Release of Growth Factors into Root Canal by Irrigations in Regenerative Endodontics

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Abstract

Introduction: The aim of this study was to investigate the release of growth factors into root canal space after the irrigation procedure of regenerative endodontic procedure. **Methods:** Sixty standardized root segments were prepared from extracted single-root teeth. Nail varnish was applied to all surfaces except the root canal surface. Root segments were irrigated with 1.5% NaOCl + 17% EDTA, 2.5% NaOCl + 17% EDTA, 17% EDTA, or deionized water. The profile of growth factors that were released after irrigation was studied by growth factor array. Enzyme-linked immunosorbent assay was used to validate the release of transforming growth factor (TGF)-β1 and basic fibroblast growth factor (bFGF) at 4 hours, 1 day, and 3 days after irrigation. The final concentrations were calculated on the basis of the root canal volume measured by cone-beam computed tomography. Dental pulp stem cell migration on growth factors released from root segments was measured by using Transwell assay. **Results:** Total of 11 of 41 growth factors were detected by growth factors array. Enzyme-linked immunosorbent assay showed that TGF-β1 was released in all irrigation groups. Compared with the group with 17% EDTA (6.92 $\pm$ 4.49 ng/mL), the groups with 1.5% NaOCl + 17% EDTA and 2.5% NaOCl + 17% EDTA had significantly higher release of TGF-β1 (69.04 $\pm$ 30.41 ng/mL and 59.26 $\pm$ 3.37 ng/mL, respectively), with a peak release at day 1. The release of bFGF was detected at a low level in all groups (0 ng/mL to 0.43 $\pm$ 0.22 ng/mL). Migration assay showed the growth factors released from root segments induced dental pulp stem cell migration. **Conclusions:** The root segment model in present study simulated clinical scenario and indicated that the current irrigation protocol released a significant amount of TGF-β1 but not bFGF. The growth factors released into root canal space induced dental pulp stem cell migration. *(J Endod 2016;42:1760–1766)*

Key Words

Cell migration, growth factors, irrigation, regenerative endodontics, root canal surface

Significance

Growth factors play an important role in regenerative endodontics. Until now, few studies have explored the release of growth factors into root canal by irrigations used in regenerative endodontic treatment. Our study provides the direct evidence that multiple growth factors are released from dentin after irrigations, and the study furthers the current knowledge of growth factors in regenerative endodontics.

Endodontic therapy for an immature permanent tooth with pulp necrosis and apical periodontitis is a challenge. Conventional root canal therapy is limited because of the thin dentinal walls and open apex (1). Apexification provides an alternative treatment modality by inducing the formation of apical barrier for later obturation of the canal. In the 1960s, Nygaard-Ostby (2) raised the concept of tissue regeneration inside the root canal. In 2004, Banchs and Trope (3) introduced a modified clinical protocol that involved minimal instrumentation, copious irrigation, and placement of antimicrobial intracanal medicament, followed by inducing bleeding inside the root canal. In the last decade, numerous case series and several retrospective and prospective studies showed that continued root development and apical closure were achieved in regenerative endodontic cases (4–8). However, the success rate of regenerative endodontic treatment compared with apexification is still inconclusive because of the variable study designs and limited sample sizes (9–13). In addition, the histologic results from animal studies and human case reports showed that the regenerated tissues inside the root canal were not pulp tissues but periodontal tissues instead (14, 15).

To improve the outcome of regenerative endodontic therapy and regenerate a functional pulp-dentin complex, tissue engineering technology has been applied in the field of regenerative endodontics (16–18). The successful regeneration of a pulp-dentin complex needs all components of the tissue engineering triad: stem cells, growth factors, and scaffolds. Studies have proved that stem cells were delivered into the

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root canal with bleeding in regenerative endodontic treatment (19, 20). These stem cells could be stem cells from apical papilla (SCAPs), inflamed periapical progenitor cells, periodontal ligament cells, and bone marrow stromal cells (21). The blood clot, platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and various bioscaffolds have also been studied and tested (22–24). The remaining question is whether the growth factors are present in this regeneration system.

Growth factors play a critical role in dental stem cell recruitment, migration, proliferation, and differentiation (25–27). In regenerative endodontics, growth factors may come from different sources: blood clot, PRP, or PRF (28, 29), and dentin matrix has been found to be a reservoir of growth factors. During tooth development, a variety of bioactive molecules are secreted by odontoblasts and embedded in dentin matrix (30, 31), including transforming growth factor (TGF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and epidermal growth factor (32, 33). It has been shown that these growth factors could be released on the damage to dentin or during repair processes to promote dentin regeneration (34–36). It is important to know whether these growth factors could also be released from dentin matrix into the root canal space after the canal is treated with current regenerative endodontic protocol, which involves minimal instrumentation and copious irrigation with NaOCl and EDTA. Up to now, there has been no study directly addressing this question. In some previous studies, growth factors were either extracted from dentin powder (32) or extracted from EDTA in which the dentin disks were submerged (37). None of these studies simulated clinical scenario of regenerative endodontics in which the growth factors released after irrigation could contribute to the regeneration process.

