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BASIC RESEARCH:

Effects of a Benzalkonium Chloride Surfactant-Sodium Hypochlorite Combination on Microhardness and Mineral Content of Dentin

Efectos de una combinación de tensioactivo de cloruro de benzalconio e hipoclorito de sodio en la microdureza y el contenido mineral de la dentina

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ABSTRACT: This study aimed to investigate the impact of the combination of Benzalkonium chloride (BAC) and sodium hypochloride (NaOCl) and its application after ethylenediaminetetraacetic acid (EDTA) in root canal irrigation procedures on the microhardness and mineral content of dentin. Distal roots of mandibular third molars were embedded in auto-polymerizing acrylic resin, sectioned coronally to apically resulting in fifty-four root sections. Thirty sections underwent microhardness evaluation, and twenty-four sections were randomly chosen for mineral analysis. The microhardness assessment comprised three groups: Group 1 (2.5% NaOCl), Group 2 (2.5% NaOCl with 0.084% BAC), and Group 3 (2.5% NaOCl with 0.084 BAC post-17% EDTA). Initial and post-irrigation microhardness values were measured for each group. For mineral analysis, samples were categorized into four groups: distilled water, 2.5% NaOCl, 2.5% NaOCl with 0.084% BAC, and 17% EDTA+2.5% NaOCl with 0.084% BAC. X-ray photoelectron spectroscopy immediately measured magnesium, phosphorus, and calcium mineral contents on dentin surfaces post-irrigation. Results indicated a significant reduction in root dentin microhardness for all solutions ($p<0.05$). However, no statistically significant difference in the percentage of reduction was observed among the groups ($p>0.05$). XPS analysis revealed no significant disparity in dentin surface mineral content among the groups ($p>0.05$). In conclusion, the addition of Benzalkonium chloride to sodium hypochloride, either alone or after EDTA, did not induce a significant alteration in dentin microhardness or mineral content. These findings contribute to a nuanced understanding of dental irrigation protocols and their effects on dentin properties during endodontic procedures.

KEYWORDS: Benzalkonium chloride; Dentin; EDTA; Microhardness; Mineral; Sodium hypochloride.

RESUMEN: Este estudio tuvo como objetivo investigar el impacto de la combinación de cloruro de benzalconio (BAC) e hipocloruro de sodio (NaOCl) y su aplicación después del ácido etilendiaminotetraacético (EDTA) en procedimientos de irrigación del conducto radicular sobre la microdureza y el contenido mineral de la dentina. Las raíces distales de los terceros molares mandibulares se incluyeron en resina acrílica autopolimerizable y se seccionaron de coronal a apical, lo que dió como resultado cincuenta y cuatro secciones de raíz. Treinta secciones se sometieron a una evaluación de microdureza y veinticuatro secciones fueron elegidas al azar para el análisis mineral. La evaluación de la microdureza comprendió tres grupos: Grupo 1 (2,5% NaOCl), Grupo 2 (2,5% NaOCl con 0,084% BAC) y Grupo 3 (2,5% NaOCl con 0,084 BAC post-17% EDTA). Para cada grupo se midieron los valores de microdureza inicial y post-irrigación. Para el análisis mineral, las muestras se clasificaron en cuatro grupos: agua destilada, 2,5% NaOCl, 2,5% NaOCl con 0,084% BAC y 17% EDTA+2,5% NaOCl con 0,084% BAC. La espectroscopía fotoelectrónica de rayos X midió inmediatamente el contenido de minerales de magnesio, fósforo y calcio en las superficies de la dentina después de la irrigación. Los resultados indicaron una reducción significativa en la microdureza de la dentina radicular para todas las soluciones ($p<0,05$). Sin embargo, no se observó diferencia estadísticamente significativa en el porcentaje de reducción entre los grupos ($p>0,05$). El análisis XPS no reveló ninguna disparidad significativa en el contenido mineral de la superficie de la dentina entre los grupos ($p>0,05$). En conclusión, la adición de cloruro de benzalconio al hipocloruro de sodio, solo o después de EDTA, no indujo una alteración significativa en la microdureza o el contenido mineral de la dentina. Estos hallazgos contribuyen a una comprensión matizada de los protocolos de irrigación dental y sus efectos sobre las propiedades de la dentina durante los procedimientos de endodoncia.

PALABRAS CLAVE: Cloruro de benzalconio; Dentina; EDTA; Microdureza; Mineral; Hipocloruro de sodio.

