Effectiveness of etidronate in root canal smear layer removal: A study with scanning electron microscopy

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ABSTRACT

Introduction: We evaluated the effect of individual and combined use of 18% etidronate (HEBP) in root canal smear layer removal using scanning electron microscopy (SEM). **Methods:** Thirty human single-rooted teeth were used. The roots were prepared with ProTaper Universal System rotary files and randomly divided into three groups according to the irrigation protocol used. G1: 2.5% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetraacetic acid (EDTA); G2: 2.5% NaOCl and 18% HEBP; and G3: 2.5% NaOCl + HEBP 18%, mixed in equal parts. The roots were sectioned longitudinally and metallized for SEM analysis. The photomicrographs obtained from the cervical, middle, and apical thirds were evaluated by three calibrated examiners, who assigned scores from 1 to 5 to the images. The experimental groups were compared using the Kruskal-Wallis test and the Student-Newman-Keuls test. Root thirds were compared by the Friedman test. The level of significance was set at 5%. **Results:** The capacity for removal of the smear layer by 2.5% NaOCl and 18% HEBP (G2) was similar to that achieved with 2.5% NaOCl and 17% EDTA (G1) in the cervical and middle thirds of the root; the cleansing level of the apical third was similar, regardless of the irrigation protocol used. **Conclusion:** HEBP is a promising solution for use in endodontic treatments.

Keywords: Etidronic Acid. Sodium Hypochlorite. Dissolution. Organic Matter.

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Introduction

The mechanical instrumentation of the root canal produces an amorphous irregular smear layer that may interfere with endodontic treatment success. This smear layer consists of organic and inorganic substances, including fragments from odontoblastic processes, microorganisms, and necrotic materials. It has been demonstrated that the smear layer prevents the penetration of irrigating solutions[1] and endodontic sealer[2] into the dentinal tubules, which may result in the compromised sealing of the canal root.[3,4]

Sodium hypochlorite (NaOCl) is an irrigating solution that is widely used in endodontic treatment due to its excellent antimicrobial action and its ability to dissolve organic matter. In dentistry, the concentration of the NaOCl solution used has been increased to 5.25%[5,6] because of evidence that it is more effective in removing organic matter. A known disadvantage of NaOCl is its inability to eliminate the smear layer. Therefore, a substance is needed which promotes better root canal cleaning by dentin surface decalcification, i.e. removing part of the inorganic content.[5,7,8]

The most commonly used chelating agents which promote the surface decalcification of dentin are ethylenediaminetetraacetic acid (EDTA), citric acid, and, more recently, etidronate (HEBP).[9] The use of EDTA or citric acid as chelating agents in irrigation protocols in combination with hypochlorite solutions has been questioned because these agents react strongly with NaOCl, rendering it ineffective.[9]

Etidronate solution (HEBP), also known as 1-hydroxyethylidene-1, 1-bisphosphonate or etidronic acid, is a substance used in the prevention of bone resorption and has been employed for the treatment of patients with osteoporosis and Paget's disease.[10] HEBP has been indicated for the removal of the smear layer because it has chelating properties, has minimal effects on the dentin structure, and offers the possibility of being mixed with the NaOCl solution without altering the antimicrobial properties of this substance.[11] It has also been suggested that when the mixture is used during biomechanical preparation, no smear layer appears to form.[11,12]

An irrigation solution that could act on the organic and inorganic portion of the root dentin, promoting cleansing and allowing better sealing of the dentinal tubules, would be a great contribution to endodontic therapy. Thus, this study evaluated the efficacy of HEBP and HEBP mixed with NaOCl as a single solution in the cleansing and removal of the smear layer of the root canal in vitro and by scanning electron microscopy (SEM) analysis.

Materials and methods

This study was approved by the Ethics Committee on Human Research of the Health Sciences Institute; Protocol no. 850.737.

Sample Selection

Thirty, recently extracted, single-rooted human teeth were collected. Tissue and debris remnants on the root surfaces were removed and the teeth were decontaminated by immersion in 1% NaOCl solution for 30 minutes. After cleaning, the teeth were stored in saline solution at 9°C until use. After obtaining periapical radiographs, all teeth with an incomplete apex, previous root canal treatment, internal or external root resorption, calcifications, root caries, complicated root canal anatomy, or accentuated curvatures were excluded from the sample.

