Title

Effect of different endodontic regeneration protocols on wettability, roughness and chemical composition of surface dentin

Authors

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***Highlights (for review)**

- We investigated the contact angle between a blood analogue and dentin surface after various endodontic regeneration treatments.
- All endodontic regeneration protocols caused significant increase in contact angle (reduction in wettability).
- Calcium hydroxide regeneration protocol caused significant reduction in dentin wettability compared to the use of triple antibiotic paste.

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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 four treatment groups and one untreated control group (n=10). One treatment group was irrigated with sodium hypochlorite (NaOCl) for five minutes, followed by ethylenediaminetetraacetic acid (EDTA) for 10 minutes. The other three treatment groups were irrigated with NaOCl, treated for four weeks with either triple antibiotic paste (TAP), diluted triple antibiotic paste (DTAP), or calcium hydroxide [$Ca(OH)_2$], then irrigated with EDTA. After treatment, contact angles between a blood analogue and dentin surfaces were evaluated. Surface roughness and chemical composition were characterized using optical profilometry and energy dispersive X-ray spectroscopy, respectively. One-way ANOVA, followed by Fisher's Least Significant Difference tests were used for statistical analyses. **Results:** All treatment groups showed significant reduction in wettability and significant increase in surface roughness, when compared to untreated dentin. Dentin treated with $Ca(OH)_2$ had significantly lower wettability compared to all other groups. No significant difference in wettability was found between dentin treated with NaOCl+EDTA, DTAP or TAP protocols. Dentin treated with TAP had significantly higher surface roughness compared to all other groups. Untreated dentin and NaOCl+EDTA treated dentin had significantly higher calcium and phosphorus, as well as significantly lower carbon compared to 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 significant reduction in dentin wettability compared to TAP or DTAP protocols.

Keywords: Endodontic regeneration, Triple antibiotic paste, Calcium hydroxide, Dentin, wettability, surface roughness.

Introduction

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 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 utilizing intracanal irrigants, mainly sodium hypochlorite, 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 minocyclin present in TAP (6), as well as the development of antimicrobial resistance and 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 ethylenediaminetetraacetic acid (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, increase in surface wettability of a substrate was associated with significant improvement in cellular attachment and protein adsorption (11). The topography and chemical structure of 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 significant effect on the attachment and proliferation of dental pulp stem cells (12-15). This study aimed to investigate the changes in wettability, roughness, and chemical structure of surface dentin after various endodontic regeneration protocols.

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 IRB 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 non-pulpal 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 was flattened using 1,200-, 2,400- and 4,000-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 min. Deep coronal dentin, rather than radicular dentin, was used to provide adequate surface area for multiple measurements of the various outcomes of the study. Previous work 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 g/mL) was prepared by mixing 1000 mg of United States Pharmacopeia grade antibiotic powders comprising equal portions of metronidazole, ciprofloxacin, and minocycline (Champs Pharmacy, San Antonio, TX) with 1 mL of sterile water (4, 17). A diluted paste-like consistency of 1 mg/mL TAP (DTAP) was prepared as described in recent studies (18, 19). In summary, 50 mg of TAP antibiotic powders were dissolved in 50 mL of sterile water. Then, 4 g of methylcellulose powder (Methocel 60 HG, Sigma-Aldrich, St Louis, MO) was incorporated into the 50 mL of 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 four treatment groups and an untreated control group (n=10 per group). Samples in the control groups were stored for four weeks at 37 °C in a sealed 2 mL conical sample cup (Fisher Scientific, Pittsburgh, PA, USA) at approximately 100% humidity. In the first treatment group, the pulpal side of each dentin disc was slowly irrigated with 20 mL of 1.5% NaOCl for five minutes using a 27-gauge needle. Samples were then stored for four 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 of 17% EDTA for 10 minutes. For the other three treatment groups, the pulpal side of each dentin disc was irrigated with 20 mL of 1.5% NaOCl for five minutes and treated with 0.1 mL of either TAP, DTAP, or $Ca(OH)_2$ for four weeks at 37 °C in a sealed 2 mL conical sample cup at approximately 100% relative humidity. After four weeks, the treated side of each dentin specimen was irrigated with 20 mL of 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 analogue preparation and contact angle measurement

To evaluate dentin wettability within the context of endodontic regeneration, contact angle measurements between a blood analogue 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 of distilled water was mixed with 100 mL of 100% glycerol (Sigma-Aldrich) at room temperature (22 ºC) for 30 minutes under a magnetic stirrer to create a blood analogue with a viscosity of 3.2 centipoise.

