooth.com/)
Stemade Biotech Pvt., India (http://www.stemade.com/)
The Norwegian Tooth Bank, Norway (http://www.fhi.no/morogbarn)

Although the autologous transplantation of banked teeth has been successfully achieved in the clinic, stem cell-based therapies involving stem cell banking have not yet been reported. Moreover, many ethical controversies and legal and social questions need to be addressed before banked dental stem cells become clinically available. Therefore, the utility of stem cell banking should be carefully evaluated, and proper maintenance of the cryopreserved cells and tissues to ensure good quality for future use in transplantation should be ensured. In addition, legislation of the banking system is essential, as it may provide bio-insurance for future use.

Conclusions and Perspectives

Current research on dental stem cells is expanding at an unprecedented rate. Stem cells derived from teeth are easily accessible and can be obtained in a convenient and minimally invasive way. According to the above discussion, these new sources of stem cells could be beneficial for cellular therapy and the eventual development of regenerative treatment. These cells guarantee a donor match (autologous transplant) for life that, to a certain extent, may also be useful for close relatives of the donor. In addition, because these are adult stem cells, ethical concerns are not an issue.

However, several main objectives need to be addressed for future research, especially concerning the following issues:

(I) Identifying specific surface markers and understanding the mechanisms of self-renewal. This understanding would allow us to purify, regulate, and expand dental stem cell growth in the lab to generate sufficient numbers of cells for therapeutic use.

(II) Understanding the regulation of dental MSCs during differentiation and the generation of specific tissue. The formation of certain tissues requires the expression of particular genes and involves sequential signal activation. Controlling these signals in the correct temporal pattern may facilitate regeneration of the desired tissue both in vitro and in vivo.

(III) Clarifying the long-term fate of transplanted dental MSCs in the recipient. The capacity and efficiency of dental MSCs for homing to and transdifferentiating into a particular tissue and their ability to find the optimum "niche" have been major concerns.

(IV) Understanding how the various functional attributes of MSCs are specified at the population level. MSC populations display considerable phenotypic and functional heterogeneity [121]. BMMSCs are the most extensively investigated population, and these cells are heterogeneous [122]. We assume that intrinsic heterogeneity may also exist in dental MSCs. Multiple methods such as single-cell sequencing and digital PCR can be used to understand the molecular basis of heterogeneity and the impact of heterogeneity on the clinical development and therapeutic potency of MSCs. This understanding will allow future studies to enhance the clinical efficacy of MSC-based therapies.

(V) Clarifying the mechanisms underlying the immunomodulatory properties of dental MSCs. Dental MSCs offer a fascinating new cell source for clinical applications; however, the immune responses of the recipient should be noted. Further research will explore the critical role of dental MSCs in immunomodulation and the secretion of dental MSCs as well as the interactions between dental MSCs and the immune system for future applications.

(VI) Identifying whether dental MSCs are subject to the effects of aging due to intrinsic factors or the somatic environment. Age-related changes can impinge on the activity of MSCs, as shown in previous studies [123]. Hence, discovering biomarkers to assess the cellular senescent state, establishing reliable methods to measure age-induced effects, and identifying the molecular basis of dental stem cell aging will undoubtedly affect their future clinical use. Understanding the relationships between MSC senescence and organismal aging and developing approaches to reverse the effects of aging on MSCs are recommended for future studies.

(VII) Developing safe and reproducible catheter-based delivery systems for depositing dental MSCs into the recipient and designing a comprehensive and global strategy for dealing with a multiplicity of issues involving the safety, harmonization, privacy, and transparency. The emerging trend of establishing human dental stem cell banks to support basic research and translational applications requires international collaboration. It will be important to address harmonization and standardization processes for banking dental stem cells and establish an international banking network based on common fundamental norms and standards, which will undoubtedly ensure the legitimacy of these banks.

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Disclosure Of Potential Conflicts Of Interest

The authors indicate no potential conflicts of interest.

