Cell Homing for Pulp Tissue Engineering with Endogenous Dentin Matrix Proteins

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Abstract

Introduction: Compelling evidence pinpoints that pulp tissue engineering after the transplantation of stem cells is possible. Although intriguing, severe problems regarding clinical feasibility remain. Cell homing has been proposed as a viable alternative in which dentin-derived growth factors in a conducive scaffold may attract resident cells to form pulplike tissue. In this study, an ectopic animal model for *in situ* dental pulp tissue engineering was developed to evaluate whether pulplike tissue formation in empty root canals after the attraction of stem cells was possible and whether this could be enhanced by dentin-derived growth factors. **Methods:** Three types of fibrin (custom-made fibrin, fibrin sealant, and plasma rich in growth factors [PRGF]) as well as a self-assembling peptide were evaluated *in vivo* in a modified tooth root model using human teeth. Root canal dentin was conditioned with EDTA, tooth roots were filled with growth factor–laden scaffolds, and dental pulp stem cells in collagen were placed at the root tip. Constructs were implanted into immunocompromised mice for 4 weeks and subsequently analyzed histologically. Differential interference contrast and second harmonic generation imaging were performed for selected sections. **Results:** For custom-made fibrin and fibrin sealant with dentin matrix proteins, migration into the roots and the formation of a pulplike tissue were observed, whereas the peptide-based scaffold appeared less suitable. PRGF supported tissue formation regardless of the addition of dentin matrix proteins. In the test groups with dentin matrix proteins and EDTA conditioning, differentiated odontoblastlike cells extended cellular processes into the dentinal tubules, which coincided with the deposition of the newly formed collagenous dentin matrix. **Conclusions:** This new cell homing model provides evidence that fibrin derivatives make applicable scaffolds and that dentin-derived proteins induce chemotaxis and pulplike tissue formation. *(J Endod 2018;■:1–7)*

Key Words

Cell homing, fibrin, plasma rich in growth factors, pulp regeneration, self-assembling peptide, tissue engineering

Significance

After irreversible damage to dental pulp, a tooth can remain functional after root canal filling with a synthetic material. Studies show that dental pulp can be regenerated after stem cell transplantation, but this approach is not feasible for dental offices. However, fibrin-based materials enriched with endogenous, dentin-derived growth factors appear suitable for *in situ* tissue engineering because they allow for cell ingrowth and pulplike tissue formation.

Conventional root canal treatment involves the permanent loss of pulpal tissue and a replacement with synthetic materials. In recent years, regenerative approaches have gained increasing interest in endodontics. Vital pulp therapy is currently advocated for teeth diagnosed with reversible pulpitis. However, traditional diagnostic schemes are up for discussion, and preservation of the healthy parts of the pulp rather than complete extirpation is under consideration also for teeth diagnosed with irreversible pulpitis (1). In immature teeth with pulp necrosis in which thin and fracture-prone dentin walls make obturation difficult, revitalization or guided endodontic repair has been established as a treatment alternative to apexification (2). After disinfection, induction of bleeding into the root canal can lead to an influx of stem cells from the apical papilla (3) and new tissue formation. Blood coagulation results in the formation of a 3-dimensional fibrin network containing not only blood cells but also cytokines and growth factors that initiate wound healing (4). Those signaling molecules may promote chemotaxis, proliferation, and differentiation of the aforementioned stem cells inside the root canal and lead to generation of new tissue. An extension of the respective protocol to mature teeth is currently being debated (5). In cases of immature and mature teeth, patients might benefit from less invasive and biology-based treatment approaches in which tissue function is restored to exert an immune response and generate a mineralized tissue barrier. Although current clinical protocols show high success rates for this treatment (6, 7), histologic analysis in animal studies (8) and occasional patient cases (9) show that pulp tissue may not regenerate to its original architecture and function but rather repair by the formation of fibrous tissue, cementum, or bone.

Application of the classic principles of tissue engineering (TE) might allow us to better control the involved cells and tissues and lead to more predictable outcomes.

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Accordingly, the formation of new pulp in empty root canals was shown in animal models by the insertion of scaffolds, cells, and growth factors (10). This approach is impeded by several critical obstacles concerning translation and chairside application, namely the laborious isolation, storage, and expansion of cells along with costly procedures to follow good manufacturing practice guidelines. Hence, cell homing (or *in situ* TE) (ie, the process in which a primarily cell-free scaffold delivers bioactive cues to recruit resident stem cells and induce their differentiation) has gained increasing attention recently (11). *In situ* TE has been investigated for various possible applications including vascular grafts and nerve and hard tissue regeneration (12).

This straightforward approach might be a clinically relevant alternative to enable dental pulp regeneration in the near future. Resident cell sources include mesenchymal stem cells from dental pulp (13), apical papilla in immature teeth (14), and the periapical area of teeth with complete root formation (15).

In this study, an ectopic animal model for *in situ* TE of dental pulp was developed to mimic the clinical situation; human tooth roots were filled with a scaffold laden with dentin matrix proteins, and dental pulp stem cells were placed only at the tip of each root. Four weeks after subcutaneous implantation into mice, the degree and quality of tissue formation were assessed. It was hypothesized that (1) pulplike tissue formation inside tooth roots is possible by the migration of mesenchymal stem cells into the canal, their proliferation and differentiation, and (2) tissue formation can be improved by the exploitation of dentin-derived growth factors, which includes both exposition on canal walls as well as supplementation to the scaffold.

