Regenerative Endodontic Procedures: A Perspective from Stem Cell Niche Biology

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Abstract

Introduction: Endodontics uses cell therapy strategies to treat pulpal and periapical diseases. During these therapies, surgeons aim to reconstruct the natural microenvironments that regulate the activity of dental stem cells. **Methods:** We searched for more than 400 articles in PubMed using key words from regenerative endodontics and dental stem cell biology. In 268 articles, we reviewed what factors may influence histologic results after preclinical dental treatments that use regenerative endodontic procedures after pulpectomy. **Results:** Several factors, such as the origin of stem cells, the biomimicry of scaffolds used, and the size of lesions, are considered to influence the histologic appearance of the regenerated pulp-dentin complex after treatments. Information is accumulating on transcription factors that generate the pulp-dentin complex and survival/trophic factors that would benefit niche recovery and histologic results. **Conclusions:** In this article, we discuss the noninterchangeability of stem cells, the influence of dentin-entrapped molecule release on pulp regeneration and survival of stem cells, and the need of positional markers to assess treatments histologically. The *ex vivo* amplification of appropriate dental stem cells, the search for scaffolds storing the molecular diversity entrapped in the dentin, and the use of positional transcription factors as histologic markers are necessary to improve future preclinical experiments. *(J Endod 2017;43:52–62)*

Key Words

Biomaterial, endodontics, equivalence, regenerative endodontic procedures, stem cell niche, translational research

Significance

Clinical results of REPs will be improved with new approaches to reconstruct DPSC niches and microenvironments, which take into account the noninterchangeability of stem cells and include the use of scaffolds storing survival/trophic factors entrapped in the dentin.

Regenerative dentistry aims to restore tooth anatomy and function after dental damage (1). In this discipline, regenerative endodontics is a set of biology-based procedures designed to heal periapical lesions and replace cells and dentin of the pulp-dentin complex in a damaged tooth (2). Regenerative endodontic procedures (REPs) are bioengineering therapies that seek to restore the physiological functions of the dental pulp (3–9). These techniques involve a triad of elements: stem cells, growth factors, and biomaterials, also named scaffolds or templates (10–14).

A dental stem cell is a self-renewable cell type in the tooth involved in the maintenance of adult or developing dental tissues (12). These dental stem cells and their daughter cells grow and differentiate dependent on growth factors released by their surrounding tooth microenvironments (15, 16), the dental stem cell niches (1, 17). During REP therapies, the triad aims to reconstruct these microenvironments (18, 19).

Although these techniques clearly induce revascularization after pulpectomy to resolve pulp necrosis and apical periodontitis successfully, scarce results have been published on actual pulp-dentin regeneration (20–25). Applied to the root canal space in animals, REP may generate cementumlike, bonelike (20, 26–30), or periodontal-like (31) tissues instead of a normal dental pulp. Furthermore, REPs applied to human teeth with open apices may also induce the formation of tissues of the periodontium (connective tissue, bone, and cementum) into the root canals (32). Scaffolds impregnated with growth factors and stem cells from other tissues normally induce tissue growth, but its matching to healthy functional tissue is not fully accomplished (33, 34). These nonsatisfactory results suggest that some important factors are not being considered in current REPs.

Although research has paid special attention to the technical expertise of dental stem cell isolation and scaffold developments (12, 29, 35–39), the dental microenvironments that generate the pulp-dentin complex have been much less investigated. The nonsatisfactory results obtained may be caused by the partial reconstruction of these microenvironments, and this could be solved by stimulating either exogenous or endogenous stem cells or growth factors (1).

In this review, we discuss preclinical histologic results of REP treatments and *in vivo* REP-related experiments under this hypothesis of microenvironmental

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2016.09.011

regulation. From this, some experimental improvements and translational developments are discussed to speed the application of REPs to humans.

Dental Stem Cells under the Developmental Biology Paradigm

To better understand technical failures in REPs, the triad of elements used will be first evaluated under the scope of modern developmental biology. Potential metabolic causes of poor dental pulp regeneration will not be discussed here. "Nonequivalence," "hierarchical combinatory of transcription factors," and "positional memory" (40) are cell biology concepts that highlight how signal molecules from scaffolds are influencing stem cells. As a result, the noninterchangeability of dental stem cells or the extreme heterogeneity of released signal molecules are revisited in this article for future progress. Although anti-inflammatory or immunomodulatory treatments are very important topics in REP treatments (13, 41, 42), they are not discussed in this review.