Prior to contact angle measurements, each dentin specimen was air-dried for three seconds. A Goniometer (Fibro system ab, Stockholm, Sweden) was then used to measure the static contact angles between the chemically treated dentin surfaces and the blood analogue, utilizing the sessile drop method. Three drops (2 µl/drop) of the blood analogue were vertically dispensed on each treated dentin surface at three 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 ºC). The three 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 of 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, Venture Way, Taunton, UK) and three randomly selected areas (1x1 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 were at 100 on both X and Y axes. Two surface roughness outcomes (Ra and Rq) were then obtained using dedicated software (Proscan, 2000). Both Ra (arithmetic average roughness) and Rq (geometric average roughness obtained by calculating the root mean square roughness) were measured in this study, in order to have an enhanced understanding of the dentin surface profile after various treatments. The three roughness measurements obtained from each dentin specimen were averaged to obtain a single value for each sample.

Energy dispersive X-ray (EDX) measurement

After roughness measurement, five samples were randomly selected from each group for EDX analyses. Each selected sample was dried for 48 h 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, Peabody, MA) equipped with EDX spectroscopy (EDAX Octane Super detector, Mahwah, NJ). The samples were not sputter coated to insure the precise identification of all selected elements. The EDX system was operated at 15 kV accelerated voltage and 1000 x magnification. EDX analyses were performed on five randomly selected spots for each treated surface. The relative contribution of the four measured elements was automatically normalized to a total of 100%. The five 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, phosphate, and calcium data was performed to satisfy the normality assumptions. The effects of various endodontic regeneration protocols on measured outcomes were examined using one-way ANOVA and Fisher's 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 ($p=0.0003$) as well as dentin treated with DTAP ($p<.0001$), TAP ($p<.0001$), and $Ca(OH)_2$ ($p<.0001$) protocols. Dentin treated with NaOCl+EDTA had a significantly lower contact angle than dentin treated with TAP ($p=0.03$) and $Ca(OH)_2$ ($p<.0001$) protocols. Furthermore, dentin treated with DTAP or TAP protocols had a significantly lower contact angle than dentin treated with $Ca(OH)_2$ ($p=0.005$, $p=0.008$).

Untreated dentin had significantly lower Ra and Rq than dentin treated with NaOCl+EDTA ($p=0.0001$) as well as dentin treated with $Ca(OH)_2$ ($p=0.03$ and $p=0.009$), DTAP ($p=0.01$ and $p=0.008$), and TAP ($p<.0001$) protocols (Figure2A-B). Furthermore, dentin treated with TAP had significantly higher Ra and Rq than dentin treated with NaOCl+EDTA ($p=0.01$ and $p=0.02$) as well as dentin treated with $Ca(OH)_2$ ($p=0.0001$ and $p=0.0003$) or DTAP ($p=0.0001$ and $p=0.0003$).

Table 1 shows that dentin treated with NaOCl+EDTA had significantly higher Ca and P compared to all other groups ($p<0.0001$). Additionally, untreated dentin had significantly higher Ca and P compared to dentin treated with $Ca(OH)_2$ ($p<0.0001$), DTAP ($p<0.0001$), and TAP ($p<0.0001$). Furthermore, dentin treated with $Ca(OH)_2$ had significantly higher Ca and P compared to that treated with TAP ($p<0.0001$).

Dentin treated with NaOCl+EDTA had significantly lower C and N ($p<0.0001$) compared to dentin treated with $Ca(OH)_2$, DTAP, and TAP (Table 1). Untreated dentin had significantly lower C ($p<0.0001$) than dentin treated with $Ca(OH)_2$, DTAP, and TAP. Dentin treated with $Ca(OH)_2$ and DTAP had significantly lower carbon than dentin treated with TAP

($p<0.0001$). Dentin treated with TAP had significantly lower N than untreated dentin ($p=0.03$) and dentin treated with $Ca(OH)_2$ ($p=0.001$) or DTAP ($p=0.004$).