INTRODUCTION

Both mechanical and chemical preparation is important to achieve a clean and debris-free root canal, especially in inaccessible areas, for successful root canal treatment. Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution (1), but has high surface tension (2), which might prevent its penetration into dentinal tubules and reduce its antibacterial effectiveness. Studies have reported that bacterial components are present in dentinal tubules up to 300-500 μm (3,4). Using various irrigation systems and adding surface modifiers (surfactants) to irrigates is recommended for reaching canal irregularities, to penetrate the dentinal tubules better, and to increase their antibacterial activity (5-7). The surface-modifying agent benzalkonium chloride (BAC) is a quartener ammonium compound that is most commonly used in medicine as a biocide and preservative of eye and nasal solutions (8). In previous studies, it has been reported that the addition of BAC to NaOCl reduced the surface energy, contact angle, and the number of bacteria remaining in the root canal compared with using NaOCl alone (9,10).

Endodontic irrigation solutions might alter the physical properties and chemical composition of dentin (11-13). These changes can reduce microhardness of root canal dentin and increase its permeability and solubility, thereby increasing susceptibility to tooth fracture. Furthermore, these chemicals can alter the interaction between dentin and root canal sealer. Studies have shown that NaOCl and ethylene diamine tetra acetic acid (EDTA) irrigation decreases dentin microhardness and change the calcium (Ca) to phosphorus (P) ratio (14,15). Despite these changes caused by solutions in the dentin, NaOCl and EDTA removes the smear layer and better penetration of the sealer into the dentinal tubules (16).

XPS (X-ray photoelectron spectroscopy) permits the analysis of polished or rough surfaces of various materials. In the literature, the cytotoxicity, antibacterial properties, and surface tension of BAC has been determined and it has been shown favorable results. However, the chemical and mechanical effects of BAC on dentin surfaces have not been determined sufficiently. In this study, we evaluated the effects of the use of NaOCl with and without BAC, or following EDTA use on the microhardness and mineral content of root canal dentin.

MATERIALS AND METHODS

Ethical approval was obtained from the Hacettepe University Human Subjects Ethics Review Committee (GO 17/589-04.07.17) for tooth samples to be used in the study. Signed informed consent was obtained from all participants to be included in the study. The distal roots of forty freshly extracted, caries-free lower third molars taken from the patients were used. The teeth were obtained from patients aged 20-30 years. Following extraction, remnants of soft tissue and debris were cleaned with a periodontal scaler and the teeth were washed with 0.9% sterile saline solution (Isolyte; Eczacıbaşı Baxter, Türkiye). The teeth were then stored in 0.5% chloramine-T aqueous solution until the experiments were performed. Teeth were decoronated and distal roots were split and embedded in auto-polymerizing acrylic resin. The distal roots were sectioned from the coronal part to the apical with a thickness of 1.5mm (Isomet, Buehler Ltd., Lake Bluff, NY, USA) horizontally. Coronal and apical 1.5mm were discarded and 54 root sections were obtained. Sections were polished under distilled water with silicon carbide abrasive papers to remove surface scratches (180, 320, and 600 grit) with 0.25mm diamond polishing papers (Metkon, Bursa, Türkiye). 30 root sections were used to test microhardness, and 24 were used for mineral analysis.

EVALUATION OF DENTIN MICROHARDNESS

The pulp-dentin interface was determined using an optical microscope attached to a Vickers microhardness tester device (Shimadzu HMV-2, Tokyo, Japan). Microhardness values of the 30 root sections were measured initially by using a Vickers microhardness tester at a depth of 100 from the pulp-dentin interface as four separate indentations on buccal, lingual, mesial, and distal parts of the root, at a magnification of 50x. Indentations were made before and after the surface treatment by using a 300 g load and a 20 second dwell time. After initial indentation, root sections were randomly divided into 3 groups (n=10):

- Group 1: 2.5% NaOCl for 5 minutes.
- Group 2: 2.5% NaOCl with 0.084% BAC for 5 minutes.
- Group 3: 17% EDTA for 5 minutes, 2.5% NaOCl with 0.084% BAC for 5 minutes.

Samples were immersed in each of the solutions for 5 minutes. For group 3, samples were firstly immersed in 17% EDTA for 5 minutes, rinsed with 5 ml distilled water, and immersed in 2.5% NaOCl with 0.084% BAC for 5 minutes. After surface treatment, cotton pellets were used to dry the samples. The dentin sample microhardness was measured at locations closest to the initial indentation areas.

