Effect of Different Endodontic Regeneration Protocols on Wettability, Roughness, and Chemical Composition of Surface Dentin

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Abstract

Introduction: We investigated the changes in physiochemical properties of dentin surfaces after performing different endodontic regeneration protocols. **Methods:** Human dentin slices were randomized into 4 treatment groups and 1 untreated control group ($n = 10$). One treatment group was irrigated with sodium hypochlorite (NaOCl) for 5 minutes followed by EDTA for 10 minutes. The other 3 treatment groups were irrigated with NaOCl; treated for 4 weeks with triple antibiotic paste (TAP), diluted triple antibiotic paste (DTAP), or calcium hydroxide ($Ca[OH]_2$); and then irrigated with EDTA. After treatment, contact angles between a blood analog and dentin surfaces were evaluated. Surface roughness and chemical composition were characterized using optical profilometry and energy-dispersive X-ray spectroscopy, respectively. One-way analysis of variance followed by Fisher least significant difference tests were used for statistical analyses. **Results:** All treatment groups showed a significant reduction in wettability and a significant increase in surface roughness when compared with untreated dentin. Dentin treated with $Ca(OH)_2$ had significantly lower wettability compared with all other groups. No significant difference in wettability was found between dentin treated with DTAP and TAP protocols. Dentin treated with TAP had significantly higher surface roughness compared with all other groups. Untreated dentin and NaOCl + EDTA–treated dentin had significantly higher calcium and phosphorus as well as significantly lower carbon compared with dentin treated with $Ca(OH)_2$, DTAP, and TAP. **Conclusions:** Endodontic regeneration protocols had a significant effect on wettability, surface roughness, and chemical composition of surface dentin. The $Ca(OH)_2$ protocol caused a significant reduction in dentin wettability compared with TAP or DTAP protocols. *(J Endod 2015;41:956–960)*

Key Words

Calcium hydroxide, dentin, endodontic regeneration, surface roughness, triple antibiotic paste, wettability

Endodontic regeneration procedures are contemporary, biologically based therapies that manage immature teeth with necrotic pulps. These procedures may offer several advantages over traditional treatments of necrotic immature teeth, such as a shorter treatment time (1) and continuous root development (1, 2). The first critical aspect of endodontic regeneration procedures includes the disinfection of root canal systems using intracanal irrigants, mainly sodium hypochlorite (NaOCl), and medicaments (3). The most commonly used medicaments during endodontic regeneration are triple antibiotic paste (TAP) and calcium hydroxide ($Ca[OH]_2$) (3). However, recent recommendations suggest the use of low concentrations of TAP, ranging from 0.1–1 mg/mL, to avoid cytotoxic effects against human stem cells of the apical papilla (4, 5). Furthermore, concerns have been raised regarding the dental discoloration effect of minocycline present in TAP (6) as well as the development of antimicrobial resistance and an allergic reaction to antibiotic medicaments (7). The other important aspect of endodontic regeneration procedures includes creating a regenerative biological environment inside the root canal system through irrigation with EDTA and the initiation of a blood clot (3, 8).

Evoking bleeding and efficiently wetting root canal dentin may improve the interaction between stem cells and the dentin surface. Indeed, the induced bleeding step in regenerative procedures was found to convey a significant amount of stem cells into the canal space (9). The wettability of radicular dentin was suggested to modify the attachment of dental pulp cells to dentin (10). Furthermore, an increase in the surface wettability of a substrate was associated with a significant improvement in cellular attachment and protein adsorption (11). The topography and chemical structure of the dentin surface are surface properties that may play an important role in modifying dentin wettability during endodontic regeneration. These surface properties were also suggested to have a significant effect on the attachment and proliferation of dental pulp stem cells (12–15). This study aimed to investigate changes in the wettability, roughness, and chemical structure of surface dentin after various endodontic regeneration protocols.

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Materials and Methods

Sample Preparation

Fifty intact human third molars were collected and stored in 0.1% thymol solution at 4°C after obtaining local institutional review board approval. A dentin disc (1.5 mm) was cross-sectioned from each molar parallel to the occlusal surface and close to the pulp chamber using a low-speed saw (IsoMet; Buehler, Lake Bluff, IL) under constant irrigation. The nonpulpal side of each disc was flattened with 500-grit silicon carbide paper (Struers, Cleveland, PA) using a polishing unit (Struers). The pulpal sides of each disc were flattened using 1200-, 2400-, and 4000-grit silicon carbide paper (Struers) followed by 0.3 μm diamond polishing spray (Struers). The polished specimens were then sonicated in deionized water for 3 minutes. Deep coronal dentin, rather than radicular dentin, was used to provide an adequate surface area for multiple measurements of the various outcomes of the study. A previous study found no significant differences between radicular and deep coronal dentin in density and cross-sectional areas of dentin tubules, even after various acidic challenges (16).