Discussion

The presence of an intimate contact between stimulated bleeding and root canal dentin during endodontic regeneration may improve the formation of a blood clot-dentin natural scaffold, accelerate the interaction between dentin growth factors and blood containing stem cells, and improve the deposition of new dentin. Wettability can be expressed in terms of contact angle, which measures the ability of a liquid to spread on a plane solid surface. The contact angle has an inverse relationship with wettability. Furthermore, dentin wettability is greatly dependent on chemical structure and surface roughness of the surface (22, 23). Therefore, the wettability, topography and chemical composition of surface dentin were investigated in the current study.

Our results demonstrated a significant decrease in dentin wettability among all treatment groups when compared with untreated control dentin. This could be explained by the significant increase in surface roughness outcomes reported among all treatment groups. Recent studies suggested that lower dentin wettability was associated with higher surface roughness (22, 24). The significantly lower dentin wettability after TAP, DTAP and $Ca(OH)_2$ treatment can also be explained by the significant reduction in the inorganic phase represented by Ca and P and the significant increase in the organic phase represented by C among these groups. The acidic nature of TAP (pH=2.9) (25) and EDTA's calcium chelating ability may be responsible for the net surface demineralization effect of dentin surfaces after various regeneration protocols. It is well established that the inorganic hydroxyapatite phase of dentin has high wettability while the organic collagen phase of dentin has low wettability (26). Indeed, a previous study reported a correlation between increases in dentin wettability and higher concentrations of calcium on the dentin surface (27).

An interesting finding of this study is that dentin treated with $Ca(OH)_2$ showed significantly lower wettability compared to all other groups. This relatively poor adherence may support the finding of a recent clinical study that found that endodontic regeneration cases disinfected with $Ca(OH)_2$ had significantly thinner root walls compared to regeneration cases disinfected with TAP (2). The results of dentin surface topography and chemical composition in our study may not be enough to completely justify the reported low dentin wettability after $Ca(OH)_2$ treatment. One additional explanation is the effect of $Ca(OH)_2$ on surface dentin modulus of elasticity. A recent study proposed that dentin wettability was affected by changes in surface dentin modulus of elasticity (23) and $Ca(OH)_2$ was found to significantly change dentin modulus of elasticity after one week of application (28).

In the current study, a dentin surface treated with NaOCl+EDTA showed significantly higher inorganic phase (Ca and P) and significantly lower organic phase (C and N) compared to untreated control dentin. This agrees with previous work that reported significantly higher inorganic composition of dentin treated with EDTA and NaOCl compared to untreated dentin (29). This could be explained by the presence of smear layer on untreated control dentin that is rich with organic debris represented by N and C (30). On the other hand, the final 10 minutes of irrigation with EDTA in dentin-treated NaOCl+EDTA is expected to remove the smear layer. A relatively recent study suggested that root canal irrigation with NaOCl followed by EDTA caused complete removal of the smear layer (31). Despite the high inorganic phase in surface dentin treated with NaOCl+EDTA, a significantly lower wettability of dentin irrigated with NaOCl+EDTA compared to untreated control dentin was observed in this study. This agrees with a previous study that suggested significantly lower wettability of dentin irrigated with EDTA compared to untreated dentin (32). This can be explained by the ability of EDTA to increase surface roughness, completely remove the smear layer and create wide open dentinal tubules (33, 34). Open dentin tubules with larger diameters have been proposed to significantly decrease the dentin wettability due to the effect of small air pockets inside the dentin tubules (22-24).

A Newtonian blood surrogate was used in the current study instead of non-Newtonian human blood to detect the wettability of dentin. This standardized approach was used in an attempt to explore the influence of change in physiochemical surface properties of dentin after various regeneration protocols on the static contact angle. The measurement of dynamic contact angles using actual human blood in future studies may provide further understanding of the spreading behavior of blood on dentin surfaces.

Collectively, our study showed that the endodontic regeneration protocols had a significant effect on dentin surface wettability, roughness, and chemical composition. The use of $Ca(OH)_2$ during endodontic regeneration caused a significant reduction in dentin wettability compared to the use of TAP or DTAP intracanal medicaments. Furthermore, TAP caused significant increase in surface roughness compared to other tested intracanal medicaments.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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Figure 1. Mean (SE) of contact angles measured on dentin surfaces after various endodontic regeneration protocols.

Figure 2A. Mean (SE) of Ra roughness outcome measured on dentin surfaces after various endodontic regeneration protocols.

