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Abbreviations and Acronyms

ALP = alkaline phosphatase

BM = basal membrane

BMPs = bone morphogenetic proteins

DFPCs = dental follicle progenitor cells

DP = dental papilla

DPSCs = dental pulp stem cells

DSPP = dentin sialophosphoproteins

ES = embryonic stem cells

FGF = fibroblast growth factor

GFs = growth factors

GTR = guided tissue regeneration

HA = hydroxyapatite

HERS = Hertwig's epithelial root sheath

(continued)

Application of Stem Cell Technology in Dental Regenerative Medicine

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Significance: In this review, we summarize the current literature regarding the isolation and characterization of dental tissue-derived stem cells and address the potential of these cell types for use in regenerative cell transplantation therapy.

Recent Advances: Looking forward, platforms for the delivery of stem cells via scaffolds and the use of growth factors and cytokines for enhancing dental stem cell self-renewal and differentiation are discussed.

Critical Issues: We aim to understand the developmental origins of dental tissues in an effort to elucidate the molecular pathways governing the genesis of somatic dental stem cells. The advantages and disadvantages of several dental stem cells are discussed, including the developmental stage and specific locations from which these cells can be purified. In particular, stem cells from human exfoliated deciduous teeth may act as a very practical and easily accessible reservoir for autologous stem cells and hold the most value in stem cell therapy. Dental pulp stem cells and periodontal ligament stem cells should also be considered for their triple lineage differentiation ability and relative ease of isolation. Further, we address the potentials and limitations of induced pluripotent stem cells as a cell source in dental regenerative.

Future Directions: From an economical and a practical standpoint, dental stem cell therapy would be most easily applied in the prevention of periodontal ligament detachment and bone atrophy, as well as in the regeneration of dentin-pulp complex. In contrast, cell-based tooth replacement due to decay or other oral pathology seems, at the current time, an untenable approach.

SCOPE AND SIGNIFICANCE

DISEASES THAT DESTROY the cellular composition and structure of teeth and surrounding tissue, such as periodontitis and pulpitis compromise patients' standard of living. Once tissue injury occurs in the oral cavity, structures are either lost permanently or heal with little scar formation. Stem cells have the ability to regenerate various differentiated cell types and thus, may be applied to promote the regeneration of functional tissue. This article compares and contrasts somatic dental stem cells and pluripotent stem cells and

discusses their regenerative potential and practicality. Homing of these stem cells is essential for their regenerative potential to take effect, so the methods of delivery, proliferation, and differentiation of the stem cells are also discussed.

TRANSLATIONAL RELEVANCE

Gaining a strong fundamental understanding of the molecular mechanisms that govern dental tissue ontogeny during development is paramount for effective stem cell-based regenerative medicine. Successful

manipulation of self-renewal, differentiation, mechanotransductive, and homing mechanisms will be critical for moving the field of dental regeneration medicine forward.

CLINICAL RELEVANCE

The current treatment plans for dental related diseases, such as periodontitis that have shown some promise in tissue regeneration are bone grafting and guided tissue regeneration (GTR). However, they are performed infrequently and are less reliable than other, more traditional periodontitis treatments. Currently, the government database (ClinicalTrials.gov) describes four registered clinical trials in different stages aimed at the advancement of periodontal ligament stem cells (PDLSCs) in regenerative therapy. Success of the clinical trials indicates that PDLSCs, which are discussed in this review, hold a great potential in treatment of periodontitis.

BACKGROUND

Developmental origins of dental tissues

Craniofacial development is a complex process involving the combined efforts of a cohort of stem cells with varying developmental origins. The teeth alone have at least two embryonic origins. Ectoderm-derived oral epithelium gives rise to dental enamel, while the neural crest give rise to the remaining dental structures, including pulp, dentin and cementum.¹ However, other craniofacial bones, including the flat bones of the skull, are derived from mixtures of progenitor cells, primarily mesodermal cells and neural crest cells.² Stem cell therapy has garnered much attention in the dental community because of the spectrum of opportunity for autologous cell-based therapies. Limitations of current procedures have led researchers

to explore the possible use of stem cells for the regeneration of lost dental structures.

Any effort to advance stem cell therapy into the therapeutic arena will require advances in directed differentiation protocols that can effectively recapitulate the embryological developmental processes of dental tissue. Thus, advancing such efforts necessitates an in-depth understanding of the normal development of dental structures. Odontogenesis starts around the 5th week of embryonic development and continues until all the permanent teeth have replaced primary teeth. After ~5 weeks of gestation, the primary epithelial bands form and thicken at the upper and lower jaws of the future dental arches. Invagination of the oral epithelium around the epithelial bands on both arches result in vestibular lamina and dental lamina. Odontogenesis initiates under the dental lamina ushering in the three stages of the dental development: bud, cap, and bell stages. During the bud stage, the epithelial cells move into the underlying ectomesenchyme, and ectomesenchymal cells pack closer together around the bud (Fig. 1A). In the next, cap stage, the bud splits into a cap-like structure due to proliferation. The epithelium forms the enamel organ, and the ectomesenchymal cells aggregate beneath the enamel organ to form dental papilla (DP) (Fig. 1B). The dental follicle forms around the papilla like a sac. During bell stage, the undersurface of the enamel organ deepens, and cells starts to differentiate (Fig. 1C). Cells of inner dental epithelium (IDE) and DP communicate with each other through the basal membrane (BM). IDE cells and DP cells stop proliferating, and their nuclei move in opposition to the BM and the cells become elongated. Cells of IDE become preameloblasts and they send

