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Comparison of EDTA and Boric Acid Irrigation in Terms of Root Canal Dentin Microhardness and Sealer Penetration Depth

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ORIGINAL ARTICLE

Comparison of EDTA and Boric Acid Irrigation in Terms of Root Canal Dentin Microhardness and Sealer Penetration Depth

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ABSTRACT

Chemo mechanical preparation is one of the important stages for successful endodontic treatment, and Boric acid has been proposed as an alternative irrigation solution for root canal treatment. **Objective:** The purpose of this study was to evaluate the effect of irrigation with boric acid (BA) and EDTA on the mineral content and microhardness of dentin and penetration of the AH Plus root canal sealer. **Methods:** 63 single-rooted teeth were instrumented. Microhardness analysis, 45 teeth were cut longitudinally and then transversely to obtain dentin. Pre- and post-treatment microhardness values were measured. The samples were randomly assigned into 3 groups (n = 15) for irrigation solution: distilled water, 17% EDTA, and 5% BA. The 18 roots were irrigated with three solutions (n = 6). Nine of the roots were filled with AH-Plus for confocal laser scanning microscopy analysis, and the others were used for XRD analysis. **Results:** The results were evaluated with the ANOVA test. BA caused a significant decrease in microhardness value in apical and coronal parts ($p < 0.05$), while EDTA reduced only in coronal parts ($p < 0.05$). **Conclusion:** BA provided higher sealer penetration ability. BA reduced the microhardness and provided better sealer penetration.

Key words: boric acid, confocal laser microscopy analysis, dentin microhardness, EDTA, XRD analysis

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INTRODUCTION

Chemomechanical preparation is one of the important stages for successful endodontic treatment, but there are more factors that affect the success of root canal treatment.[1] The prepared dentine surface is coated with a smear layer, consisting of amorphous organic and inorganic debris.[2] Sodium hypochlorite (NaOCl) is the most widely used as a disinfectant irrigation solution for the chemomechanical preparation of root canals.[3] Chelating agents, such as Ethylenediaminetetraacetic acid (EDTA), and decalcifying solutions, such as phosphoric acid, have also been shown to remove the smear layer.[4-6] The most commonly used method of removing the smear layer is the use of EDTA and NaOCl solutions in succession.[7]

Boric acid has been proposed as an alternative irrigation solution for root canal treatment.[8,9] It has been reported that boric acid has antibacterial, anti-inflammatory, and mild antiseptic effects.[10,11] Many studies have examined the potential medical uses of boric acid and mentioned the benefits of this solution.[12,13] It has been used in the treatment of recurrent vaginal yeast infections, the treatment of Otitis externa, and as an antiseptic solution for minor burns and cuts.[14] Although local use of boric acid has been shown to be efficient in treating periodontal disease.[7]

A limited number of studies have been performed on the use of boric acid in dentistry.[8,9,12] The purpose of this study was to evaluate the in vitro effectiveness of 5% boric acid as an irrigation solution in the chemomechanical preparation of root dentin. The null hypothesis of this study was there would be no differences between experimental groups on the microhardness of root canal dentin, mineral contents and stiffness of dentin, and also the sealer penetration into the dentin tubules.

METHODS

This study was approved by the Ethical Commitee of Ankara Yıldırım Beyazıt University (Approval number: 2018-243).

Collection of teeth and preparation of root canals

Sixty-three caries-free, single-rooted human mandibular anterior teeth extracted for orthodontic and periodontal purposes were used in this study. After collection, debris calculus and soft tissue remnants on the root surfaces were removed, and all teeth were stored in a sterile saline solution for 7 days until experiments. The crowns of the teeth were removed with a diamond disk (Carborondum, Carbodent-Gysi Buenos Aires, Argentina). The lengths of the root canals were measured with a 15-K file (Dentsply, Sirona, Baillagues, Switzerland) until the tip of the instrument was visible from the apical foramen.The root canal lengths of all teeth were adjusted to 16 ± 1 mm. The root canals were instrumented with One Shape Files (MicroMega, Besançon, France), and irrigated with 2 ml of 2.5% NaOCl solutions (Werax, Izmir, Turkiye). 45 teeth were randomly assigned for microhardness testing.

The other 18 teeth were randomly divided into 3 groups for irrigation solution (n = 6) distilled water, 17% EDTA, 5% BA. And then, each six teeth were randomly assigned into two groups for analysis methods: XRD analysis and CLSM.