X-RAY PHOTON SPECTROMETER (XPS) ANALYSIS

24 samples were used for mineral analysis using XPS. Samples were randomly divided into four groups (n=6):

- Group A: control (distilled water) for 5 minutes.
- Group B: 2.5% NaOCl for 5 minutes.
- Group C: 2.5% NaOCl with 0.084% BAC for 5 minutes.
- Group D: 17% EDTA for 5 minutes, 2.5% NaOCl with 0.084 BAC for 5 minutes.

Samples were dried after surface treatment using cotton pellets and XPS analysis was performed immediately after. The specimens were introduced into a monochromatic XPS (PHI-Versaprobe 5000, MN, USA) equipped with an Al monochromatic X-ray radiation source. The power of the X-ray was limited to 25W and the energy was fixed at 58.70eV, at an angle of 45°. The magnesium, phosphorus, and calcium mineral content of dentin slab surfaces were measured using wide-scanned XPS spectra.

STATISTICAL ANALYSIS

All data were analyzed with the SPSS for Windows 22 program. The Shapiro-Wilk test was used to decide the normality of the distribution. The differences between the microhardness values before and after the treatment were analyzed with the Student's T-test and $p<0.05$ was considered significant. Comparisons between experimental groups were made by using a one-way analysis of variance and Welch statistics at a=0.05. XPS surveys of the experimental groups were converted into atomic percentages using MultiPak Data Reduction Software for XPS and AES (Lake Drive East, Chanhassen, MN, USA). The percentage of atomic values were analyzed by the Kruskal-Wallis test with $p<0.05$.

RESULTS

MICROHARDNESS

Table 1 contains the descriptive statistical results of changes in root canal dentin after treatment with the solutions. Comparison of microhardness values before and after treatment showed that treatment with NaOCl, NaOCl with 0.084% BAC, and EDTA + NaOCl with 0.084% BAC significantly decreased the microhardness of root dentin compared with intact controls ($p<0.05$). Differences between pre-treatment and post-treatment values was calculated as a percentage and comparisons were made between the groups; no statistical difference was found between the groups ($p>0.05$; Table 1).

XPS ANALYSIS

One representative graph of XPS measurements from each group is shown in Figure 1. Mineral content of dentin slabs determined by XPS were converted into atomic percentages using MultiPak software. The atomic percentages of magnesium (Mg), P, and Ca mineral content on the surface of dentin samples are given in Table 2. There was no statistical difference between the groups in terms of mineral content ($p>0.05$).

Table 1. Mean vickers microhardness values of root dentin specimens with respect to the type of treatment.

	Before treatment	After treatment	Percentage decrease
	Mean (±SD)	Mean (±SD)	
Distilled water	60,48 (±3,2)	58,56 (±6,42)	3%
NaOCl	63,48 (±5)*	54,53 (±4,32)*	14%
NaOCl with 0,084% BAC	67,77 (±4,14)^	58,37 (±4,21	11%
EDTA+ NaOCl with 0,084% BAC	64,02 (±4,36)"	57,90 (±4,3)"	10%

Pre-treatment and post-treatment Vickers Microhardness values with the same marked pair indicates the statistical difference between the groups at p=.05 (paired t-test).

Table 2. Percentage mineral values of dentin determined by XPS after various treatments.

	Sodium (Na) %	Magnesium (Mg) %	Phosphorus (P) %	Calcium (Ca) %
Distilled water	0	0,04	7,93	11,51
NaOCl	1,36	0,18	5,61	7,35
NaOCl with 0,084% BAC	2,09	0,24	6,09	8,75
EDTA+NaOCl with 0,084% BAC	2,06	0,36	6,23	8,53

There was no statically difference between groups in terms of mineral content (p>.05).