References

1 Friedenstein AJ, Gorskaja J, Kulagina N. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 1976;4:267-274.
2 Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641-650.
3 Pittenger MF, Mackay AM, Beck SC et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284: 143-147.
4 Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999;96:10711-10716.
5 Sato Y, Araki H, Kato J et al. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. Blood 2005;106: 756-763.
6 Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-317.
7 Gronthos S, Zannettino AC, Hay SJ et al. Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow. J Cell Sci 2003; 116:1827-1835.
8 Williams JT, Southerland SS, Souza J et al. Cells isolated from adult human skeletal muscle capable of differentiating into multiple mesodermal phenotypes. Am Surg 1999;65:22-26.
9 Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. Br J Haematol 2000;109:235-242.
10 De Bari C, Dell'Accio F, Tylzanowski P et al. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928-1942.
11 Campagnoli C, Roberts IA, Kumar S et al. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001; 98:2396-2402.
12 Zuk PA, Zhu M, Mizuno H et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng 2001;7:211-228.
13 Noort WA, Kruisselbrink AB, Kruger M et al. Mesenchymal stem cells promote engraftment of human umbilical cord blood–derived $CD34^+$ cells in NOD/SCID mice. Exp Hematol 2002;30:870-878.
14 Scherjon SA, Kleijburg-van der Keur C, Noort WA et al. Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood 2003;102:1548-1549.
15 Salingcarnboriboon R, Yoshitake H, Tsuji K et al. Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property. Exp Cell Res 2003;287:289-300.
16 Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 2004; 22:1338-1345.
17 Shih DTb, Lee DC, Chen SC et al. Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. Stem Cells 2005;23:1012-1020.
18 Patki S, Kadam S, Chandra V et al. Human breast milk is a rich source of multipotent mesenchymal stem cells. Hum Cell 2010;23:35-40.
19 Gronthos S, Mankani M, Brahim J et al. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 2000;97:13625-13630.
20 Miura M, Gronthos S, Zhao M et al. SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003;100:5807-5812.
21 Seo B-M, Miura M, Gronthos S et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149-155.
22 Morsczeck C, Götz W, Schierholz J et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 2005;24:155-165.
23 Matsubara T, Suardita K, Ishii M et al. Alveolar bone marrow as a cell source for regenerative medicine: Differences between alveolar and iliac bone marrow stromal cells. J Bone Miner Res 2005;20:399-409.
24 Sonoyama W, Liu Y, Fang D et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PloS One 2006;1:e79.
25 Ikeda E, Yagi K, Kojima M et al. Multipotent cells from the human third molar: Feasibility of cell-based therapy for liver disease. Differentiation 2008;76:495-505.
26 Zhang Q, Shi S, Liu Y et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009;183:7787-7798.
27 Gronthos S, Brahim J, Li W et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531-535.
28 Peng L, Ye L, Zhou X-D. Mesenchymal stem cells and tooth engineering. Int J Oral Sci 2009;1:6.
29 Laino G, D'Aquino R, Graziano A et al. A new population of human adult dental pulp stem cells: A useful source of living autologous fibrous bone tissue (LAB). J Bone Miner Res 2005;20:1394-1402.
30 d'Aquino R, Graziano A, Sampaolesi M et al. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: A pivotal synergy leading to adult bone tissue formation. Cell Death Differ 2007;14:1162-1171.
31 Armiñán A, Gandía C, Bartual M et al. Cardiac differentiation is driven by NKX2. 5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev 2009;18:907-918.
32 Arthur A, Rychkov G, Shi S et al. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 2008;26:1787-1795.

