Perspectives for Cell-homing Approaches to Engineer Dental Pulp

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Abstract

Sufficient proof is available today to demonstrate that dental pulp tissue engineering is possible. The body of evidence was generated mainly on cell transplantation; however, because of several severe problems afflicted with this approach, it might not be feasible for a clinical setting in the near future. More recently, cell homing has been proposed as a viable alternative. We suggest a modification of the tissue engineering paradigm, where resident cells are attracted by endogenous, dentin-derived growth factors that further induce cell proliferation and differentiation and a bioactive scaffold material laden with these growth factors that serves as a template for tissue formation. This article highlights the latest developments regarding scaffold materials, stem cells, and dentin-derived growth factors specifically for a cell-homing approach to engineer dental pulp and summarizes new ideas. *(J Endod 2017;43:S40–S45)*

Key Words

Cell-homing, dental pulp, dental pulp stem cells, dentin matrix proteins, scaffold, tissue engineering

Significance

The development of biology-based approaches to regenerate or repair dental pulp is possible today because of recent advances in tissue engineering and biomaterials. Cell homing offers a feasible strategy for dental offices, and the described envisioned treatment protocol has the potential to become part of the therapeutic spectrum in endodontics in the near future.

With the isolation of dental pulp stem cells in 2000 (1), the ball was set rolling for tissue engineering approaches to generate dental pulp. Stem cells have been seeded into different scaffold materials and inserted into tooth-derived constructs. Early studies developed a tooth slice model with subcutaneous implantation into mice (2–4). Later, slices were extended to dentin cylinders and eventually whole tooth roots to better mimic the clinical situation of a long and narrow root canal (5–7). Formation of pulp-like tissue, vascularization, differentiation of stem cells into odontoblasts, and generation of tubular dentin by transplanted cells were proven (2–5, 7). In parallel, animal models were established to mimic regenerative endodontic treatment procedures. In a canine model, pulpotomies were conducted where the void was filled with a scaffold material containing stem cells and recombinant growth factor (8). In subsequent studies, the pulpal tissue was extirpated and similarly replaced with scaffold, cells, and growth factor (9, 10). These last experiments provide proof of principle that generation of a pulp-like tissue is possible after stem cell transplantation following a tissue engineering approach. A currently ongoing clinical trial (11) is evaluating clinical practicability and outcome. However, for a clinical setting in a dental office, transplantation of stem cells for pulp regeneration does not appear feasible at the moment. Banking of autologous cells, storage, later expansion, and culture are required. Handling and transplantation of cells must follow good manufacturing practice guidelines, which makes this approach cumbersome and costly. Pulp necrosis is not a life-threatening condition, and root canal treatment is a therapy that offers fairly high success rates (12, 13).

These cell-based experiments follow the classic tissue engineering paradigm, which involves the delivery of stem cells and recombinant growth factors in a carrier/scaffold material. Because of the above-mentioned hurdles, primarily cell-free approaches to dental pulp tissue engineering have been proposed previously (14, 15). Following the principle of cell homing, we suggest a modification of the classic tissue engineering paradigm where resident stem cells are recruited into a specifically designed and custom-made scaffold via endogenous, dentin-derived growth factors (Fig. 1). Evidence is accumulating that a regenerative endodontic procedure following the principle of cell homing might be a feasible and affordable alternative to cell transplantation. Recent work demonstrates that dentin-derived growth factors can be isolated and have beneficial effects on chemotaxis, differentiation, and mineralization of human dental pulp cells (16). Interestingly, preparations of extracellular matrix from pulp or dentin also induce chemotaxis and differentiation selectively in

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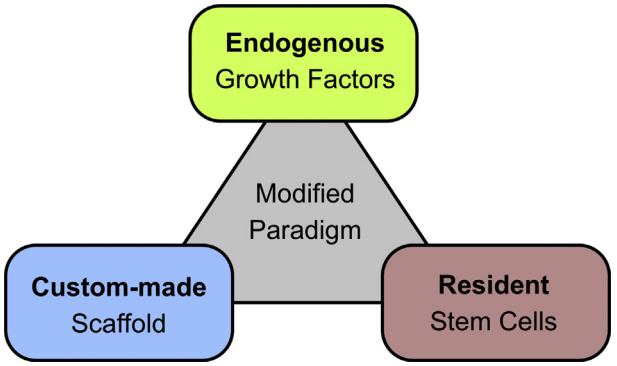

Figure 1. Tissue engineering paradigm modified for cell-homing approaches of pulp regeneration.

pulp-derived cells (17–19). Pulp-like tissue formation was observed in tooth roots filled with collagen and a cocktail of recombinant growth factors after transplantation into the dorsum of mice (15), similarly in tooth roots filled with fibrin gels transplanted on top of the calvarial bone in rats (20).