Materials and Methods

Extraction of Dentin Matrix Proteins and Preparation of Tooth Roots

The extraction of dentin matrix proteins (eDMP) was performed as described previously (16). Transforming growth factor beta 1 (TGF-β1) as a consistent and representative growth factor in dentin was quantified via enzyme-linked immunosorbent assay (ELISA) (Quantikine ELISA Kit; R&D Systems Inc, Minneapolis, MN). For *in vivo* experiments, biomaterials in the test groups were supplemented by phosphate-buffered saline (PBS) containing eDMP in a final concentration of 500 pg/mL TGF-β1. In the control groups, PBS without eDMP was used.

Caries-free, extracted human teeth were collected by local oral surgeons after written consent of the patients and stored in 0.5% chloramine (chloramine T hydrate; Sigma-Aldrich, St Louis, MO) at 4°C to avoid bacterial growth and contamination. Roots were prepared with a length of 9 mm, and canals were enlarged to a diameter of 1 mm with a diamond bur under water cooling to ensure removal of the odontoblast layer and subjacent predentin. Specimens were stored in chloramine and transferred to deionized water 24 hours before experimentation. Before insertion of the scaffold materials, tooth roots for the test groups only were immersed in 10% EDTA for 5 minutes; all specimens received a final rinse with PBS (Fig. 1*A*).

All human-derived materials, like tooth roots, dentin, pulp cells, or venous blood, were obtained with informed consent of the patients and in accordance with an ethical vote (ethics number: 14-101-0358; University of Regensburg, Regensburg, Germany). The study conformed to ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines.

Sample Preparation and Implantation

For animal experiments, 4 test and 4 control groups with scaffold materials as described later were established. Tooth roots in the test groups received EDTA conditioning before seeding as well as eDMP in the scaffold material, whereas constructs in the control groups did not receive EDTA treatment or eDMP. For the test groups, all materials were prepared with eDMP at a final concentration of 500 pg/mL TGF-β1 and with PBS only for the control groups. Twelve tooth roots were prepared per group, resulting in a total of 96 constructs.

The following 4 scaffold materials were tested:

1. Custom-made fibrin from fibrinogen and thrombin from bovine plasma (Sigma-Aldrich); final concentrations amounted to 10 mg/mL fibrinogen in PBS and 75 U/mL thrombin in 2.5 mmol/L $CaCl_2$
2. Fibrin sealant, a commercially available product (TISSUCOL Duo S 1 ml Immuno; Baxter, Deerfield, IL)
3. Plasma rich in growth factors (PRGF) was prepared with a commercially available system from BTI (Vittoria-Gasteiz, Spain) using venous blood from a 35-year-old healthy male donor
4. A bioactive self-assembling peptide (SAP) as described previously (17)

Tooth roots were filled with primarily cell-free scaffold materials where gelation occurred (Fig. 1*A*). For cell delivery, a collagen gel (10 μL) was placed at each root tip containing 5×10^5 STRO-1–sorted human dental pulp stem cells of passage 5 as used previously (18). Immunodeficient mice (8- to 10-week-old females, NMRI nu/nu, University of Regensburg) were used as subcutaneous implant recipients according to specifications of an approved small-animal protocol (University of Regensburg). The allocation of roots from 4 different groups per animal, either test or control, resulted in the retrieval of independent samples. Specimens were explanted 4 weeks later, fixed, demineralized, sectioned, and mounted to glass slides.

Data Generation and Evaluation

Sections were stained with hematoxylin-eosin and Masson trichrome. Immunohistochemistry was performed to distinguish mouse and human cells using an antibody against the human nuclear envelope marker lamin A/C. The sections were evaluated by light microscopy, and selected sections underwent differential interference contrast and second harmonic generation (SHG) imaging.

To quantify the dimension of tissue formation by transplanted cells in the test and control groups, a coding system was developed in which code 0 describes no tissue ingrowth and codes 1 to 3 represent ingrowth up to one third, two thirds, or the whole length, respectively (Fig. 1*B*). Two independent examiners analyzed 20 hematoxylin-eosin–stained sections per construct. The highest amount of ingrowth determined the code if variations throughout the roots were evident depending on the cutting plane. Pair-wise statistical analysis was performed using the Mann-Whitney *U* test (SPSS Version 24.0; IBM Corp, Armonk, NY) to compare the test and control groups as well as different materials at an $\alpha = 0.05$ level of significance.

More detailed information regarding the methodology and materials is provided in the supplemental information.

Results

Histology and Immunohistochemistry

Histologic analysis revealed that all codes were present throughout the constructs. If dentin matrix proteins were added (the test group), more tissue ingrowth was present for all materials. A statistically significant difference between the test and control groups existed for fibrin sealant ($P = .015$). For PRGF, considerable tissue ingrowth was observed also in the control groups without eDMP with significant differences compared to fibrin sealant and SAP (Table 1). The least