Influence of Root Canal Disinfectants on Growth Factor Release from Dentin

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Abstract

Introduction: During dentinogenesis, growth factors become entrapped in the dentin matrix that can later be released by demineralization. Their effect on pulpal stem cell migration, proliferation, and differentiation could be beneficial for regenerative endodontic therapies. However, precondition for success, as for conventional root canal treatment, will be sufficient disinfection of the root canal system. Various irrigation solutions and intracanal dressings are available for clinical use. The aim of this study was 2-fold: to identify a demineralizing solution suitable for growth factor release directly from dentin and to evaluate whether commonly used disinfectants for endodontic treatment will compromise this effect. **Methods:** Dentin disks were prepared from extracted human teeth and treated with EDTA or citric acid at different concentrations or pH for different exposure periods. The amount of transforming growth factor-β1 (TGF-β1), fibroblast growth factor 2, and vascular endothelial growth factor were quantified via enzyme-linked immunosorbent assay and visualized by gold labeling. Subsequently, different irrigation solutions (5.25% sodium hypochloride, 0.12% chlorhexidine digluconate) and intracanal dressings (corticoid-antibiotic paste, calcium hydroxide: water-based and oil-based, triple antibiotic paste, chlorhexidine gel) were tested, and the release of TGF-β1 was measured after a subsequent conditioning step with EDTA. **Results:** Conditioning with 10% EDTA at pH 7 rendered the highest amounts of TGF-β1 among all test solutions. Fibroblast growth factor 2 and vascular endothelial growth factor were detected after EDTA conditioning at minute concentrations. Irrigation with chlorhexidine before EDTA conditioning increased TGF-β1 release; sodium hypochloride had the opposite effect. All tested intracanal dressings interfered with TGF-β1 release except water-based calcium hydroxide. **Conclusions:** Growth factors can be released directly from dentin via EDTA conditioning. The use of disinfecting solutions or medicaments can amplify or attenuate this effect. *(J Endod 2015;41:363–368)*

Key Words

Dentin, growth factors, intracanal dressing, irrigation solutions, regenerative endodontics

During tooth development, neural crest–derived cells of the dental papilla undergo terminal differentiation into dentin-forming odontoblasts. On completion of cytodifferentiation, the odontoblasts start their secretory phase and produce dentin, the major structural component of teeth. Similarly as in bone, dentin formation first requires an organic template that later mineralizes with hydroxyapatite crystals to form calcified tissue. During this synthesis process, the odontoblasts not only lay down the unmineralized predentin but also express various bioactive molecules, which are secreted into the extracellular space (1–5). During mineralization, these bioactive factors become embedded and immobilized in the dentin matrix. Because active proteins and growth factors have a short half-life, binding to extracellular matrix components may be required to maintain their bioactivity by protecting them from proteolytic degradation and thus prolonging their life span. Among growth factor–binding compounds are proteoglycans, mainly heparin sulfate (6, 7), also specific binding proteins (8), glycoproteins (9), and different types of collagen (10, 11).

Because there is no turnover in dentin extracellular matrix, regulatory molecules can be reactivated much later in life on release from their bond. During caries, bacterial lactate exposes the organic component of dentin and releases bioactive factors, which may modify immunoresponse, cell recruitment, and differentiation (12). Application of dental materials, namely calcium hydroxide or mineral trioxide aggregate but also self-etching dental adhesives, releases bioactive factors (13–15). Organic acids or chelating agents such as EDTA are also suitable for dentin demineralization. A variety of growth factors are present in the EDTA-soluble fraction of demineralized human dentin extracellular matrix, including transforming growth factor β1 (TGF-β1), fibroblast growth factor 2 (FGF-2), bone morphogenetic protein 2, platelet-derived growth factor, placenta growth factor, and epidermal growth factor, in addition to angiogenic factors such as vascular endothelial growth factor (VEGF) (1–3). These molecules are effective at very low concentrations and still elicit cellular responses at the picogram level, modifying immunodefense, angiogenesis, cell recruitment, proliferation, differentiation, and mineralization (16–18). Regenerative endodontic procedures are based on exactly these cellular reactions. After provocation of bleeding into the root canal, stem cells from the apical papilla are flushed in (19). For these cells to regenerate pulp tissue, they need to proliferate, differentiate, and produce dentin, and a vascular network is essential to maintain cellular metabolism. The exposure of growth factors from

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root canal dentin via dentin conditioning might be a contributing factor to successful regenerative endodontic treatment by directing cellular fate.

Previous studies have mainly used powdered dentin and the EDTA-soluble fraction to extract growth factors (1–3, 7, 13, 14, 17). In regenerative endodontic therapies, the direct release of growth factors from intact dentin would be more feasible. To our knowledge, this has not been investigated or quantified. Thus, the first objective of this study was to develop a protocol for growth factor release from human dentin, to quantify the amount of 3 different growth factors, and to visualize their exposure on the dentin surface.

Yet another key factor to success of endodontic treatment is sufficient elimination of microorganisms from the root canal system (20). It can be assumed that this holds true also for regenerative approaches. In conventional root canal treatment, decontamination with antimicrobial solutions and intracanal dressings are essential. Commonly used disinfectants for irrigation during root canal preparation include sodium hypochlorite (21–25), chlorhexidine (22, 25, 26), or EDTA (21, 24, 25) in various concentrations. Intracanal medicaments, which remain in the root canal between visits, include calcium hydroxide–based, chlorhexidine-based, antibiotic-based, and corticosteroid-based gels and pastes (27). The second objective of this study was to test whether different irrigation solutions and intermediate intracanal dressings influence the amount of growth factor released from dentin.

