Cell-free Approaches for Dental Pulp Tissue Engineering

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Abstract

The standard treatment modality for teeth with irreversibly damaged dental pulp is root canal therapy, which involves complete removal of the soft tissue and obturation with a synthetic material. So far, research studies show that the combination of stem cells with a suitable scaffold material and transplantation into the root canal may result in the generation of pulplike tissue and the formation of tubular dentin. Because of the technical challenges associated with such a procedure, cell-free alternatives that take advantage of the dental pulp's inherent regenerative capacity because of endogenous stem cell populations and bioactive dentin matrix components need to be considered and explored. Following the tissue engineering approach, this includes (1) a bioactive scaffold, (2) growth and differentiation factors from dentin, and (3) the recruitment of stem cells from resident populations within the pulp or from the periapical region. If this concept proved to be successful, cell-free therapies may be a safer, more practical, feasible, and affordable approach to dental pulp regeneration. *(J Endod 2014;40:S41–S45)*

Key Words

Dentin conditioning, dentin matrix, growth factors

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A healthy dental pulp fulfills several different tasks, namely nociception as a warning system to indicate damage (1), immunoresponse and formation of dentin as active defense mechanisms against invading toxins and bacteria (2, 3), and, in the special cases of young patients, completion of root formation (4). Irritation caused by caries or trauma induces an inflammatory tissue response termed pulpitis. Initially, this reaction may be fully reversible, and healing is possible. Without immediate therapeutic intervention and with increasing intensity of the stimulus, the inflammation will likely progress to an irreversible state. Patients distressed with pain will seek help from their dentist at this point, who, through defined clinical parameters, can diagnose the offending tooth. Traditionally, the therapeutic consequence is the complete removal of pulp tissue and the initiation of root canal treatment, disregarding the fact that dental pulp may possess regenerative capacity.

The terminally differentiated odontoblast keeps a basic secretory activity throughout our lifetime and produces dentin at a very slow rate (5). Upon stimulation, the production rate can be increased and lead to the rapid deposition of reactionary tubular dentin (6). Even after pulp exposure, when the layer of odontoblasts is lost, a reparative dentin barrier can still be formed within 2–6 weeks (7, 8). In this case, cells from within the pulp migrate toward the site of injury, differentiate, and begin to lay down a collagenous matrix, which will later mineralize. This fact has been known for several decades (9), and researchers have concluded that progenitor or stem cells have to be present in the dental pulp, which are able to take over the function of differentiated odontoblasts. This hypothesis was confirmed after the discovery of stem cells in dental pulp of permanent teeth (10).

Cell-based Approaches to Dental Pulp Regeneration

The isolation of dental pulp–derived stem cells has opened new avenues for regenerative dentistry. Stem cells from permanent or deciduous teeth have been used in studies by several research groups (11–17). Sufficient evidence exists by now that dental pulp tissue engineering after the transplantation of dental pulp stem cells in a suitable carrier system is possible (11–17). Models using tooth slices and dentin cylinders laden with scaffolds and dental stem cells have been developed in which vascularized tissue similar to dental pulp formed after several weeks *in situ*; cells adjacent to the dentin had differentiated and begun to deposit tubular dentin (11–13). Animal models in dogs in which dental pulp stem cells were transplanted into root canals after pulpotomy or pulpectomy show the development of pulpal tissue, which cannot be distinguished from the original (14–16). These approaches provide proof of principle that transplantation of stem cells can result in regeneration of the dentin-pulp complex by generation of a pulplike tissue within the root canal. Stem cells could be acquired from either permanent or deciduous teeth, but other cell sources of mesenchymal stem cells might be considered, such as adipose tissue or bone marrow (17). However, the approach of pulp regeneration by stem cell transplantation is afflicted with several problems, including limited availability of stem cell sources, cell harvest and expansion, logistics, excessive cost, and regulatory hurdles for approval and clinical translation. Such a treatment modality appears not to be feasible for a condition such as pulpitis, which is not life-threatening. Therefore, it might be interesting to explore alternative options to stem cell transplantation.

Clinical Regeneration of Dental Pulp

In search of such alternative options, cell-free approaches for dental pulp tissue engineering have to be considered. Clinical case reports show that pulp regeneration

without the transplantation of stem cells is possible in young patients with immature teeth and incomplete root formation. In cases of severe pulpitis or even pulp necrosis with periapical lesions, the consistent elements of the regenerative treatment included the following (12–14):

1. Disinfection of the root canal via irrigation
2. Application of an intracanal dressing, usually with triple antibiotic paste
3. Provocation of bleeding into the root canal by mechanical irritation of remaining pulp or the periapical tissues
4. Placement of mineral trioxide aggregate onto the blood clot

A completion of root formation and healing of periapical lesions was observed in several cases over follow-up periods of 12–24 months (18). How can regeneration in these cases be explained? In the special case of a young patient with incomplete root formation, the presence of a stem cell niche residing in the apical papilla may provide a plausible explanation (19). During root development, the stem cells residing in this niche are responsible for the formation of root dentin and pulp and are guided and instructed by the Hertwig epithelial root sheath (20). With the influx of blood, stem cells from the apical papilla and other possible stem cells derived from the apical region are delivered into the root canal, leading to an enrichment of stem cells (21). Comparisons of systemic blood with intracanal blood in those cases show a significantly higher amount of cells displaying stem cell markers in the intracanal blood compared with systemic blood (21). It is likely that regeneration and completion of root formation described in the previously mentioned case reports is caused by this cell population. Therefore, the clinical regenerative protocol is a cell-free approach without stem cell transplantation in which advantage is taken of resident stem cells in the surrounding tissues.