Materials and Methods

Customized Scaffold Materials

In general terms, scaffold materials should facilitate the attachment, proliferation, migration, and three-dimensional spatial organization of the cell population required for structural and functional replacement of the target tissue. A large variety of biomaterials are available to the tissue engineer (21). In the early years of the tissue engineering era, scaffold materials were sought to be bioinert and were mainly used as a vehicle to deliver cells. These materials fulfilled a mere carrier function, and regeneration occurred more or less uncontrolled as a consequence of the intrinsic capability of the respective stem cells to form new tissue. With advances and new developments regarding the variety, design, and individual properties of biomaterials, the current approach is to use biomimetic, tailor-made scaffolds for specific applications, which closely mimic the natural environment of the target cells and provide mechanical and biochemical cues to promote specific interactions between cells and matrix. Thus, the material properties can control different aspects of cellular behavior both temporally and spatially. Biomimicry can be introduced into a scaffold material by a variety of means including nature-oriented chemistry, composition, and structure; tissue-like rigidity; degradability by cells; possibility for modification (eg, incorporation of antibacterial activity or stimulation of mineralization); bioactive motifs (eg, cell adhesion and cleavage); and delivery of growth and differentiation factors.

Table 1 shows the requirements, both general and specific, for a scaffold material for dental pulp tissue engineering following a cell-homing approach and describes means of biomimicry that can be introduced into scaffold materials.

In past years, several different biomaterials have been used for dental pulp tissue engineering. An overview of different materials tested in this context is provided in Table 2. Our own work with different natural and custom-made synthetic hydrogels enabled us to design synthetic materials that offered high control over the synthesis process and thus material properties and modifications (36), but test series revealed that natural materials are more cytocompatible. Viability of primary dental pulp cells in fibrin or collagen as natural materials and functionalized polyethylene glycol or functionalized self-assembling peptides as synthetic materials are depicted in Figure 2. For this experiment, primary dental pulp cells were isolated as described previously (40), and 1×10^5 cells of passage 3 were seeded into 100 μL of the respective biomaterial in 96-well plates. After gelation, 200 μL cell culture medium was added. MTT assays were performed after 1, 3, 5, and 7 days in culture (Thiazolyl Blue Tetrazolium Bromide; Sigma-Aldrich, St Louis, MO), where absorbance was measured spectrophotometrically at 570 nm (Infinite 200; Tecan, Männedorf, Switzerland). Three independent experiments with samples in triplicates were performed ($n = 9$). Data were treated nonparametrically, and pairwise analysis by Mann-Whitney U test showed significant differences ($\alpha = 0.05$) between natural (fibrin, collagen) and functionalized synthetic materials (self-assembling peptide, polyethylene glycol) during measurement period (unpublished data).

An interesting approach might be the use of decellularized dental pulp, which naturally offers a high degree of biomimicry. Recent work demonstrated the proliferation and differentiation of stem cells from the apical papilla in pulp extracellular matrix after optimization of the decellularization protocol (19).

Resident Stem Cells

In a clinical procedure of pulp regeneration, 2 scenarios are possible:

1. Remnant and healthy pulp tissue can remain after a pulpotomy
2. The pulp, irreversibly inflamed or necrotic, has to be removed completely, and no vital tissue is left inside the root canal.

For the first situation, the remnant tissue presents as a source of resident stem cells. It has been shown that stem cells in the dental pulp reside in their respective niche around blood vessels (perivascular niche) inside the pulpal tissue (41). These cells can be activated after tissue damage and are capable of migration to the site of injury after stimulation. They will differentiate and be able to take over the function of odontoblasts and form mineralized tissue. The situation after complete removal of the pulp tissue might be more challenging; however, recent work has demonstrated elegantly that the periapical tissues harbor stem cells that can migrate into the root canal and differentiate into pulp cells (42, 43). Thus, both scenarios appear feasible for a regenerative endodontic procedure using a cell-homing approach.

TABLE 1. General and Specific Requirements for a Scaffold Material in Terms of Dental Pulp Tissue Engineering Following a Cell-homing Approach

General requirements	Specific requirements
• Simple design and synthesis • Mimicry of extracellular matrix • Biocompatibility • Biodegradability • Penetrability for nutrients and metabolic products • Control over mechanical parameters (rigidity, setting time)	• Injectability • Facilitation of migration, attachment, proliferation, and mineralization of cells • Bioactivity by functional motifs and growth factors • Binding, stabilization, and slow release of growth factors • Antimicrobial property