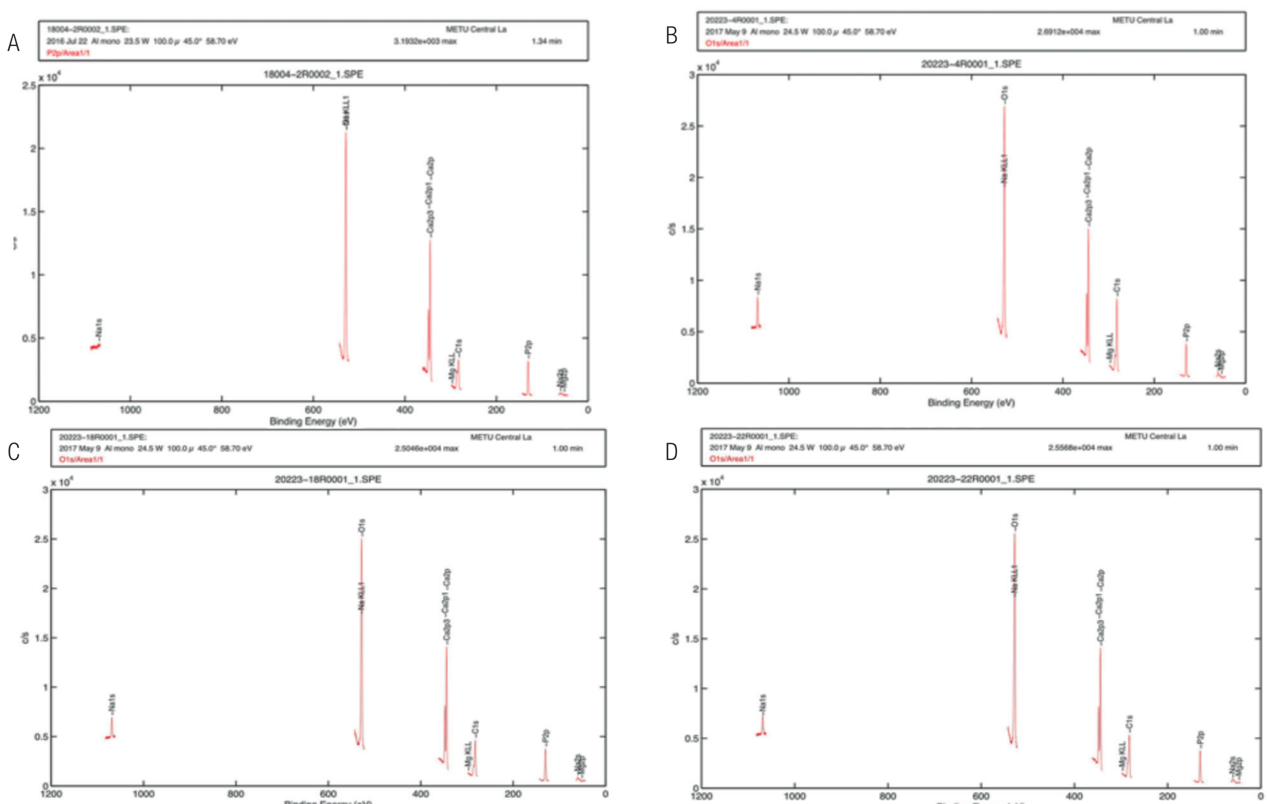

Figure 1. One representative graph of XPS measurements from each group.
PES spectrums are graphs of photoelectron count vs. binding energy.
A: Distilled water B: NaOCl %2,5 C: NaOCl %2,5+ 0,084% BAC D: 17% EDTA+ NaOCl %2,5+ 0,084% BAC.

DISCUSSION

Irrigation of the root canal plays a crucial role in clinical endodontic success. It is expected that the irrigate is able to reach all irregularities within the root canal. To achieve deeper penetration, surfactants have been added to irrigation solutions. It was reported that the addition of 0.084% BAC to NaOCl reduced the contact angle and the surface energy of the solution, without affecting the cytotoxicity, free chloride content, or antibacterial properties of NaOCl (10). In another study, addition of BAC to NaOCl caused a decrease in the number of bacteria in the canal compared with using NaOCl alone (9). Based on these findings, 0.084% BAC was added to 2.5% NaOCl in this study.

It has been reported that there is an increase in the mineral content and microhardness of dentin with aging (17). For this reason, freshly extracted third molar teeth of individuals between the ages of 20-30 were used in our study. Another study showed that the microhardness of dentin depends on location and microhardness decreased as the indentations moved closer to the pulp (18). Less resistance might have occurred to the indenter due to the increased number and width of dentinal tubules near the pulp. As such, in this study the microhardness measurements were made at the middle root level at a depth of 100µm from the pulp-dentin interface.

In clinical endodontic procedures, the smear layer is also removed with 17% EDTA solution followed by NaOCl, which is used at rates ranging from 1-6% to provide antimicrobial and tissue-dissolving effects (19). Therefore, 2.5% NaOCl, 2.5% NaOCl with 0.084% BAC, and 17% EDTA were used in this study.