Materials and Methods

Quantification of TGF-β1 Release from Dentin

For all experiments, dentin disks were prepared from human extracted molars with a wheel saw (SP/600; Leitz GmbH, Wetzlar, Germany) under constant water flow at 600 rpm (quantification of growth factors: 8-mm diameter, 200-μm thickness; gold labeling: 6-mm diameter, 500-μm thickness). Disks were stored in 0.5% chloramine solution (Chloramin T trihydrate; Merck, Darmstadt, Germany), which was exchanged with aqua bidest 24 hours before experimentation.

To test the ability of different solutions to release TGF-β1 from the dentin surface, each disk was immersed in 100 μL test solution for 5, 10, or 20 minutes: (group A) EDTA, 268 mol/L (10 w%), pH 6; (group B) EDTA, 268 mol/L (10 w%), pH 7; (group C) EDTA, 456 mol/L (17 w %), pH 7; (group D) citric acid, 0.476 mol/L (10 w%), pH 2; (group E) citrate buffer (citric acid 0.476 mol/L and trisodium citrate dihydrate 1.55 mol/L), pH 5; and (group F) citric acid phosphate buffer (citric acid 0.476 mol/L, trisodium citrate dehydrate 0.68 mol/L, trisodium phosphate 1.09 mol/L), pH 7.

The pH values were adjusted by using hydrochloric acid or sodium hydroxide. After dentin treatment, the irrigation solution was removed, immediately frozen in liquid nitrogen, and stored at −80°C. After completion of sample collection, all samples were thawed and subjected to growth factor quantification by using an enzyme-linked immunosorbent assay (ELISA) test system for TGF-β1 (Human TGF-beta 1 Quantikine ELISA Kit; R&D Systems, Wiesbaden, Germany). Three independent experiments were performed, each containing triplicate samples. Interference of the test solutions with antibody-binding on the ELISA plates was excluded in pilot experiments, where kit standards of known concentrations of recombinant TGF-β1 were dissolved in the solutions used in groups A–F. The resulting absorption values were in accordance with those of the standards delivered with the ELISA kit. Sodium hypochloride (NaOCl) was also tested; this solution was not compatible with the ELISA test.

Effect of EDTA on the Release of FGF-2 and VEGF

To measure the release of the growth factors FGF-2 and VEGF, dentin disks were treated with EDTA, 10% at pH 7 for 5, 10, and 20 minutes. This irrigation solution was chosen on the basis of the results from experiments with TGF-β1 described above. Analog to the previous experiments, the amount of growth factor was determined by using ELISA test systems (Human FGF basic Quantikine ELISA Kit and Human VEGF Quantikine ELISA Kit; R&D Systems). Three independent experiments with triplicate samples were performed.

Visualization of Growth Factor Exposure

Growth factor exposure on the dentin surface after EDTA conditioning was visualized by gold labeling and subsequent scanning electron microscopy (SEM) imaging. Primary antibodies were used for collagen type I (Acris, Herford, Germany), as well as for TGF-β1, FGF-2, and VEGF (Abcam, Cambridge, UK). Dentin disks were immersed in EDTA (10%, pH 7) for 10 minutes, blocked with 2% goat serum for 2 hours, and incubated with primary antibodies overnight at 4°C. Secondary antibodies with gold nanoparticles (25 nm; Aurion, Wageningen, Netherlands) were applied for 2 hours at 4°C. Gold labeling with antibodies for collagen type I was performed for comparison. Negative controls included untreated dentin, which showed a smear layer, NaOCl-treated dentin, and EDTA-treated dentin without primary antibody treatment; none of the controls showed gold labeling.

Dentin disks were fixed in 2.5% glutaraldehyde at 4°C overnight, left to dry, mounted onto aluminum stubs by using self-adhesive carbon disks (Leit-Tabs; Provac, Oestrich-Winkel, Germany), and prepared for SEM analysis after carbon coating. An FEI Quanta 400 FEG environmental scanning electron microscope with a field emitter diode was used (FEI Europe B.V., Eindhoven, Netherlands) and operated in HVSEM imaging mode.

TGF-β1 Release after the Use of Irrigation Solutions

For further release studies, 10% EDTA at pH 7 was chosen for dentin conditioning and TGF-β1 as a representative growth factor. Two commonly used disinfection solutions were tested for interference with EDTA-induced growth factor release: chlorhexidine (Gum Paroex, chlorhexidine digluconate 0.12 %; Sunstar, Etoy, Switzerland) and sodium hypochloride (5.25%; Speiko, Münster, Germany).

Dentin disks were immersed in either solution for 5 or 10 minutes. Subsequently, the disks were treated with EDTA for 5, 10, or 20 minutes, and the release of TGF-β1 into the EDTA solution was quantified via ELISA.

TGF-β1 Release after the Use of Intracanal Medicaments

The following intracanal medicaments were tested: corticoid-antibiotic paste: Ledermix (Riemser Pharma GmbH, Greifswald, Germany); calcium hydroxide, water-based: Calxyl (OCO-Präparate, Dirmstein, Germany); calcium hydroxide, oil-based (sample from DMG, Hamburg, Germany); triple antibiotic paste: 20 mg ciprofloxacin, 20 mg cefuroxime, 40 mg metronidazole in macrogol propylene glycol (Apotheke Dr. Hörmann, Weinfelden, Switzerland); and chlorhexidin-bis (D-gluconate) gel: Chlorhexamed 1% gel (GlaxoSmithKline, Brentford, UK).

Dentin disks were coated with a layer of intracanal dressing and incubated at 37°C in a humidified atmosphere for 48 hours. Subsequently, the disks were rinsed with phosphate-buffered saline 3 times and immersed in EDTA for 5, 10, or 20 minutes as described above, and TGF-β1 release was quantified via ELISA.