The aim of this study was to investigate the release of growth factors into the root canal space after the irrigation procedure following current American Association of Endodontists (AAE) regenerative endodontic protocol (38). The type and amount of growth factors were evaluated and validated by using a root segment model. Functionality of growth factors released from root segments was also studied with migration of dental pulp stem cells (DPSCs).

Materials and Methods

Preparation of Root Segment Model

Total of 60 extracted teeth were collected from the oral surgery clinic. The teeth met the following criteria: permanent teeth, single root, and teeth without fractures, artificial alterations, and anatomic aberrations. The study was approved by the Institutional Review Board of Temple University.

The freshly extracted teeth were rinsed with phosphate-buffered saline, and periodontal tissues were removed by scrapping the root surface with a scalpel blade (Fig. 1*A*). After the coronal portions of teeth were removed, root segments were prepared and standardized by cutting 12 mm from apex (Fig. 1*B*). Because previous studies indicated that 1 mm was the critical apical size for revascularization (39, 40), all root segments were instrumented with hand files up to size 100 to achieve a standardized truncated cone-shaped canal with open apex of 1 mm in diameter (Fig. 1*C*). The external root surfaces were covered with nail varnish, and only the inner root canal surface was left uncovered (Fig. 1*D* and *E*).

Growth Factor Array

Three root segment samples were irrigated with 1.5% NaOCl (20 mL/5 min), followed by 17% EDTA (20 mL/5 min). Medium was collected at day 1 and subjected to growth factor array (Human 41 Growth Factor Glass Factor Antibody Array; Creative Proteomics, Shirley, NY). Total of 41 targets were measured (Table 1). The negative control was the medium collected from 1 root segment that had all surfaces of root segment (including internal root canal surface) wrapped with nail varnish. The fluorescent signals of the target growth factors were imaged and captured by using GenePix4000 B Microarray Scanner (Axon Instruments, Sunnyvale, CA). The expression of targets was normalized to the control group in consideration of the background effect from each target. The targets with detectable expression were those that had higher expression than threshold according to the manufacturer's manual (Creative Proteomics).

Irrigation Protocols, Sample Collection, and Enzyme-linked Immunosorbent Assay

Forty-eight prepared root segments were randomly allocated to 4 groups with different irrigation protocols:

1. 1.5% NaOCl (20 mL/5 min) followed by 17% EDTA (20 mL/5 min),
2. 2.5% NaOCl (20 mL/5 min) followed by 17% EDTA (20 mL/5 min),
3. 17% EDTA (20 mL/5 min), and
4. deionized water (20 mL/5 min). After the irrigation, the segments were placed into 1 mL alpha-minimum essential medium (α-MEM) (HyClone, Logan, UT) supplemented with 100 U/mL penicillin and 100 U/mL streptomycin (HyClone). The samples were kept at 37°C for 4 hours, 1 day, or 3 days. At each time point, medium from 4 samples was collected and filtered. The amount of TGF-β1 and bFGF released into collecting medium was quantified by using enzyme-linked immunosorbent assay (ELISA) following the protocol provided by the manufacturer (R&D Systems, Minneapolis, MN).

Calculation of Growth Factor Concentration in Root Canal

Considering the significant difference between the volume of root canal space and the volume of medium used in ELISA, previous studies that used the volume of solution in ELISA to calculate growth factor concentration may have underestimated the actual concentration of growth factors inside the canal. The volume of prepared root canal space in each root segment was measured by cone-beam computed tomography (CBCT) (OP300; Instrumentarium Imaging Dental, Milwaukee, WI), and the volume of canal space was calculated as a truncated cone. The parameters including the length (L), coronal diameter (D), and apical diameter (d) were measured by using software Invivo Version 5.2 (Anatomage Inc, San Jose, CA). Each measurement was repeated 3 times. The volume (V) of canal space was calculated as the following:

$$V_{(canal)} = \pi L\left\{(D/2)\hat{}2 + (D/2)(d/2) + (d/2)\hat{}2\right\}\Big/3$$

The final concentration of growth factors in root canal space (C_{canal}) was calculated as the following:

$$C_{(canal)} = C_{(ELISA)} \times V_{(collecting\ Medium)}\Big/V_{(canal)}$$

Migration of DPSCs

The capability of growth factors inducing DPSC migration was measured by using Transwell assay (Corning, Kennebunk, ME). The third passage of human DPSCs (AllCells, Alameda, CA) were cultured in α-MEM supplemented with 15% fetal bovine serum (FBS) (HyClone), 100 U/mL penicillin, and 100 U/mL streptomycin at 37°C in a moist environment with 5% CO_2 atmosphere. These DPSCs expressed