33 Stevens A, Zuliani T, Olejnik C et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev 2008;17:1175-1184.
34 Patil R, Kumar BM, Lee W-J et al. Multilineage potential and proteomic profiling of human dental stem cells derived from a single donor. Exp Cell Res 2014;320:92-107.
35 Batouli S, Miura M, Brahim J et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 2003;82:976-981.
36 Takeda T, Tezuka Y, Horiuchi M et al. Characterization of dental pulp stem cells of human tooth germs. J Dent Res 2008;87:676-681.
37 Wang J, Ma H, Jin X et al. The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. Biomaterials 2011;32:7822-7830.
38 Zhang W, Ahluwalia IP, Literman R et al. Human dental pulp progenitor cell behavior on aqueous and hexafluoroisopropanol based silk scaffolds. J Biomed Mater Res Part A 2011;97:414-422.
39 Graziano A, d'Aquino R, Angelis MGCD et al. Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. J Cell Physiol 2008;214:166-172.
40 Kerkis I, Ambrosio CE, Kerkis A et al. Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic. J Transl Med 2008;6:1-13.
41 Pierdomenico L, Bonsi L, Calvitti M et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 2005;80:836-842.
42 Wada N, Menicanin D, Shi S et al. Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 2009;219:667-676.
43 Tomic S, Djokic J, Vasilijic S et al. Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. Stem Cells Dev 2010;20:695-708.
44 Zhao Y, Wang L, Jin Y et al. Fas ligand regulates the immunomodulatory properties of dental pulp stem cells. J Dent Res 2012;91:948-954.
45 Parner E, Heidmann JM, Kjaer I et al. Biological interpretation of the correlation of emergence times of permanent teeth. J Dent Res 2002;81:451-454.
46 Huang G-J, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. J Dent Res 2009;88:792-806.
47 Pivoriūnas A, Surovas A, Borutinskaitė V et al. Proteomic analysis of stromal cells derived from the dental pulp of human exfoliated deciduous teeth. Stem Cells Dev 2009;19:1081-1093.
48 Kerkis I, Kerkis A, Dozortsev D et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 2007;184:105-116.
49 Cordeiro MM, Dong Z, Kaneko T et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008;34:962-969.
50 Ishkitiev N, Yaegaki K, Calenic B et al. Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features *in vitro*. J Endod 2010;36:469-474.
51 Seo B, Sonoyama W, Yamaza T et al. SHED repair critical-size calvarial defects in mice. Oral Dis 2008;14:428-434.
52 Sakai V, Zhang Z, Dong Z et al. SHED differentiate into functional odontoblasts and endothelium. J Dent Res 2010;89:791-796.
53 Galler KM, Cavender AC, Koeklue U et al. Bioengineering of dental stem cells in a PEGylated fibrin gel. Regen Med 2011;6:191-200.
54 Yamaza T, Kentaro A, Chen C et al. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther 2010;1:5.
55 Bartold P, Mcculloch CA, Narayanan AS et al. Tissue engineering: A new paradigm for periodontal regeneration based on molecular and cell biology. Periodontology 2000 2000;24:253-269.
56 Shi S, Bartold P, Miura M et al. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8:191-199.
57 Xu J, Wang W, Kapila Y et al. Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. Stem Cells Dev 2008;18:487-496.
58 Ding G, Liu Y, Wang W et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells 2010;28:1829-1838.
59 Liu D, Xu J, Liu O et al. Mesenchymal stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol 2012;39:1174-1182.
60 Kémoun P, Laurencin-Dalicieux S, Rue J et al. Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) in vitro. Cell Tissue Res 2007;329:283-294.
61 Völlner F, Ernst W, Driemel O et al. A two-step strategy for neuronal differentiation *in vitro* of human dental follicle cells. Differentiation 2009;77:433-441.
62 Yagyuu T, Ikeda E, Ohgushi H et al. Hard tissue-forming potential of stem/progenitor cells in human dental follicle and dental papilla. Arch Oral Biol 2010;55:68-76.
63 Morsczeck C, Völlner F, Saugspier M et al. Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation in vitro. Clin Oral Investig 2010;14:433-440.
64 Mason S, Tarle S, Osibin W et al. standardization and safety of Alveolar bone-derived stem cell Isolation. J Dent Res 2014;93:55-61.
65 Park JC, Kim JC, Kim YT et al. Acquisition of human alveolar bone-derived stromal cells using minimally irrigated implant osteotomy: In vitro and in vivo evaluations. J Clin Periodontol 2012;39:495-505.
66 Kim B-S, Kim Y-C, Zadeh H et al. Effects of the dichloromethane fraction of Dipsaci Radix on the osteoblastic differentiation of human alveolar bone marrow-derived mesenchymal stem cells. Biosci Biotechnol Biochem 2011;75:13-19.
67 Kim B-S, Kim H-J, Kim JS et al. IFITM1 increases osteogenesis through Runx2 in human alveolar-derived bone marrow stromal cells. Bone 2012;51:506-514.
68 Kim B-S, Kim S-J, Kim H-J et al. Effects of nicotine on proliferation and osteoblast differentiation in human alveolar bone marrow-derived mesenchymal stem cells. Life Sci 2012;90:109-115.
69 Lim K, Hexiu J, Kim J et al. Effects of electromagnetic fields on osteogenesis of human alveolar bone-derived mesenchymal stem cells. BioMed Res Int 2013;2013:296019.
70 Lim K, Kim J, Seonwoo H et al. In vitro effects of low-intensity pulsed ultrasound stimulation on the osteogenic differentiation of human alveolar bone-derived mesenchymal stem cells for tooth tissue engineering. BioMed Res Int 2013;2013:269724.
71 Lim K-T, Kim J, Seonwoo H et al. Enhanced osteogenesis of human alveolar bone-derived mesenchymal stem cells for tooth tissue engineering using fluid shear stress in a rocking culture method. Tissue Eng Part C Methods 2012;19:128-145.
72 Lim KT, Hexiu J, Kim J et al. Synergistic effects of orbital shear stress on in vitro growth and osteogenic differentiation of human alveolar bone-derived mesenchymal stem cells. BioMed Res Int 2014;2014:316803.
73 Kim BS, Kim JS, Chung YS et al. Growth and osteogenic differentiation of alveolar human bone marrow-derived mesenchymal stem cells on chitosan/hydroxyapatite composite fabric. J Biomed Mater Res Part A 2013;101:1550-1558.
74 Pekovits K, Kröpfl JM, Stelzer I et al. Human mesenchymal progenitor cells derived from alveolar bone and human bone marrow stromal cells: A comparative study. Histochem Cell Biol 2013;140:611-621.
75 Sonoyama W, Liu Y, Yamaza T et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: A pilot study. J Endod 2008;34:166-171.
76 Ding G, Wang W, Liu Y et al. Effect of cryopreservation on biological and immunological properties of stem cells from apical papilla. J Cell Physiol 2010;223:415-422.
77 Abe S, Yamaguchi S, Amagasa T. Multilineage cells from apical pulp of human tooth with immature apex. Oral Sci Int 2007;4:45-58.
78 Ding G, Liu Y, An Y et al. Suppression of T cell proliferation by root apical papilla stem cells in vitro. Cells Tissues Organs 2010;191:357-364.
79 Yalvac M, Ramazanoglu M, Rizvanov A et al. Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: Implications in neovascularization, osteo-, adipo-and neurogenesis. The Pharmacogenomics J 2010;10:105-113.
80 Yalvaç ME, Ramazanoglu M, Tekguc M et al. Human tooth germ stem cells preserve neuro-protective effects after long-term cryo-