Preparation of Medicaments Used in the Study

A clinically recommended concentration of TAP (1000 mg/mL) was prepared by mixing 1000 mg United States Pharmacopeia grade antibiotic powders comprising equal portions of metronidazole, ciprofloxacin, and minocycline (Champs Pharmacy, San Antonio, TX) with 1 mL sterile water (4, 17). A diluted pastelike consistency of 1 mg/mL TAP (DTAP) was prepared as described in recent studies (18, 19). In summary, 50 mg TAP antibiotic powder was dissolved in 50 mL sterile water. Then, 4 g methylcellulose powder (Methocel 60 HG; Sigma-Aldrich, St Louis, MO) was incorporated into the 50-mL TAP solution under magnetic stirring for 2 hours to obtain a homogenous DTAP. A commercial $Ca(OH)_2$ intracanal medicament (UltraCal XS; Ultradent, South Jordan, UT) was also used in this study.

Treatment Procedure

The dentin discs were randomized into 4 treatment groups and an untreated control group (n = 10 per group). Samples in the control groups were stored for 4 weeks at 37°C in a sealed 2-mL conical sample cup (Fisher Scientific, Pittsburgh, PA) at approximately 100% humidity. In the first treatment group, the pulpal side of each dentin disc was slowly irrigated with 20 mL 1.5% NaOCl for 5 minutes using a 27-G needle. Samples were then stored for 4 weeks at 37°C in a sealed 2-mL conical sample cup at approximately 100% relative humidity. After that, the pulpal side of each dentin disc was irrigated with 20 mL 17% EDTA for 10 minutes. For the other 3 treatment groups, the pulpal side of each dentin disc was irrigated with 20 mL 1.5% NaOCl for 5 minutes and treated with 0.1 mL TAP, DTAP, or $Ca(OH)_2$ for 4 weeks at 37°C in a sealed 2-mL conical sample cup at approximately 100% relative humidity. After 4 weeks, the treated side of each dentin specimen was irrigated with 20 mL 17% EDTA for 10 minutes. The application time of intracanal medicaments as well as the irrigation time and volume of both NaOCl and EDTA were selected according to recent clinical recommendations for the endodontic regeneration procedures (8).

Blood Analog Preparation and Contact Angle Measurement

To evaluate dentin wettability within the context of endodontic regeneration, contact angle measurements between a blood analog and dentin were performed. A solution that falls within the normal range of human blood viscosity (3–4 centipoise) was prepared as described in previous studies (20, 21). In summary, 200 mL distilled water was mixed with 100 mL 100% glycerol (Sigma-Aldrich) at room temperature (22°C) for 30 minutes under a magnetic stirrer to create a blood analog with a viscosity of 3.2 centipoise.

Before contact angle measurements, each dentin specimen was air-dried for 3 seconds. A PGX goniometer (Fibro Systems AB; Stockholm, Sweden) was then used to measure the static contact angles between the chemically treated dentin surfaces and the blood analog using the sessile drop method. Three drops (2 μL/drop) of the blood analog were vertically dispensed on each treated dentin surface at 3 different locations using a goniometer manual dispensing unit (Fibro System AB). Images were captured immediately after deposition using a microvideo system, and contact angles were automatically provided. All measurements were performed at room temperature (22°C). The 3 contact angle measurements obtained from each dentin specimen were averaged to obtain a single value for each sample.

Surface Roughness Measurement

After contact angle measurements, each specimen was washed with 5 mL sterile water and left to dry for 10 minutes before conducting roughness analyses. Each specimen was then horizontally positioned in an optical profilometer (Proscan 2000; Scantron, Taunton, UK), and 3 randomly selected areas (1×1 mm^2) from the treated side of each specimen were scanned. The step size was set at 0.01 mm, and the number of steps was at 100 on both the x- and y-axes. Two surface roughness outcomes (arithmetic average roughness [Ra] and geometric average roughness obtained by calculating the root mean square roughness [Rq]) were then obtained using dedicated software (Proscan 2000). Both Ra and Rq were measured in this study to have an enhanced understanding of the dentin surface profile after various treatments. The 3 roughness measurements obtained from each dentin specimen were averaged to obtain a single value for each sample.

Energy Dispersive X-ray Measurement

After roughness measurement, 5 samples were randomly selected from each group for energy-dispersive X-ray (EDX) analyses. Each selected sample was dried for 48 hours, and the weight percentages of calcium (Ca), phosphorus (P), carbon (C), and nitrogen (N) were measured from treated dentin surfaces using scanning electron microscopy (JEOL 7800F; JEOL, Peabody, MA) equipped with EDX spectroscopy (Octane Super Detector; EDAX, Mahwah, NJ). The samples were not sputter coated to ensure the precise identification of all selected elements. The EDX system was operated at 15 kV accelerated voltage and 1000× magnification. EDX analyses were performed on 5 randomly selected spots for each treated surface. The relative contribution of the 4 measured elements was automatically normalized to a total of 100%. The 5 measurements of each detected element from a treated dentin surface were averaged to obtain a single value.

Statistical Analyses

All data were checked for normality using the Kolmogorov-Smirnov test, and a natural logarithm transformation of surface roughness, P, and Ca data was performed to satisfy the normality assumptions. The effects of various endodontic regeneration protocols on measured outcomes were examined using 1-way analysis of variance and Fisher protected least significant differences to control the overall significance level at 5%.

Results

Figure 1 shows that untreated dentin had a significantly lower contact angle than dentin treated with NaOCl + EDTA (P = .0003) as well as dentin treated with DTAP (P < .0001), TAP (P < .0001), and