Figure 2B. Mean (SE) of Rq roughness outcome measured on dentin surfaces after various endodontic regeneration protocols.

Figure 1

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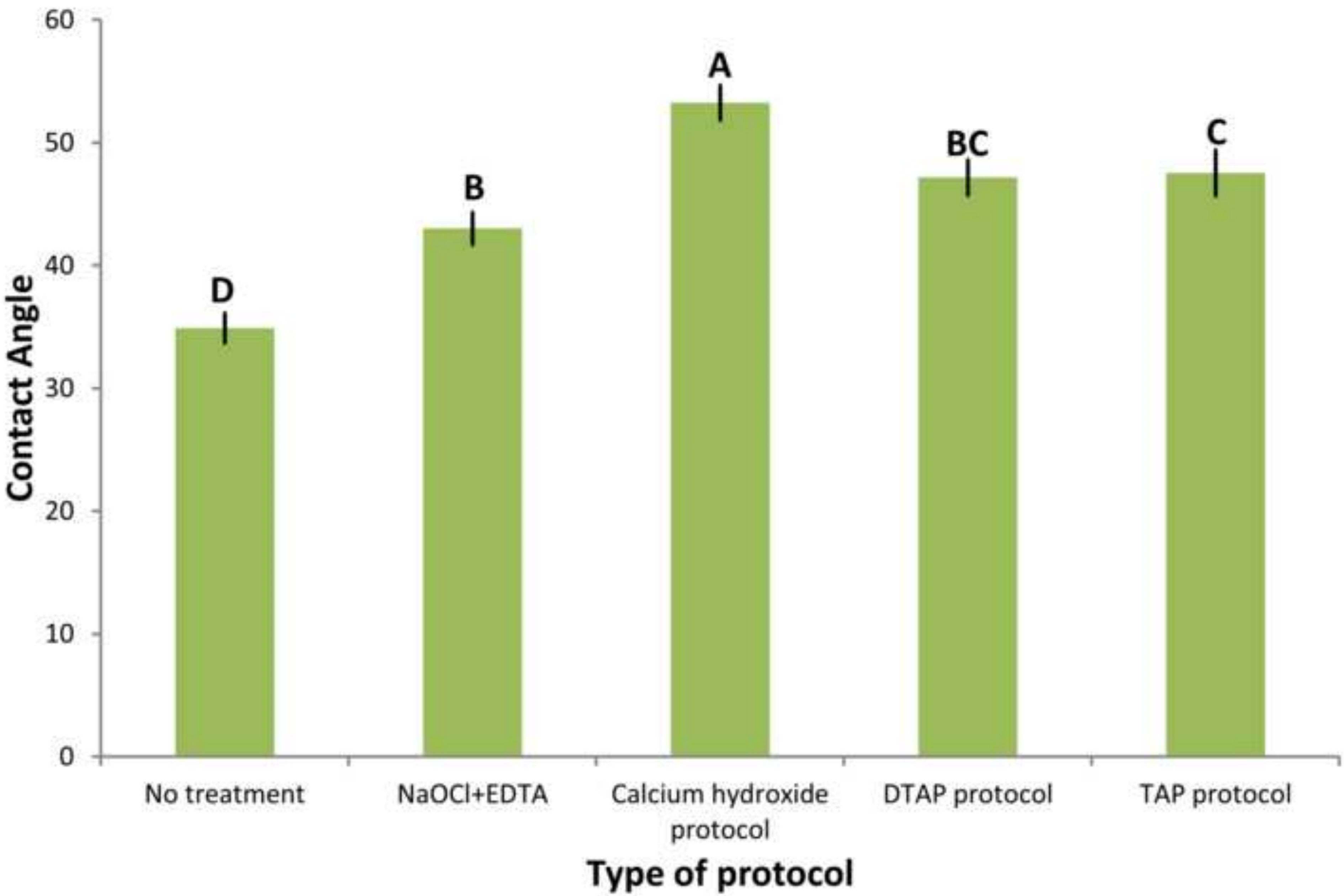