preservation. Curr Neurovasc Res 2010;7:49-58.
81 Yalvaç ME, Yilmaz A, Mercan D et al. Differentiation and neuro-protective properties of immortalized human tooth germ stem cells. Neurochem Res 2011;36:2227-2235.
82 Dogan A, Yalvac ME, Sahin F et al. Differentiation of human stem cells is promoted by amphiphilic pluronic block copolymers. Int J Nanomed 2012;7:4849-4860.
83 Palmer R, Lubbock M. The soft connective tissues of the gingiva and periodontal ligament: Are they unique? Oral Dis 1995;1: 230-237.
84 Tang L, Li N, Xie H et al. Characterization of mesenchymal stem cells from human normal and hyperplastic gingiva. J Cell Physiol 2011;226:832-842.
85 Wang F, Yu M, Yan X et al. Gingiva-derived mesenchymal stem cell-mediated therapeutic approach for bone tissue regeneration. Stem Cells Dev 2011;20:2093-2102.
86 Marynka-Kalmani K, Treves S, Yafee M et al. The lamina propria of adult human oral mucosa harbors a novel stem cell population. Stem Cells 2010;28:984-995.
87 Zhang QZ, Su WR, Shi SH et al. Human gingiva-derived mesenchymal stem cells elicit polarization of M2 macrophages and enhance cutaneous wound healing. Stem Cells 2010;28:1856-1868.
88 Su WR, Zhang QZ, Shi SH et al. Human gingiva-derived mesenchymal stromal cells attenuate contact hypersensitivity via prostaglandin E2-dependent mechanisms. Stem Cells 2011;29:1849-1860.
89 Zhang Q, Nguyen AL, Shi S et al. Three-dimensional spheroid culture of human gingiva-derived mesenchymal stem cells enhances mitigation of chemotherapy-induced oral mucositis. Stem Cells Dev 2011; 21:937-947.
90 Chen M, Su W, Lin X et al. Adoptive transfer of human gingiva-derived mesenchymal stem cells ameliorates collagen-induced arthritis via suppression of Th1 and Th17 cells and enhancement of regulatory T cell differentiation. Arthritis Rheum 2013;65: 1181-1193.
91 Abe S, Yamaguchi S, Watanabe A et al. Hard tissue regeneration capacity of apical pulp derived cells (APDCs) from human tooth with immature apex. Biochem Biophys Res Commun 2008;371:90-93.
92 Honda MJ, Imaizumi M, Tsuchiya S et al. Dental follicle stem cells and tissue engineering. J Oral Sci 2010;52:541-552.
93 Huang GT-J, Yamaza T, Shea LD et al. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 2009;16:605-615.
94 d'Aquino R, De Rosa A, Lanza V et al. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 2009;18:75-83.
95 Huang GT-J, Sonoyama W, Liu Y et al. The hidden treasure in apical papilla: The potential role in pulp/dentin regeneration and bioroot engineering. J Endod 2008;34: 645-651.
96 Yang B, Chen G, Li J et al. Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix-based scaffold. Biomaterials 2012;33:2449-2461.
97 Lee J-H, Lee D-S, Choung H-W et al. Odontogenic differentiation of human dental pulp stem cells induced by preameloblast-derived factors. Biomaterials 2011;32:9696-9706.
98 Gotlieb EL, Murray PE, Namerow KN et al. An ultrastructural investigation of tissue-engineered pulp constructs implanted within endodontically treated teeth. J Am Dent Assoc 2008;139:457-465.
99 Saito N, Takaoka K. New synthetic biodegradable polymers as BMP carriers for bone tissue engineering. Biomaterials 2003;24: 2287-2293.
100 Feng F, Akiyama K, Liu Y et al. Utility of PDL progenitors for in vivo tissue regeneration: A report of 3 cases. Oral Dis 2010;16: 20-28.
101 Gault P, Black A, Romette JL et al. Tissue-engineered ligament: Implant constructs for tooth replacement. J Clin Periodontol 2010;37:750-758.
102 Washio K, Iwata T, Mizutani M et al. Assessment of cell sheets derived from human periodontal ligament cells: A preclinical study. Cell Tissue Res 2010;341:397-404.