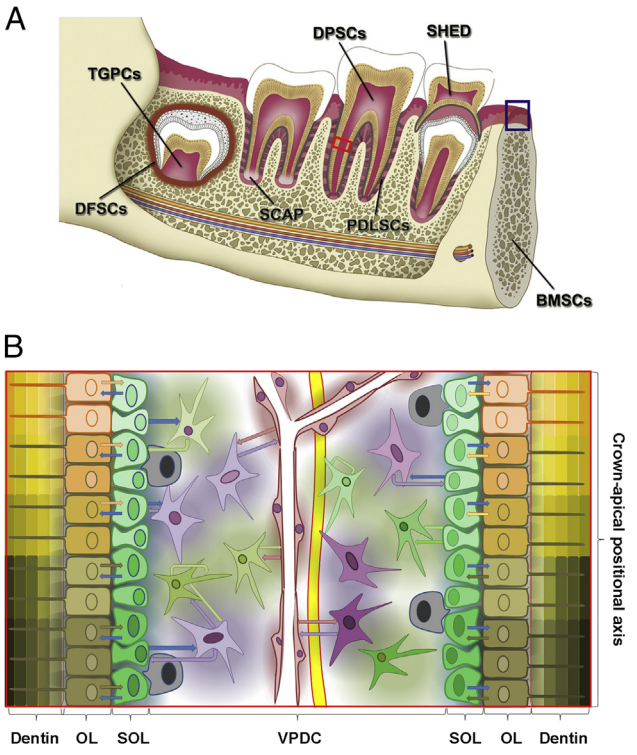

Figure 1. Dental stem cells and signal molecules. (*A*) A drawing of a mandible that shows the most important dental stem cells. Dental stem cell initials are described in the text. Reproduction after *Journal of Prosthodontic* research (Egusa et al, 2012;56:151–165; http://dx.doi.org/10.1016/j.jpor.2012.06.001) with permission from Elsevier (http://creativecommons.org/licenses/by-nc-nd/4.0/) (12). (*B*) A schematic of signal molecule distributions in the pulp-dentin complex. Positional identities of cells are in different colors. Graded colors around cells are specific compositions of released signal molecules. Colors in the margins are different compositions of dentin-entrapped signal molecules. Both well-documented dentin-derived signal molecules transforming growth factor beta-1, bone morphogenetic protein-2, dentin matrix protein 1 and hepatocyte growth factor, and mesenchymal stem cell survival, proangiogenic, morphogens, trophic factors, or cytokines would comprise these compositions. Variations in colors along the crown-apical axis are potential changes in positional identities. OL, odontoblast layer; SOL, subodontoblast layer; VPDC, vascularized pulp-dentin complex. The *arrows* are suggested interactions in the literature. The crown-apical positional axis is shown in the margin. The scheme corresponds to the *red rectangular region* indicated in Figure 1*A*.

Several populations of adult stem cells have been identified in the tooth (Fig. 1*A*). Dental pulp stem cells (DPSCs) (11, 12, 38, 43) and stem cells from human exfoliated deciduous teeth (SHEDs) have been isolated from the pulp-dentin complex. Cells derived from them differentiate *in vitro* into odontoblasts, adipocytes, osteoblasts, or chondroblasts and form dentin/pulplike tissues after *in vivo* transplantation (11, 12). Periodontal ligament stem cells or stem cells of the developing root apical papilla (SCAPs) have also been isolated. Cells derived from these cells can differentiate into odontoblasts, cementoblastlike cells, adipocytes and connective tissue, both *in vitro* and *in vivo* (11, 12). Finally, dental follicle stem cells (DFSCs), potential progenitor cells of periodontal ligament stem cells, tooth germ progenitor cells (12) (Fig. 1*A*), inflammatory periapical progenitor cells, and dental mesenchymal stem cells (DMSCs) (44, 45) (not shown in Fig. 1*A*) are other dental stem cells isolated from the tooth. Stem cells from other mandible positions have also been isolated (eg, bone marrow stem cells [BMSCs]) (Fig. 1*A*). Both DMSCs and BMSCs are considered similar to mesenchymal stem cells (MSCs) (12). Also useful for therapies, induced pluripotent stem cells (iPSs) could be genetically modified to acquire a pluripotent dental-like state (1, 13).

The regenerative potential of these dental cells is clarified by both natural and experimental conditions. Differentiated "dentinoblasts," also named secondary odontoblasts, produce new dentin in response to dental pulp injury. This regenerativelike process is named "reparative tertiary dentinogenesis." Recruitment of endogenous dental stem cells has been proposed to underlie this type of dentinogenesis (46, 47). Furthermore, cells derived from isolated DPSCs (Fig. 1*A*) and DMSCs are sufficient for the generation of a tooth with normal histology but nonsatisfactory morphology (44, 48). Also, epithelial cells and either dentinogenic mesenchymal cells in collagen drops (48, 49) or iPS-derived neural crest cells in reaggregates (50) give functional teeth in mice after coimplantation in the mandible. Both the natural regenerative capacity of the tooth and the behavior of cells derived from isolated dental stem cells or developing dental tissues are of paramount importance in REP preclinical research. New advances at the cellular and molecular levels are providing interesting information to better understand this regenerative potential.

In developmental biology terminology, "equivalence" is a condition by which 2 cells from different embryonic origins (eg, DPSCs and BMSCs) could be interchanged without any developmental perturbation (1, 51, 52). Although iliac, tibia, femur, and orofacial BMSCs may regenerate bone, they are molecularly and functionally different in both human and animal models (33, 53–55). Similar evidence is also accumulating in favor of the "nonequivalence," and thus noninterchangeability, of dental stem cells and MSCs (13, 56–58). This could also be applied to their responses to the niches surrounding them (1, 17, 57, 59, 60). These differences would reside in the diverse developmental origins of these cells.

During animal development or regeneration, cells orchestrate the generation of tissues and organs by the deployment of a "genetic program" (61). This program comprises a hierarchical, combinatorial network (62, 63) of specific transcription factors (TFs). In the cell nuclei, the specific TFs regulate the transcription of genes coding for signal molecules. These molecules would be released to surrounding cells, which in turn would regulate gene transcription in neighbor cells by activating new TFs (Fig. 1*B*) (17). Besides this, other molecular mechanisms may also regulate this basic process (eg, acetylation/deacetylation [64] or microRNAs [65–69]). TFs provide positional prepatterning, size control, and cell differentiation (57). Once an initial pattern of cell specifications is achieved, a "positional memory"