Cell-free Approaches

An alternative to transferred stem cells are populations already present in the patient's body that can be recruited to the site of injury to stimulate self-healing mechanisms and unlock the innate powers of regeneration. Methods of *in situ* tissue regeneration relying on endogenous cell homing, functional stimulation, and local tissue responses hold great promise because recent proof-of-concept studies document success (22). Regarding pulp regeneration, this approach involves the following:

1. Endogenous stem cells from resident tissue, either dental pulp or the periapical region
2. An injectable, bioactive scaffold, which will eventually undergo cell-mediated degradation to be replaced with natural extracellular matrix
3. Potent chemoattractants and growth factors to induce cell migration, proliferation, and differentiation

Thus, *in situ* tissue regeneration of dental pulp follows the classical tissue engineering approach in contrast to the "regenerative protocol" described in the previously mentioned case reports, which do not involve exogenous scaffolds or growth factors.

Endogenous Stem Cells

In many postnatal tissues, stem cells are present that are responsible for tissue maintenance during regular turnover as well as repair after injury. They reside in a specific stem cell niche, a microenvironment that regulates their survival, self-renewal, and differentiation (23). In this milieu, stem cells may remain quiescent for long periods of time. Upon insult, however, they exit the niche, migrate, proliferate extensively, and differentiate to regenerate tissue damage (23). In most tissues and organs, stem cells are localized around the blood vessel walls, the perivascular niche (23). This holds true also for dental pulp in which colocalization of endothelial and mesenchymal stem cell markers has been shown (24). Dental pulp stem cells are capable of giving rise to a variety of cell types (25, 26). Because of their ability to differentiate into odontoblasts and produce tubular dentin (10), they appear to be a suitable cell source for dental pulp regeneration or tissue engineering. Furthermore, they might contribute to the establishment of a vascular network in newly formed pulplike tissue (11, 12).

TABLE 1. Bioactive Dentin Matrix Components

Growth factors
Transforming growth factor beta (TGF-β) (isoforms 1, 2, and 3)
Basic fibroblast growth factor (bFGF)
Vascular endothelial growth factor (VEGF)
Bone morphogenetic proteins (BMPs)
Insulinlike growth factor (IGF-1 and -2)
Platelet-derived growth factor (PDGF)
Placenta growth factor (PlGF)
Hepatocyte growth factor (HGF)
Epidermal growth factor (EGF)

Data from Smith et al (34), Roberts-Clark and Smith (36), and Tomson et al (37).

In clinical cases of pulpitis, vital tissue is still present in the root canal and can serve as a source of endogenous stem cells. Histologic images have shown that inflammation starts where bacterial invasion takes place, usually in the coronal portion at 1 of the pulpal horns; ongoing stimulation leads to spreading toward the central and apical areas (27). Inflammatory processes can also appear restricted to a defined area, such that healthy pulp coexists with inflamed tissue in a compartmentalized abscess (27). In deciduous teeth, a treatment modality termed pulpotomy exists in which the coronal portion of the pulp is removed, whereas the apical portion is left *in situ* and can remain vital after it has been covered with a suitable material. This procedure is not recommended in permanent teeth at this point because of a limited regenerative capacity after the completion of root formation. Clinical studies in permanent teeth show that pulpotomy does not appear superior in terms of success rates when compared with capping procedures (28). However, considering pulpotomy as part of a

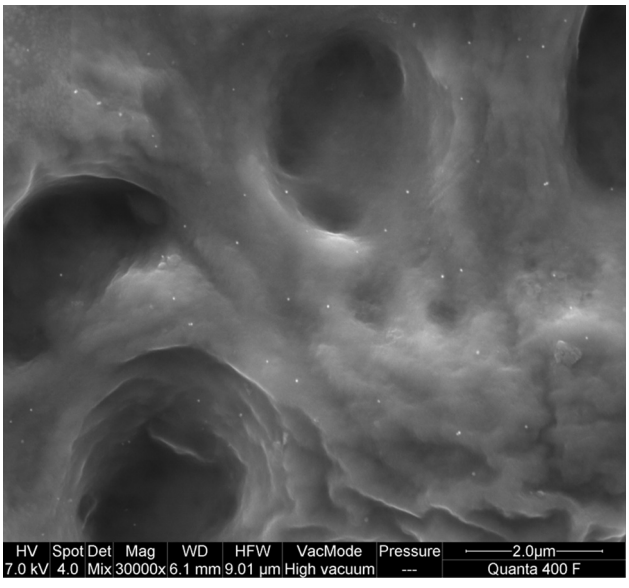

Figure 1. Visualization of transforming growth factor beta 1 exposure on human dentin after treatment with 10% EDTA for 10 minutes. Scanning electron microscopic imaging after immunhistochemistry with secondary antibodies labeled with gold nanoparticles.