Abbreviations and Acronyms (*continued*)

IDE	= inner dental epithelium
iPS	= induced pluripotent stem cells
MSC	= mesenchymal stem cell
PDGF	= platelet-derived growth factors
PDL	= periodontal ligament
PDLSCs	= periodontal ligament stem cells
PRP	= platelet-rich plasma
SCAP	= apical papilla progenitor cells
SHED	= stem cells from human exfoliated deciduous teeth
TGF β	= transforming growth factor beta family

Figure 1. Odontogenesis initiates under the dental lamina ushering in the three stages of the dental development: bud (A), cap (B), and bell stages (C). (A) Bud stage: epithelial cells move into the underlying ectomesenchyme, and ectomesenchymal cells pack closer together around the bud. (B) Cap stage: the bud splits into a cap-like structure and ectomesenchymal cells aggregate beneath the enamel organ to form dental papilla. (C) Bell stage: the undersurface of the enamel organ deepens, and cells start to differentiate. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

signals for cells of DP to become odontoblasts. Odontoblasts subsequently secrete predentin, an organic matrix, around the area underneath the IDE, and form odontoblast processes as they move toward the center of the tooth. The secretion of hydroxyapatite (HA) and mineralization convert predentin to dentin. The presence of dentin signals the preameloblasts to make enamel and become ameloblasts.

Dental roots develop separately from crowns. The internal and external dental epithelial cell layers grow downward from the cervical loop to

form Hertwig's epithelial root sheath (HERS). HERS cells induce the DP to differentiate into odontoblasts producing root dentin. The three cell types that support and stabilize teeth in their sockets are cementoblasts, periodontal ligament (PDL) fibroblasts and alveolar bone osteoblasts. These cells are derived from the dental follicle and together they are called periodontium. It is from this periodontium that stem and progenitor cells with various potential for self-renewal and differentiation have been isolated. In this review, we compare the advantages and limitations of several of the well-characterized periodontium-derived stem cells.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

Cell sources for dental regenerative medicine

When considering cell sources for regenerative medicine, it is widely appreciated that autologous transplantation will be required to effectively circumvent immune-rejection issues. For the purposes of cell replacement in the dental arena, there exist several potentially viable sources of stem/progenitor cells. First, dental follicle cells, which originate from neural crest-derived mesenchyme, give rise to cells that form PDL. Periodontium is vulnerable to attack by bacterial, chemical and mechanical trauma,³ and the results of such damage are different forms of periodontitis.

Figure 2. Periodontal disease. Accumulation of periodontal bacteria trigger neutrophils to migrate into the gingival crevice. Cytokines released by the neutrophils and the bacteria attract macrophages that induce fibroblasts to degrade collagens and the underlying alveolar bone and osteoclasts to degrade the alveolar bone as well. Red arrow indicates attachment loss. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Periodontitis manifests in redness of the gingival tissue, deep periodontal pockets, and movement of teeth resulting from atrophy of alveolar bone and detachment of PDL from the teeth (Fig. 2). Patients can be genetically predisposed to periodontitis; however, environmental factors, including smoking and diabetes are major risk factors for individuals contracting periodontitis. If left untreated, these conditions may lead to loss of the affected teeth.⁴ PDLSCs can be isolated with ease from sources, such as naturally lost or surgically removed teeth.^{3,5} It has also been suggested that stem cells from an inflamed PDL still retain self-renewal and differentiation capacities.⁶ PDL cells were found to differentiate into cementoblast-like cells, osteoblast-like cells, adipocytes, and collagen-forming cells *in vitro*, and transformed into cementum/PDL-like structures when they were transplanted with a calcium HA scaffold into immunocompromised rodents.⁷ Collagen fibers from transplanted PDLSCs were able to link with the cementoblast-like structures which resemble the physiological attachment of Shapely's fibers.⁷ Currently, the clinical trial.gov database describes four registered clinical trials in different stages aimed at the advancement of PDLSCs in regenerative therapy. The search engine of the International Standard Randomized Controlled Trial Number currently has zero registered trials on PDLSCs, and there are currently no published data for assessment of efficacy of PDLSCs in humans.