103 Yoshida T, Washio K, Iwata T et al. Current status and future development of cell transplantation therapy for periodontal tissue regeneration. Int J Dent 2012;2012:307024.
104 Arthur A, Shi S, Zannettino AC et al. Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells 2009;27:2229-2237.
105 Yang K-L, Chen M-F, Liao C-H et al. A simple and efficient method for generating Nurr1-positive neuronal stem cells from human wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. Cytotherapy 2009;11:606-617.
106 Wang J, Wang X, Sun Z et al. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cells Dev 2010;19:1375-1383.
107 Király M, Kádár K, Horváthy DB et al. Integration of neuronally predifferentiated human dental pulp stem cells into rat brain in vivo. Neurochem Int 2011;59:371-381.
108 de Almeida FM, Marques SA, Ramalho BdS et al. Human dental pulp cells: A new source of cell therapy in a mouse model of compressive spinal cord injury. J Neurotrauma 2011;28:1939-1949.
109 Taghipour Z, Karbalaie K, Kiani A et al. Transplantation of undifferentiated and induced human exfoliated deciduous teeth-derived stem cells promote functional recovery of rat spinal cord contusion injury model. Stem Cells Dev 2011;21:1794-1802.
110 Sakai K, Yamamoto A, Matsubara K et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 2012;122:80.
111 Yalvaç ME, Yarat A, Mercan D et al. Characterization of the secretome of human tooth germ stem cells (hTGSCs) reveals neuroprotection by fine-tuning micro-environment. Brain Behav Immun 2013;32:122-130.
112 Fang D, Seo BM, Liu Y et al. Transplantation of mesenchymal stem cells is an optimal approach for plastic surgery. Stem Cells 2007;25:1021-1028.
113 Gandia C, Arminan A, García-Verdugo JM et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 2008;26:638-645.
114 Marchionni C, Bonsi L, Alviano F et al. Angiogenic potential of human dental pulp stromal (stem) cells. Int J Immunopathol Pharmacol 2008;22:699-706.
115 Nakashima M, Iohara K, Sugiyama M. Human dental pulp stem cells with highly angiogenic and neurogenic potential for possible use in pulp regeneration. Cytokine Growth Factor Rev 2009;20:435-440.
116 Monteiro B, Serafim R, Melo G et al. Human immature dental pulp stem cells share key characteristic features with limbal stem cells. Cell Prolif 2009;42:587-594.
117 Gomes JÁP, Monteiro BG, Melo GB et al. Corneal reconstruction with tissue-engineered cell sheets composed of human immature dental pulp stem cells. Invest Ophthalmol Vis Sci 2010;51:1408-1414.
118 Sugiyama M, Iohara K, Wakita H et al. Dental pulp-derived CD31−/CD146− side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A 2011;17:1303-1311.
119 Inoue T, Sugiyama M, Hattori H et al. Stem cells from human exfoliated deciduous tooth-derived conditioned medium enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A 2012;19:24-29.
120 Arora V, Arora P, Munshi A. Banking stem cells from human exfoliated deciduous teeth (SHED): Saving for the future. J Clin Pediatr Dent 2009;33:289-294.
121 Russell KC, Phinney DG, Lacey MR et al. In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. Stem Cells 2010;28:788-798.
122 Lee MW, Kim DS, Yoo KH et al. Human bone marrow-derived mesenchymal stem cell gene expression patterns vary with culture conditions. Blood Res 2013;48:107-114.
123 Han J, Mistriotis P, Lei P et al. Nanog reverses the effects of organismal aging on mesenchymal stem cell proliferation and myogenic differentiation potential. Stem Cells 2012;30:2746-2759.