Microhardness evaluation

The teeth were first cut longitudinally, then transversely into the cervical and apical segments under water cooling using a diamond disk (Carborondum, Carbodent-Gysi Buenos Aires, Argentina). Both sections of each root were embedded in a separate acrylic silicon device containing acrylic resin, leaving the dentin surface exposed. The dentin surfaces of the mounted specimens were glazed with silicon carbide abrasive papers (180, 320, and 600 grit) and 0.25 mm diamond polishing papers (Metkon, Bursa, Turkiye) under distilled water to remove any surface scratches.

Pre-treatment microhardness of the samples was measured on dried specimens parallel to pulp dentin complex using a Shimadzu Vickers microhardness tester (HMV-700, Kyoto, Japan) under 300 gr load for 20 sec and at x40 magnification. Three indentations with 300 µm intervals were made for each specimen, and the results were averaged.

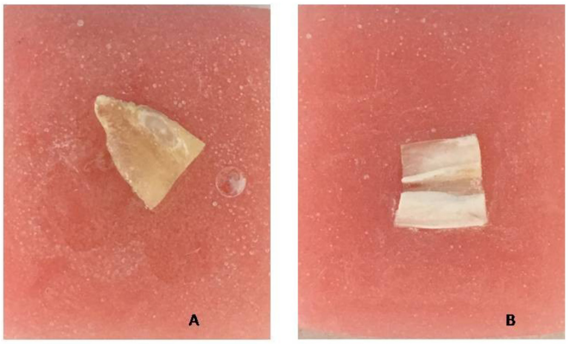

Figure 1. Apical and servical segments of the roots. A: apical segment, B: servical segment.

Table 1. Experimental groups.

Groups			
1 (n = 15)	5 ml Distilled water 5 min	5 ml %2.5 NaOCl (Werax, Izmir, Turkiye) 1min	2 ml Distilled water 1 min
2 (n = 15)	5 ml %17 EDTA (Werax, Izmir, Turkiye) 5 min	5 ml %2.5 NaOCl (Werax, Izmir, Turkiye) 1min	2 ml Distilled water 1 min
3 (n = 15)	5 ml %5 H3BO3 (Merck, Darmstadt, Germany) 5 min	5 ml %2.5 NaOCl (Werax, Izmir, Turkiye) 1min	2 ml Distilled water 1 min

The samples were then randomly assigned into 3 groups. Each group also included two subgroups consisting of the apical and the cervical segments of the roots (Figure 1). The groups were in Table 1. The samples were subjected to their respective irrigation procedures (Table 1), and the post-treatment dentin microhardness of each sample was determined as described above.

X-Ray Diffraction (XRD) analysis

One mm dentin disks were prepared using an automated precision cutter (Carborondum, Carbodent-Gysi Buenos Aires, Argentina). The disks were cleaned thoroughly under flowing water and then immersed into the different irrigation solutions according to their groups, as shown in Table 1. Then the samples were rinsed and dried under vacuum for 2 hours. The 20 x 20 x 2 mm sample holder was filled with play dough, and the specimens were embedded into the sample holder. XRD measurements were performed on a Rigaku Miniflex 600 diffractometer (RigakuCorp., Tokyo, Japan) employing 0.154056 nm Cu K-α radiation at 40 kVvoltage and 15 mAcurrent, a 0.02° step size and 1°/mins can speed.

Confocal laser microscopy (CLSM) analysis

The canals of intact, unsectioned roots were prepared

Table 2. Mean pre-treatment and post-treatment microhardness values of apical and coronal parts of the root canals (N/mm^2).

Group	Apical		Coronal	
	Before irrigation (mean ± std)	After irrigation (mean ± std)	Before irrigation (mean ± std)	After irrigation (mean ± std)
Distilled water	47.77 ± 13.28	40.70 ± 6.11	46.22 ± 10.24	42.47 ± 6.74
EDTA	43.89 ± 7.40	39.74 ± 5.49	47.41 ± 9.58	39.30 ± 4.03*
Boric Acid	48.50 ± 7.75	41.01 ± 5.08*	45.61 ± 7.34	39.16 ± 3.09*

*means significant difference between comparison pairs

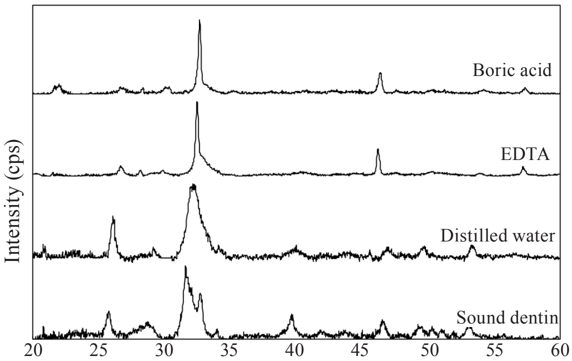

Figure 2. XRD patterns of samples after irrigation.