The current treatment plans for periodontal diseases include scaling and root planning, prescribing antibiotic medications, open flap surgery, along with bone and tissue grafting.⁸ One disadvantage of open flap surgery, scaling, and root planning is the disruption of fibrin linkage by a long junctional epithelium, resulting in periodontal repair instead of regeneration.^{3,9} However, periodontal repair doesn't fully restore the function and architecture of the original tissue.⁹ Two techniques, bone grafting and GTR, have shown much improved regeneration of the attachment apparatus in comparison to traditional open flap surgery.⁹ Bone grafts are the most widely used treatment procedures for the replacement of atrophied alveolar bone. Sources of bone can include autografts, allografts, xenografts, and alloplasts. GTR allows for periodontal regeneration by excluding gingival connective tissue cells and preventing downgrowth of epithelium into the wound in the belief that they interfere with regeneration.¹⁰ However, there are many more contraindications for GTR than there are indications, making GTR reliable in only select cases of periodontitis.^{4,9} The indications for GTR

are narrow 2- or 3- wall infrabony defects, circumferential defects, class II molar furcations, and recession defects.⁹

The inherent multipotency of PDL cells has lead researchers to study different delivery vehicles for growth factors (GFs) and cytokines to attract endogenous PDL stem cells and initiate periodontal regeneration.¹¹ A preclinical approach to GF-induced periodontal regeneration was recently attempted in dogs. Mesenchymal stem cells (MSCs) transfected with fibroblast growth factor (FGF)-2 were implanted in sites of defect, resulting in faster regeneration than the delivery of control MSCs.¹² A clinical trial of implantation of MSCs with GFs was completed in Japan on a 54-year-old female patient. Yamada *et al.* delivered a fibrin gel mixture of bone marrow stromal stem cells and platelet-rich plasma (PRP) to the root surface,¹³ and the result indicated improvements in probing depths, clinical attachment level, bleeding on probing and bone fill. Flores *et al.* demonstrated the ability of transplantable human PDL cell sheets to develop into a cementum-PDL complex *in vivo*¹⁴ (Fig. 2). Thus, these studies on delivery of stem cells combined with GFs show promise in early clinical stages; however, larger scale human studies are necessary to unequivocally determine their efficacy.

In addition to PDL cells, other progenitor cell sources exist within the craniofacial tissues, including dental follicle progenitor cells (DFPCs), apical papilla progenitor cells (SCAP), and dental pulp cells. These cells all share a number of features with the well-studied bone marrow-derived mesenchymal stem cells, including their ability to differentiate, albeit with varying efficacy, into cells of the major mesenchymal lineages: osteoblasts, chondrocytes, and adipocytes. However, these cells seem to differ in their propensity to differentiate into functional odontoblasts.¹⁵ Further, some of these cells, such as the SCAP, are isolated from specific developmental stages (in this case the apex of a developing tooth), and thus, do not serve as practical sources of cells for autologous cell-based transplantation.

Dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), SCAP, and PDLSCs have all been successfully isolated and characterized both *in vitro* and *in vivo*. Because of the nature of the deciduous teeth, guiding the development of permanent teeth and eventual replacement by the permanent teeth, deciduous teeth may act as a very practical and easily accessible reservoir for autologous stem cells and hold the most value in stem cell therapy.¹⁶ SHED are obtained by enzymatic digestion of pulp from

deciduous teeth and are more proliferative than the four other common dental stem cell types.^{15,16} SHED exhibit the capacity to undergo osteogenic and adipogenic differentiation. *In vitro*, SHED express different neural cell markers.¹⁷ Similar to DPSCs and SHEDs, SCAP has the potential to undergo odontogenic differentiation *in vitro*. SCAP is found apical to the epithelial diaphragm, and there is a cell-rich zone between the apical papilla and the pulp.¹⁸ PDLSCs can also be easily obtained and isolated from surgical waste as previously described. DPSCs are not as readily accessible as SHED or PDLSCs, because they are conventionally obtained from freshly extracted third molars (wisdom teeth) of healthy adults.¹⁹ This is problematic when patients have congenitally missing third molars, late eruption of third molars or surgical splitting of impacted teeth which renders them unfit for DPSCs isolation.^{20,21} Successful isolation of DPSCs was demonstrated from pulps of diseased dentitions (excluding pulpitis teeth); however, whether DPSCs from diseased tissues have the same proliferative and differentiation capacity as those isolated from healthy tissue remains to be clarified.²² One important feature of DPSCs is their odontoblastic differentiation potential. *In vitro*, DPSCs exhibited osteogenic, chondrogenic and myogenic differentiation potential.²³ DFPCs were previously isolated from human dental follicles of impacted third molars, and thus, suffer from the same lack of availability for some individuals as DPSCs. DFPCs have the potential to differentiate into periodontium, such as cementum, PDL and alveolar bone. Comparisons of osteo/odontogenic, neurogenic, and chondrogenic biomarkers among the five groups of dental stem cells reveal that DPSCs may most readily differentiate into cells that support dentinogenesis. Second to DPSCs are the SCAP, and then SHED, with PDLSCs and DFPCs expressing just a few biomarkers.¹⁵

Whether the more broadly studied bone marrow-derived or perivascular mesenchymal stem cells are capable of functional differentiation into odontoblasts, or whether the aforementioned dental-derived progenitor cells inherently differ from their bone marrow/perivascular-derived cousins in their being preprogrammed for odontoblastic fate specification remains a matter of some controversy. While Huang *et al.*¹⁵ propose that the differentiation programs that allow dental-derived progenitors to form odontoblasts are analogous to those that allow MSCs to generate osteoblasts based on differential transcriptome profiling of these populations,²⁴ other studies have observed pericyte (perivascular)

MSCs directly differentiating into odontoblasts *in vivo*,²⁵ suggesting that these particular MSCs might be capable of adopting both an osteogenic and odontogenic cell fate. Curiously, human dental pulp cells are the only mesenchymal cells that, to date, have been shown to harbor the ability to differentiate into osteoblasts, odontoblasts, and adipocytes *in vitro*.²⁶ Thus, these dental pulp cells might offer an invaluable experimental platform for elucidating the signals that induce odontogenesis versus osteogenesis.