Figure 2A
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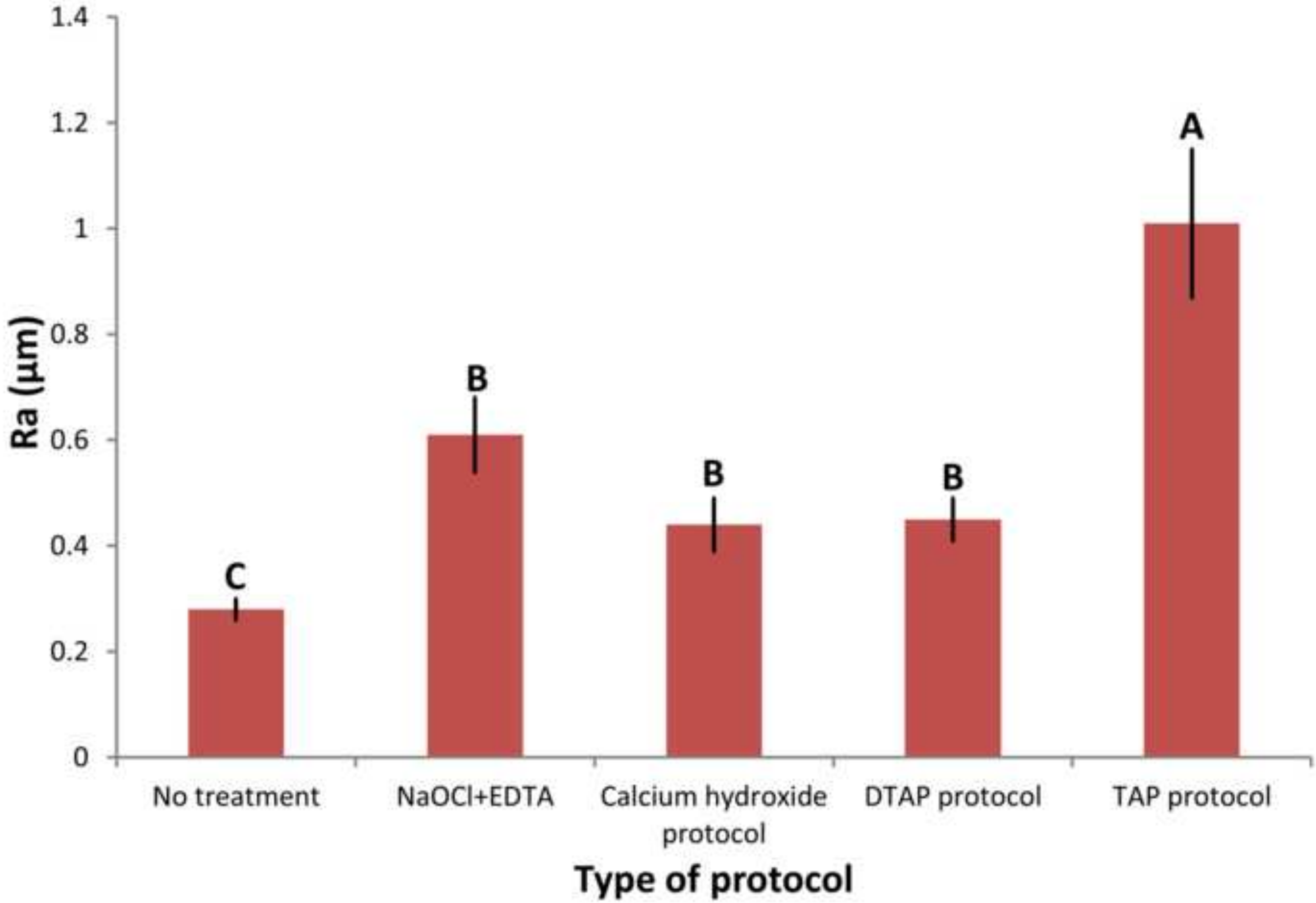