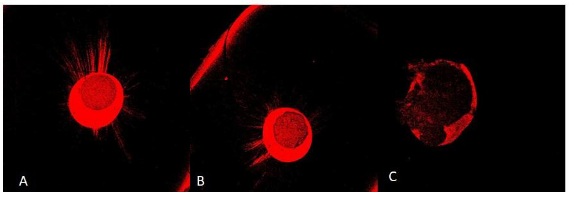

Figure 3. Confocal laser microscopy images of the sealer penetration depth after different irrigation solutions. A: 5% BA, B: 17% EDTA, C: distilled water.

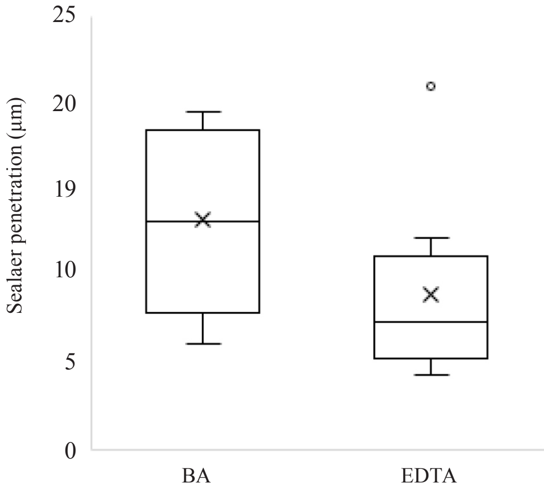

Figure 4. Sealer penetration depth measured from CLSM images.

and treated for CLSM as described above. To remove residual irrigation solutions, all teeth were irrigated with 2 ml of distilled water, and root canals were dried with paper points (Diadent Group International Inc, Burbany, BC, Canada).

AH Plus (Dentsply, Sirona, Switzerland) sealer was mixed with 0.1% rhodamine B (Sigma-Aldrich, St. Louis, MO, USA) and placed in the canal 1 mm shorter than the working length using lentulo spiral (Dentsply Sirona). The canals were then obturated with gutta-percha points (Dentsply, Sirona) using the lateral compaction technique. Temporarily filling material (Cavit G, 3M ESPE, St Paul, MN) was used for temporary restoration.

The specimens were sectioned using a 0.4-mm-thick diamond disk at low speed under water cooling (Isomet 1000, Buehler, IL, USA). A 1±0.1-mm-thick section was obtained at 5 mm from the root apex. Three sections were prepared from each tooth. These sections were then glazed with silicon carbide abrasive paper. All samples were perched on glass slides and examined using a CLSM (Carl Zeiss LSM 510; Carl Zeiss Microscopy, Jena, Germany) with a zoom fat lens at 575 nm and 4x magnification. The images were transferred to Image J analysis software (v. 1.44p; National Institutes of Health, Bethesda, MD, USA). The maximum penetration depth was measured as described elsewhere.[15]

Statistical analysis

The normality of the data was tested with the Shapiro–Wilk test. Pre- and post-treatment microhardness values were evaluated using repeated measures ANOVA, followed by Tukey post-hoc test. The data were analyzed using SPSS 22 Software (IBM, Armonk, NY, USA). The significance level was set to 0.05 in all statistical tests.

RESULTS

Mean microhardness values of the coronal and apical dentin before and after different irrigation solutions were shown in Table 2.

Repeated measures ANOVA revealed that treatment with BA caused a significant reduction in hardness on both apical ($p = 0.004$) and coronal ($p = 0.009$) sites. Treatment with EDTA caused a significant reduction in hardness only on coronal sites ($p = 0.003$) but not on apical sites ($p = 0.093$). Treatment with distilled water caused no significant difference either on apical ($p = 0.139$) or coronal ($p = 0.216$) sites.

XRD analysis showed that distilled water was not effective in removing the smear layer. Demineralization was observable in EDTA and BA groups, and both resulted in similar XRD patterns (Figure 2). Sealer penetration was evaluated via CLSM. No tubular penetration was observed in the distilled water-treated samples (Figure 3). The average penetration depth was 13.25 ± 5.14 µm in BA-treated samples and 8.95 ± 4.95µm in EDTA-treated samples (Figure 4). Better penetration depth was obtained with BA irrigation.