Gaining a comprehensive understanding of the molecular regulatory programs that specify odontoblast versus osteoblast cell fate and whether dual differentiation potential can be held by an individual progenitor cell *in vivo* is of critical importance for advancing one of the most promising stem cell types, the induced pluripotent stem (iPS) cell into the dental regenerative arena.

iPS cells: promises and limitations in dental regenerative medicine

While somatic stem cells, including MSCs or dental progenitors discussed above offer promise as sources for cell-replacement therapies, they have inherent limitations. In particular, somatic stem cells cannot always be derived from the patient for use in autologous transplantation (Table 1). Another significant concern is the limited replicative potential of these cells that may preclude their clinical use if sufficient numbers for transplantation cannot be generated *in vitro*. To overcome these issues, increasing attention has been paid to the potential use of pluripotent cells for cell-based regenerative medicine.

Pluripotent cells are characterized by their ability to differentiate into *any* cell type of an organism, unlike somatic stem cells that are regarded as multipotent due to their ability to differentiate only into cells of the tissue from which they were isolated. The pluripotent state is characterized by the activity of the pluripotency gene regulatory network, which has the transcription factors Oct4, Sox2, and Nanog at its core. Numerous reports have been published suggesting the presence of pluripotent cells in the adult stroma; however, none have been verified and strong evidence suggests that the pluripotent state, and therefore, the pluripotency regulatory network, does not exist in somatic cells *in vivo*.^{27,28} Human embryonic stem (ES) cells represent the gold standard source of pluripotent cells. ES cells can self-renew indefinitely in culture without losing their capacity to differentiate, in stark contrast to their somatic counterparts where long periods of

Table 1. Sources of somatic dental stem cells

Stem Cell Types	Locations	Advantages	Disadvantages
DPSCs	Pulp of adult teeth	Formation of dentin-pulp-like complex, odontoblastic differentiation potential, source for reparative dentin	Large range of growth rates, different cell morphologies and sizes, need to be obtained from healthy adult teeth.
SHED	Pulp of deciduous teeth	Most proliferative of the five stem cells, easily obtainable, osteoinductive capacity, odontoblastic differentiation potential	Not able to form dentin-pulp-like complex
SCAP	Apex of a developing tooth	Formation of dentin-pulp-like complex, odontoblastic differentiation potential, source for root dentin, superior cell source for tissue regeneration than DPSCs	Not easily obtainable
PDLSCs	PDL	PDL-like formation, cementum-like formation, easily obtainable	Lack odontogenic potential
DFPCs	Dental follicles of extract third molars	PDL-like formation, cementum-like formation	Lack odontogenic potential

DPSCs, dental pulp stem cells; SHED, stem cells from human exfoliated deciduous teeth; SCAP, apical papilla progenitor cells; PDLSCs, periodontal ligament stem cells; DFPCs, dental follicle progenitor cells.

in vitro culture frequently come at the cost of decreased differentiation capacity and replication-induced senescence. The obvious drawback of applying ES cells in regenerative medicine is the inability to access these cells in patients: ES cells must be isolated from preimplantation embryos, usually necessitating the destruction of that embryo. Thus, intense research has been directed at the generation of pluripotent cells from adult (somatic) tissues to fulfill the promise of an autologous, pluripotent cell source for regenerative medicine.

A breakthrough in generating pluripotent cells from somatic tissue came in the 1990s with the advent of cloning, or somatic cell nuclear transfer, in which a somatic genome was transplanted into an enucleated oocyte.²⁹ Unknown factors in the ooplasm could act upon the somatic genome and “reprogram” it to the embryonic, pluripotent state. Unfortunately, this procedure was technically challenging and inefficient in mice, and required the use of donor oocytes that are not readily available in the human population. Thus, successful somatic cell nuclear transfer was never achieved in humans.

When the prospects for generating autologous pluripotent cells looked bleak, a major breakthrough came from the laboratory of Shinya Yamanaka, who went on to win the 2012 Nobel Prize in Physiology or Medicine for his pioneering work. In 2006, Yamanaka and Takahashi reported the generation of pluripotent mouse cells (called induced pluripotent stem, or iPS cells) from a somatic cell source through the simple overexpression of four transcription factors: Oct4, Sox2, Klf4, and cMyc, delivered by retroviral transduction.³⁰ The robust nature of this methodology enabled rapid

verification in a number of laboratories, and soon the procedure was successful in humans.^{31,32} The ease of this reprogramming strategy allowed the technology to be applied widely, and soon it became clear that all nucleated somatic cells are susceptible to iPS cell generation. Further advances allowed for the generation of virus-free iPS cell lines that are functionally indistinguishable from true ES cells.³³