Figure 2B

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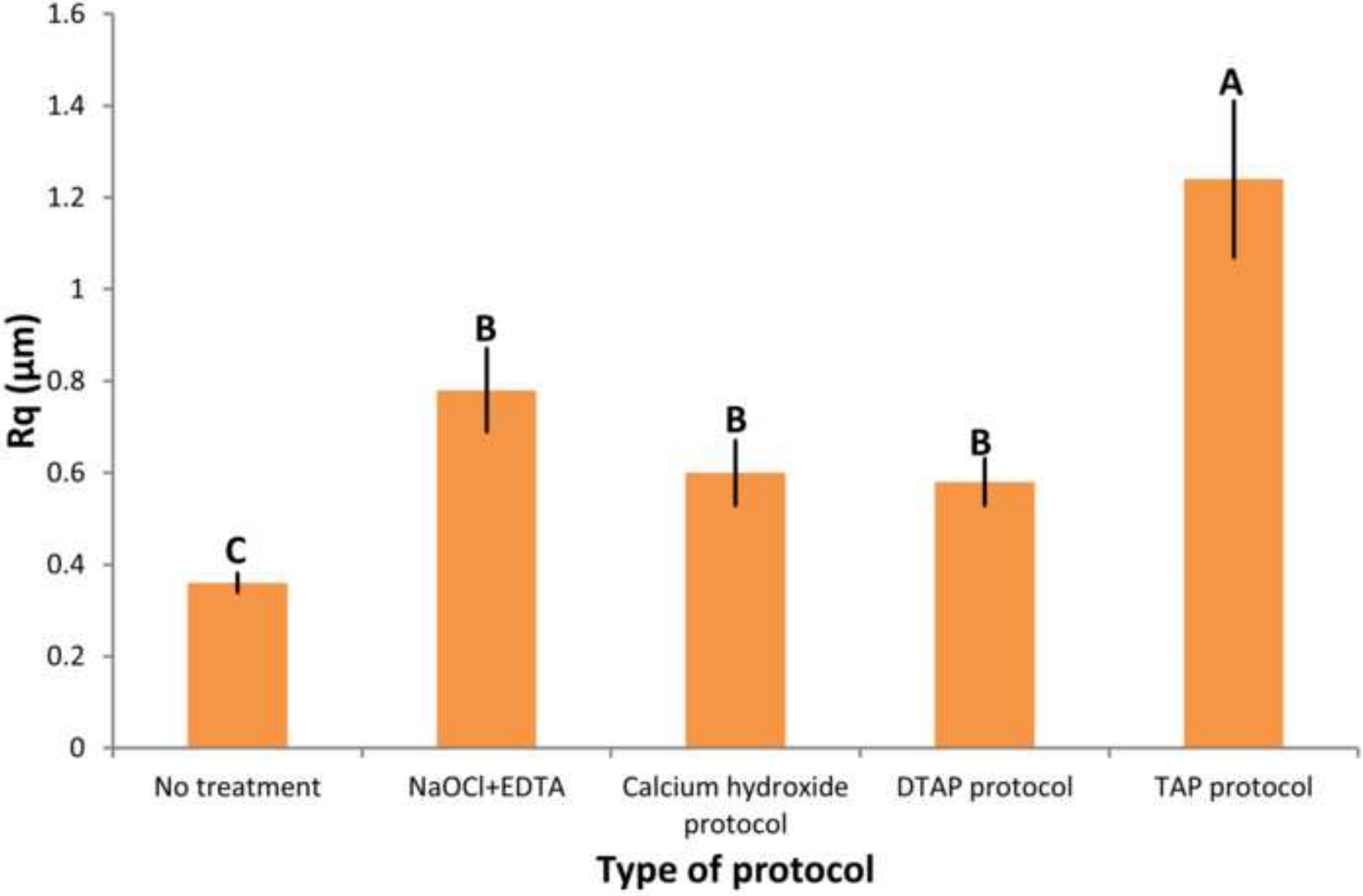

Table 1

Table 1. Mean (SE) of weight percentages of chemical elements measured on dentin surfaces after various endodontic regeneration protocols.

Type of treatment∗	Calcium	Phosphorus	Nitrogen	Carbon
NaOCl + EDTA	46.33 (3.8)A	22.1 (0.8)A	19 (3)C	12 (1.8)D
Untreated dentin	11.4 (2.2)B	8.7 (1)B	57(2.4)A	23 (0.8)C
$Ca(OH)_2$ Protocol	0.3 (0.04)C	0.6 (0.02)C	61(1.9)A	38 (1.1)B
DTAP Protocol	0.2(0.01)D	0.51(0.03)C	60(0.7)A	40 (1.3)B
TAP Protocol	0.04 (0.01)E	0.2 (0.02)D	50(1.2)B	49 (1.1)A

∗Within each element, different upper-case letter indicate a significant difference between various endodontic regeneration protocols