DISCUSSION

The extent of intra-fibrillar and inter-fibrillar mineral content determines the stiffness of the dentin.[16] The dentin stiffness may also depend on the location of the measurement. The dentin stiffness is lower in regions close to the pulp. This may be due to the increasing number of commonly opened dentin tubules near the pulp with reduced resistance, and also, expansion of tubule diameters may cause this result.[16,17] In the current study, the dentin microhardness of the samples was measured at the apical root level and at the coronal root level at the pulp-dentin interface, and the measurements were consistent with previous studies.[18,19] Many studies to date have analyzed modifications in dentin microhardness after root canal irrigation with various types of solutions.[20-22] They have evaluated initial microhardness, too, because the teeth may have different initial physical properties. In this study, three indentations with 300 µm intervals were averaged in the apical and coronal parts of the roots for irrigation to ensure a representative value of the initial microhardness of each sample.

BA resulted in a significant decrease in microhardness in both coronal and apical dentin. EDTA, otherwise, resulted in a significant decrease only in the coronal dentin but not in the apical dentin. Lo Guidice et al.[23] have reported that the average number of dentin tubules is similar in the coronal and apical zones, but the diameter of coronal dentin tubules was higher compared to apical tubules. This indicates that the density of dentin tubules is lower at coronal dentin. Therefore, the difference in microhardness values in apical and coronal dentin with BA and EDTA may be the result of the difference in dentin tubule intensity. 5% BA was effective in demineralizing both apical and coronal zones, while 17% EDTA was only effective in apical zones. The null hypothesis was partially rejected. One possible reason for this observation is the mineral density differences in the apical and coronal zones. In the coronal zone, the mineral density is higher compared to the apical zone. EDTA may have dissolved the mineral in the apical zone enough to make a difference in the microhardness while not dissolving enough minerals in the coronal zone to make a difference in microhardness.

Distilled water caused a reduction in microhardness value, even though it was not statistically significant. In the root canal preparation procedure, the root canals were irrigated with NaOCl. The reduction in the microhardness value could be related to NaOCl irrigation within the preparation procedure. Even though the distilled water does not act as a chelating agent, calcium phosphates still dissolve in distilled water depending on the pH and ionic strength of the solvent. In this case, dissolution of minerals in distilled water until the solvent is saturated with dissolved ions.[24] Empirical reduction in the microhardness may be the result of the dissolution of minerals into the distilled water.

However, this difference in mineral content was not observable with XRD. On the other hand, the CLSM results were in parallel with microhardness measurements. The sealer penetration was significantly higher in BA-treated samples.

Akman et al.[20] showed that BA, EDTA, and citric acid changed the mineral contents of the root canal dentin in SEM-EDX analysis. They were used 10%BA. In the current study, in XRD analysis, mineral contents were found similar in our experimental groups. This observation might be due to the fact that SEM-EDX analysis is more suitable for quantitative analysis compared to XRD and also the difference in the BA concentration used by Akman et al and in our study. According to using NaOCl solutions before EDTA or other chelating solutions, the hydroxyapatite covering seems to maintain the collagen fibers from the dissolving action of NaOCl. [23]

Machado et al.[25] showed that the using chelating agents increased the sealer penetration in the dentin tubules. EDTA, citric acid showed more sealer penetration compared to NaOCl and distilled water. In our study, 5% BA was more effective in removing the smear layer compared to 17% EDTA, therefore the root canal sealer penetration was higher in BA-treated samples.

BA has been used as a compound in many medicinal preparations, such as wound irrigants, eyewashes, and mouthwashes, as well as in detergents and starches, skin powders, and ointments.[26] Literature reports on medicinal and cosmetic products containing BA showed that there is no teratogenic effect in the appropriate dosage and use.[27] Topical usage of BA is effective in treating periodontal disease.[7] Gölge et al. [28] reported the local boric acid application to be efficacious in bone healing and recommended boric acid-coated implants for better and faster osseointegration. The softening efficacy of irrigation solutions on dentin walls can be clinically beneficial because it allows fast preparation and access to small-size root canal walls.[17] In addition to its advantages as an irrigation solution for removing the smear layer, it may also decrease the fracture resistance of the root. Therefore, further laboratory and clinical studies are needed on the use of BA for root canal irrigation.

CONCLUSION

According to the results of this in vitro study, 5% BA solution significantly decreased the microhardness of the coronal and apical part of the root canal dentin surface compared to 17% EDTA. These results have higher root canal sealer penetration into the dentin, as shown with CLSM.

CONFLICT OF INTEREST

The authors deny any conflict of interest.

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