Despite the great promise held by iPS cell technology, there are several limitations that must be addressed before the application of these cells in regenerative medicine. First, the reprogramming process appears to be stochastic and incomplete reprogramming can result in iPS lines with some “epigenetic memory” of their tissue of origin. While this may be a hindrance in many circumstances, it can also potentially be advantageous. For example, generation of iPS cells from a dental pulp progenitor cell may yield cultures that are more prone to odontogenic differentiation than similar iPS cells derived from leukocytes or keratinocytes. Recently, there has also been concern that the rapid cell division that occurs during the reprogramming process may render iPS cells more susceptible to replication-induced DNA mutation, although the significance and scale of this phenomenon is not well understood.³⁴ Such studies emphasize the need for stringent screening of iPS cells (or any cells for that matter) before using it in cell replacement therapy.

Possibly the largest barrier for the use of iPS cells in regenerative medicine is the paucity and inefficiency of existing directed differentiation protocols. Robust directed differentiation of pluripotent cells into the desired functional effector cell type is prerequisite for successful cell-based

therapy, and while tremendous progress is being made in differentiating iPS cells into various terminally differentiated cells, this area remains largely unexplored in dental medicine.

In the case of tooth development, where there is a complex interplay of neural crest-derived mesenchyme and oral epithelium, lineage specification protocols must first be developed to (1) specify ectoderm, (2) specify neural crest versus odontogenic epithelium, (3) initiate coculture of neural crest and epithelial derivatives on a tissue-engineered scaffold. While such stepwise, directed differentiation protocols have not yet been refined for dental tissue, similar approaches have been very successful in generating a variety of neural subpopulations and endodermal derivatives.^{35,36} Given the robust literature on oral ectoderm and odontogenic mesenchyme specification, a similar approach may be viable for derivation of dental tissues from pluripotent stem cells.

A number of classic tissue transplantation experiments have identified stages of induced competence and specification of dental tissue through interactions between craniofacial epithelium and mesenchyme. Epithelial cells of the first branchial arch are capable of instructing tooth formation when cultured with neural crest-derived mesenchyme of the second branchial arch; however, the molecular basis for this competence is not well understood.³⁷ It appears, however, that such competence may arise from the interplay of a number of signaling pathways between the two tissue types, particularly the sequential and reciprocal signaling of bone morphogenetic protein (BMP), FGF, Wnt, sonic hedgehog (SHH), and activin between the mesenchyme and epithelium.^{38–40} These studies and others (reviewed by Thesleff and Jussila⁴¹) provide a blueprint for moving forward in establishing directed differentiation protocols, possibly involving coculture of mesenchymal and epithelial components on a bioscaffold, for the derivation of mature dental tissue from pluripotent stem cells sources, such as iPS cells.

Delivery of stem/progenitor cells for regenerative dentistry

Much excitement has recently surrounded the identification and application of stem cells with therapeutic value, but equally important is the development of delivery methods for transplantation of these cells and the promotion of their efficient engraftment. Traditionally, stem cells are introduced into the body via injection into the site of interest or into the circulation. However, the efficacy of this methodology is questionable, since

studies have shown poor cell survival, engraftment, and unpredictable differentiation *in vivo*.⁴² The potential efficacy of stem cell delivery and differentiation may be improved with the adoption of tissue-engineered scaffolds for cell delivery and structural support. Delivery of dental stem cells can potentially be supported by scaffolds that provide both mechanical and molecular (GF, integrin receptor engagement) cues for differentiation. HA is a naturally occurring mineral form of calcium apatite; it can be coated onto implants and prosthesis to promote osteointegration. *In vitro* and *in vivo* studies of dental stem cells mixed with HA both showed increases in osteogenic markers, including alkaline phosphatase (ALP), Runt-related transcription factor 2 and bone sialoprotein, suggesting that HA or its derivatives may promote osteogenic differentiation of dental stem cells and may serve as a scaffold in delivering stem cells to defect sites.^{7,43} Other scaffold structures that were studied include calcium-silicate derivatives,⁴⁴ and peptide hydrogels.⁴⁵ All of these scaffolds share the common properties of being biodegradable and promoting osteogenic differentiation. Properties of scaffolds include providing cell adhesion, enabling cell proliferation and differentiation, and mimicking microenvironment observed in natural tissues and organs. Unlike injection-based delivery, scaffolds allow for superior control for stem cell delivery and allow for impregnation with time-release GFs, modulation of stiffness, pore size, and cell-substrate interaction. Some evidence suggests that in addition to directly participating in tissue regeneration, transplanted stem cells can also regulate tissue regeneration by secreting trophic factors.⁴⁶ These two properties of stem cells suggest that the proper local distribution of stem cells is critical, making bioscaffold-based delivery one of the most promising strategies in cell-based regenerative dentistry.