The results of this study showed that root canal irrigation with NaOCl, NaOCl with BAC, and EDTA+NaOCl with BAC significantly decreased the microhardness of root dentin compared with intact controls. Previous studies have reported that irrigation solutions such as EDTA, NaOCl, and chlorhexidine (CHX) reduced the microhardness of dentin (18,20,21). However, there are few studies examining the effects of surfactant agents on dentin microhardness. A recent study indicated that surfactant addition to EDTA and NaOCl did not alter the microhardness of root canal dentin compared with EDTA and NaOCl alone (12). In this study, it was also shown that the concomitant use of EDTA and sodium hypochlorite did not cause a significant reduction in dentin microhardness compared with sodium hypochlorite, with or without surfactant.

In this study, the Mg, P, and Ca mineral content of dentin slabs were evaluated using XPS. PES spectrums (Figure 1) are graphs of photoelectron count *vs*. binding energy. The peaks in a PES spectrum correspond to electrons in different subshells of an atom. The peaks with the lowest binding energies correspond to valence electrons, while the peaks with higher binding energies correspond to core electrons. When the graphics are analyzed, there appears to be no statistically significant difference in mineral levels among the groups ($p>0.05$). Many studies have reported that chemical agents used in dentin can cause changes of its chemical structure (15,22,23). Dogan and Calt showed that NaOCl and concomitant use of NaOCl and EDTA as a final flush altered the Ca/P ratio, which is the main ingredient of dental hard tissues (15). The change in the Ca/P ratio negatively affects the bonding of dental materials to dentin and sealing properties by causing a significant difference in the ratio of dentin permeability and the organic/inorganic structure (24, 25). Unlike the study of Çalt *et al.*, no significant change was observed in Ca and P content in our study. However, since it has been shown that the use of EDTA for more than 10 minutes causes erosion in the dentin, a change in the Ca/p ratio in their study is inevitable (26). In the study by Dogan and Calt, the use of chelating agents together with NaOCl caused a significant increase in Mg level (15). In our study, an increase in Mg level was observed in all irrigate groups, which was not statistically significant compared with the distilled water group. These statistical differences might have occurred due to the low number of samples due to the high cost of XPS in our study.

Use of chelating agents together with NaOCl caused a change in the Mg content of root dentin (15). Çobankara *et al*. evaluated the mineral contents of dentin following the application of different chelation agents to root canal dentin, and showed that the Ca content of dentin decreased after 5 minutes of 17% EDTA use (27). In another study by Nogo-Živanović *et al*., they showed that 2 minutes of a final rinse with QMix and EDTA changed the P mineral levels in apical and coronal thirds when compared with the no treatment group (28). In our study, similar to the aforementioned studies, although a decrease in the Ca and P content of dentin was observed in the irrigation solution groups compared with the distilled water, this change was not found to be statistically significant.

While most literature studies indicate a decrease in dentin microhardness due to irrigation solutions (13,18,21), this effect may also be associated with concentration and application duration. In this study the addition of a surfactant to the irrigation solution did not cause a significant change in this effect. Studies have suggested that the decrease in microhardness is a result of calcium loss from the dentin surface (27,29). Current study results show that irrigation solutions have caused a slight decrease in the calcium content of the dentin surface, but this decrease was not found to be statistically significant.

As a result of the study, NaOCl, NaOCl with BAC, and concomitant use of EDTA and NaOCl with BAC decreased the microhardness of dentin. However, there was no statistical difference between the groups. The aforementioned solutions did not cause a significant change in the mineral content of dentin.

CONCLUSIONS

The results of the study showed that the addition of BAC to NaOCl has no significant effect on dentin microhardness and mineral content compared with NaOCl alone or concomitantly used with EDTA. Considering the positive features of BAC in the literature, its use might be recommended during root canal irrigation and following EDTA use, before canal filling.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: E.E.D and H.A.
Literature review: E.E.D.
Methodology and validation: E.E.A., H.A. and H.D.B.
Formal analysis: E.E.A. and H.A.
Investigation and data collection: E.E.A. and H.A.
Resources: E.E.A. and H.D.B
Data analysis and interpretation: E.E.A., H.A. and H.D.B.
Writing-original draft preparation: E.E.A. and H.A.
Writing-review & editing: E.E.A. and H.A.
Supervision: E.E.A and H.D.B.
Project administration and funding acquisition: E.E.A and H.D.B.

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