Incorporation of GFs and/or cytokines has been shown to increase the efficacy of cell-based regeneration, both in the context of exogenous cell delivery, and recruitment of patients' endogenous stem cells from the local environment *in vivo*. Previously, FGF and PRP were described to enhance regeneration of PDL *in vivo*.^{12,13} GFs enhance dental stem cell activities by promoting the migration of endogenous cells and the subsequent proliferation, differentiation, angiogenesis and neuronal growth. GFs and cytokines are either autocrine or paracrine in nature and modulate cellular behavior by mediating intracellular communication. Kim *et al.* recently discussed the effects of some GFs in pulp-dentin regeneration.⁴⁷

These GFs include platelet-derived growth factors (PDGF), transforming growth factor beta (TGF β) family, vascular endothelial growth factor, FGF and *etc.* When delivered alone or with stem cells to sites of interest, they share common properties of promoting dentinogenesis, cellular proliferation and odontoblastic differentiation. PDGF, for example, normally released by platelets, induces chemotaxis and proliferation of stem cells by promoting angiogenesis and cell proliferation. PDGF has four isoform homodimers and one heterodimer: AA, BB, CC, DD, and AB. When PDGF dimers bind to their receptors, PDGFR α and PDGFR β , the two receptors dimerize and bind to different isoforms of PDGF. PDGFR α and PDGFR β are both present on odontoblasts.⁴⁸

Findings suggest that PDGF influence osteoblastic differentiation via these two receptors by stimulating the expression of dentin sialoprotein, while inhibiting ALP activity in dental pulp cells *in vitro*. BMPs, a subgroup of the TGF β superfamily, promote cellular proliferation, differentiation, and apoptosis, and are well known to have osteoinductive and chondrogenic effects.^{49,50} BMPs were found to induce elevated mRNA expression of dentin sialophosphoproteins (DSPPs) and ALP, and stimulated differentiation of dental pulp cells into odontoblasts.⁵¹ BMPs regulate DSPP expression and odontoblastic differentiation by inducing nuclear transcription factor Y activities.⁵² Choi *et al.* demonstrated *in vitro* the chondrogenesis of PDLSCs when they were treated with TGF β 3 and BMP-6.⁵³ They found upregulation of chondrogenic markers SOX9, aggrecan, and collagen type II.⁵³ The two GFs exhibited synergistic effect when given together.⁵³ Rizk and Rabie demonstrated chondrogenesis of DPSCs transplanted into nude mice on poly-L-lactic acid/polyethylene glycol electrospun fiber scaffolds. These DPSC transplants were more efficient at chondrogenesis when transduced with TGF β 3 virus, further supporting the notion that codelivery of GFs can stimulate basal levels of stem cell differentiation upon transplantation *in vivo*.⁵⁴

CONCLUSIONS

Stem cells are rapidly becoming a focus for the restoration of function and aesthetics in dentistry. Isolation of stem cells from the oral cavity has led researchers to identify five different classes of

TAKE-HOME MESSAGES

- Several somatic stem cell types are capable of generating dental tissues, including DPSCs, SHED, SCAP and PDLSCs.
- SHED may act as a very practical and easily accessible reservoir for autologous stem cells and hold the most value in stem cell therapy.
- DPSCs and PDLSCs should also be considered for their triple lineage differentiation ability and relative ease of isolation.
- iPS cells are able to differentiate into any cell types of an organism; however, efficient directed differentiation protocols for generation of dental tissues remain largely unexplored.
- Cell-based dental regeneration will likely rely heavily on biomimetic scaffolds, such as HA or others that are biocompatible and provide niches for stem cell growth.
- Incorporation of GFs and/or cytokines has been shown to increase the efficacy of cell-based regeneration

tissue-resident stem cells. Advancing cell-based therapy in dentistry will require a strong fundamental understanding of the molecular cues leading to the normal development of dental tissues. Harnessing this knowledge will enable advances in directed differentiation of stem cells and the delivery of therapeutic cell types will likely be much improved by implantation with biomimetic scaffolds and GFs. Ultimately, depending on the site of injury, clinicians need to evaluate the pros and cons of different cell sources when deciding the types of stem cells that should be employed. Stem cells should be useful in preventing detachment of PDL and bone atrophy, and regenerating dentin-pulp complex; however, the *de novo* generation of functional teeth will likely remain elusive in the foreseeable future due to the complex interactions between numerous cell types.

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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Medicine. He is also a member of the UPenn Institute for Regenerative Medicine. Dr. Lengner has authored numerous publications in the field of stem cell biology, including studies on the determinants of mesenchymal stem cell fate commitment, the molecular mechanisms underlying somatic stem cell multipotency, and the generation of iPS cells from somatic cells. The Lengner laboratory is focused on understanding the fundamental molecular pathways governing stem cell self-renewal, how these pathways can be harnessed for regenerative medicine, and how their deregulation contributes to oncogenic transformation.

REFERENCES

- Thesleff I and Tjommers M: Tooth organogenesis and regeneration. StemBook. Cambridge, MA: Harvard Stem Cell Institute, 2008.
- Jiang X, Iseki S, Maxson RE, Sucov HM, and Morriss-Kay GM: Tissue origins and interactions in the mammalian skull vault. *Dev Biol* 2002; **241**: 106.
- Lu H, Xie C, Zhao YM, and Chen FM: Review: further research and therapeutic applications of stem cell transplantation in periodontal regenerative medicine: translational considerations. *Cell Transplant* 2013; **22**: 205.
- Wolff LF, Pihlstrom BL, Liljemark WF, Schaffer EM, and Bandt CL: Distinct categories of microbial forms associated with periodontal disease. *J Periodontol Res* 1985; **20**: 497.
- Mao JJ, Robey PG, and Prockop DJ: Stem cells in the face: tooth regeneration and beyond. *Cell Stem Cell* 2012; **11**: 291.
- Park JC, Kim JM, Jung IH, Kim JC, Choi SH, Cho KS, and Kim CS: Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: *in vitro* and *in vivo* evaluations. *J Clin Periodontol* 2011; **38**: 721.
- Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahimi J, Young M, Robey PG, Wang CY, and Shi S: Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; **364**: 149.
- Greenstein G and Rethman MP: Advantages and limitations of nonsurgical periodontal therapy in the management of chronic periodontitis. *Alpha Omegan* 2000; **93**: 34.
- Wang HL and Cooke J: Periodontal regeneration techniques for treatment of periodontal diseases. *Dental Clin North Am* 2005; **49**: 637, vii.
- Kaldahl WB, Kalkwarf KL, Patil KD, Molvar MP, and Dyer JK: Long-term evaluation of periodontal therapy: I. Response to 4 therapeutic modalities. *J Periodontol* 1996; **67**: 93.
- Chen FM, An Y, Zhang R, and Zhang M: New insights into and novel applications of release technology for periodontal reconstructive therapies. *J Control Release* 2011; **149**: 92.
- Tan Z, Zhao Q, Gong P, Wu Y, Wei N, Yuan Q, Wang C, Liao D, and Tang H: Research on promoting periodontal regeneration with human basic fibroblast growth factor-modified bone marrow mesenchymal stromal cell gene therapy. *Cytotherapy* 2009; **11**: 317.
- Yamada Y, Ueda M, Hibi H, and Baba S: A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: a clinical case report. *Int J Periodontics Restorative Dent* 2006; **26**: 363.
- Flores MG, Hasegawa M, Yamato M, Takagi R, Okano T, and Ishikawa I: Cementum-periodontal ligament complex regeneration using the cell sheet technique. *J Periodontol Res* 2008; **43**: 364.
- Huang GT, Gronthos S, and 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.
- Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, and Shi S: SHED: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA* 2003; **100**: 5807.
- Miura Y, Miura M, Gronthos S, Allen MR, Cao C, Uveges TE, Bi Y, Ehrlichou D, Kortessidis A, Shi S, and Zhang L: Defective osteogenesis of the stromal stem cells predisposes CD18-null mice to osteoporosis. *Proc Natl Acad Sci USA* 2005; **102**: 14022.
- Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, and Bernad A: Spontaneous human adult stem cell transformation. *Cancer Res* 2005; **65**: 3035.
- Tirino V, Paino F, De Rosa A, and Papaccio G: Identification, isolation, characterization, and banking of human dental pulp stem cells. *Methods Mol Biol* 2012; **879**: 443.
- Bishara SE and Andreasen G: Third molars: a review. *Am J Orthod* 1983; **83**: 131.
- Pogrel MAD TB, Swift JQ, Bonine FL, Rafetto LK, Kennedy JE, and Malmquist JP: White Paper on Third Molar Data. Rosemont, IL: American Association of Oral and Maxillofacial Surgeons, 2007. Available at www.aaoms.org/docs/third_molar_white_paper.pdf
- Chen YK, Huang AH, Chan AW, Shieh TY, and Lin LM: Human dental pulp stem cells derived from different cryopreservation methods of human dental pulp tissues of diseased teeth. *J Oral Pathol Med* 2011; **40**: 793.
- Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, Pirozzi G, and Papaccio G: 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.
- Shi S, Robey PG, and Gronthos S: Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. *Bone* 2001; **29**: 532.
- Feng J, Mantesso A, De Bari C, Nishiyama A, and Sharpe PT: Dual origin of mesenchymal stem cells contributing to organ growth and repair. *Proc Natl Acad Sci USA* 2011; **108**: 6503.
- Karbanova J, Soukup T, Suchanek J, and Mokry J: Osteogenic differentiation of human dental pulp-derived stem cells under various *ex-vivo* culture conditions. *Acta Med* 2010; **53**: 79.
- Lengner CJ, Camargo FD, Hochedlinger K, Welstead GG, Zaidi S, Gokhale S, Scholer HR, Tomilin A, and Jaenisch R: Oct4 expression is not required for mouse somatic stem cell self-renewal. *Cell Stem Cell* 2007; **1**: 403.
- Lengner CJ, Welstead GG, and Jaenisch R: The pluripotency regulator Oct4: a role in somatic stem cells? *Cell Cycle* 2008; **7**: 725.

29. Gurdon JB and Colman A: The future of cloning. *Nature* 1999; **402**: 743.
30. Takahashi K and Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663.
31. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, and Yamanaka S: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861.
32. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, and Thomson JA: Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**: 1917.
33. Lengner CJ: iPS cell technology in regenerative medicine. *Ann NY Acad Sci* 2010; **1192**: 38.
34. Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J, Canto I, Giorgetti A, Israel MA, Kiskinis E, Lee JH, Loh YH, Manos PD, Montserrat N, Panopoulos AD, Ruiz S, Wilbert ML, Yu J, Kirkness EF, Izpisua Belmonte JC, Rossi DJ, Thomson JA, Eggan K, Daley GQ, Goldstein LS, and Zhang K: Somatic coding mutations in human induced pluripotent stem cells. *Nature* 2011; **471**: 63.
35. Cheng X, Ying L, Lu L, Galvao AM, Mills JA, Lin HC, Kotton DN, Shen SS, Nostro MC, Choi JK, Weiss MJ, French DL, and Gadue P: Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. *Cell Stem Cell* 2012; **10**: 371.
36. Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G, Antonacci C, Buch A, Yang L, Beal MF, Surmeier DJ, Kordower JH, Tabar V, and Studer L: Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* 2011; **480**: 547.
37. Lumsden AG: Spatial organization of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* 1988; **103 Suppl**: 155.
38. Klein OD, Minowada G, Peterkova R, Kangas A, Yu BD, Lesot H, Peterka M, Jernvall J, and Martin GR: Sprouty genes control diastema tooth development via bidirectional antagonism of epithelial-mesenchymal FGF signaling. *Dev Cell* 2006; **11**: 181.
39. Wang XP, Suomalainen M, Felszeghy S, Zelaryan LC, Alonso MT, Pliukus MV, Maas RL, Chuong CM, Schimmang T, and Thesleff I: An integrated gene regulatory network controls stem cell proliferation in teeth. *PLoS Biol* 2007; **5**: e159.
40. Akiyama T, Maeda S, Yamane S, Ogino K, Kasai M, Kajiura F, Matsumoto M, and Inoue J: Dependence of self-tolerance on TRAF6-directed development of thymic stroma. *Science* 2005; **308**: 248.
41. Jussila M and Thesleff I: Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. *Cold Spring Harb Perspect Biol* 2012; **4**: a008425.
42. Mooney DJ and Vandenburgh H: Cell delivery mechanisms for tissue repair. *Cell Stem Cell* 2008; **2**: 205.
43. van Manen EH, Zhang W, Walboomers XF, Vazquez B, Yang F, Ji W, Yu N, Spear DJ, Jansen JA, and Yelick PC: The influence of electrospun fibre scaffold orientation and nano-hydroxyapatite content on the development of tooth bud stem cells *in vitro*. *Odontology* 2012 Sep 26 [Epub ahead of print]; DOI: 10.1007/s10266-012-0087-9.
44. Gandolfi MG, Shah SN, Feng R, Prati C, and Akintoye SO: Biomimetic calcium-silicate cements support differentiation of human orofacial mesenchymal stem cells. *J Endod* 2011; **37**: 1102.
45. Cavalcanti BN, Zeitlin BD, and Nor JE: A hydrogel scaffold that maintains viability and supports differentiation of dental pulp stem cells. *Dent Mater* 2012; **29**: 97.
46. Capone C, Frigerio S, Fumagalli S, Gelati M, Principato MC, Storini C, Montinaro M, Kraftsik R, De Curtis M, Parati E, and De Simoni MG: Neurosphere-derived cells exert a neuroprotective action by changing the ischemic microenvironment. *PLoS One* 2007; **2**: e373.
47. Kim SG, Zhou J, Solomon C, Zheng Y, Suzuki T, Chen M, Song S, Jiang N, Cho S, and Mao JJ: Effects of growth factors on dental stem/progenitor cells. *Dent Clin North Am* 2012; **56**: 563.
48. Aubin JE, Liu F, Malaval L, and Gupta AK: Osteoblast and chondroblast differentiation. *Bone* 1995; **17**: 77S.
49. Blum B, Moseley J, Miller L, Richelsoff K, and Haggard W: Measurement of bone morphogenetic proteins and other growth factors in demineralized bone matrix. *Orthopedics* 2004; **27**: s161.
50. Urist MR: Bone: formation by autoinduction. *Science* 1965; **150**: 893.
51. Saito T, Ogawa M, Hata Y, and Bessho K: Acceleration effect of human recombinant bone morphogenetic protein-2 on differentiation of human pulp cells into odontoblasts. *J Endod* 2004; **30**: 205.
52. Chen S, Gluhak-Heinrich J, Martinez M, Li T, Wu Y, Chuang HH, Chen L, Dong J, Gay I, and MacDougall M: Bone morphogenetic protein 2 mediates dentin sialophosphoprotein expression and odontoblast differentiation via NF- κ B signaling. *J Biol Chem* 2008; **283**: 19359.
53. Choi S, Cho TJ, Kwon SK, Lee G, and Cho J: Chondrogenesis of periodontal ligament stem cells by transforming growth factor-beta3 and bone morphogenetic protein-6 in a normal healthy impacted third molar. *Int J Oral Sci* 2013; **5**: 7.
54. Rizk A and Rabie AB: Human dental pulp stem cells expressing transforming growth factor beta3 transgene for cartilage-like tissue engineering. *Cytotherapy* 2013; **15**: 712.