Specimen Preparation

After preparing the access cavity, the working length was determined by inserting a K-file ISO size 10 until the tip of the instrument became visible at the tip of the root; 1 mm was subtracted from this. The apices were sealed using sticky wax before the preparation of root canals. Root canals were enlarged with K-files ISO 15 and then Gates-Glidden bur II (Dentsply, Maillefer, Ballaigues, Switzerland). Subsequently, the roots were prepared with ProTaper Universal rotary instruments (Dentsply, Maillefer, Ballaigues, Switzerland) coupled to a X-Smart electric motor (Dentsply, Maillefer, Ballaigues, Switzerland) at 3N torque and a speed of 250 rpm. The SX instrument was used for cervical preparation, after S1, S2, F1, F2, and F3 instruments were used at the working length. During the instrumentation phase, the teeth were randomly divided into three groups (n=10) according to the irrigation protocol used.

Irrigation Protocol

In group G1, the root canals were irrigated between changes of files with 2.5 mL of 2.5% NaOCl;

the final irrigation was performed with 0.5 mL of 17% EDTA for 3 minutes, followed by irrigation with 2.5 mL of 2.5% NaOCl. In group G2, root canals were irrigated with 2.5 mL of 2.5% NaOCl at each instrument change; the final irrigation was performed with 0.5 mL of 18% HEBP for 3 minutes, followed by irrigation with 2.5 mL of 2.5% NaOCl. In group G3, root canals were irrigated with 2.5 mL of 2.5% NaOCl, mixed with 18% HEBP at each instrument change; the final irrigation was performed with 2.5 mL of physiological saline solution.

Root sectioning and cleaning evaluation

After instrumentation, two longitudinal grooves were prepared on the buccal and lingual surfaces of the roots by means of a disc (Microdont, São Paulo, Brazil), with no entrance into the canal space. The roots were washed externally with saline solution and sectioned longitudinally in a buccolingual direction by a chisel (Hu-Friedy, Chicago, IL, USA). The halves were prepared for scanning electron microscopy (SEM) (LEO-1430, ZEISS, Oberkochen, Germany) analysis at 900x magnification. Photomicrographs were taken of the cervical, middle and apical thirds of each specimen. Three previously calibrated examiners analyzed the images according to a modified score system proposed by Hulsmann et al.[13] (Fig. 1).

Statistical analysis.

The data were analyzed using statistical software (Bioestat 5.3® Software, Tefé, AM, Brazil) with a bilateral a of 5%. The experimental groups were compared with Kruskal-Wallis test and Student-Newman-Keuls test. The different root thirds were compared using the Friedman test.

Results

The Friedman test showed no difference in the amount of smear layer between the apical, middle, and cervical thirds of root canals in any of the experimental groups. The Kruskal-Wallis test showed statistically significant differences between groups in the amount of smear layer remaining in cervical ($p = 0.035$) and middle ($p = 0.039$) root thirds. The Student-Newman-Keuls test showed statistically significant differences in the amount of smear layer between the G1 (NaOCl 2.5% and EDTA 17%) and G3 (2.5% NaOCl and 18% HEBP) groups in the cervical ($p = 0.036$) and middle ($p = 0.026$) thirds of root canals (Tab. 1 and Fig. 2).

Table 1. Scores for smear layer removal (adapted to the classification by Hulsmmann et al.[13])

Score	Smear layer
1	Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules
2	Homogenous smear layer covers in the root canal wall, only few dentinal tubules are open
3	Homogenous smear layer covers in the root canal wall, many dentinal tubules are open
4	*Small amount of smear layer, many dentinal tubules are open*
5	No smear layer, dentinal tubules are open

Table 2. Medians (and interquartile deviation) of the root canal cleansing scores according to the root third and the irrigation protocol.

	Cervical	Middle	Apical	p*
G1: NaOCl 2,5% + EDTA 17%	4 (0)[a]	4(0)[a]	3,5 (1,75)[a]	0,153
G2: NaOCl 2,5% + HEBP 18%	3 (1)[a,b]	3,5 (1,75)[a,b]	2,5 (1)[a]	0,217
G3: NaOCl 2,5% + HEBP 18% (mixed)	3 (0,75)[b]	3 (0.75)[b]	3 (1)[a]	0,088
p†	0,036	0,039	0,157	

Friedman test. † Kruskal-Wallis test. Medians followed by different letters on the column differ statistically through Student- Newman-Keuls test at 5 %.

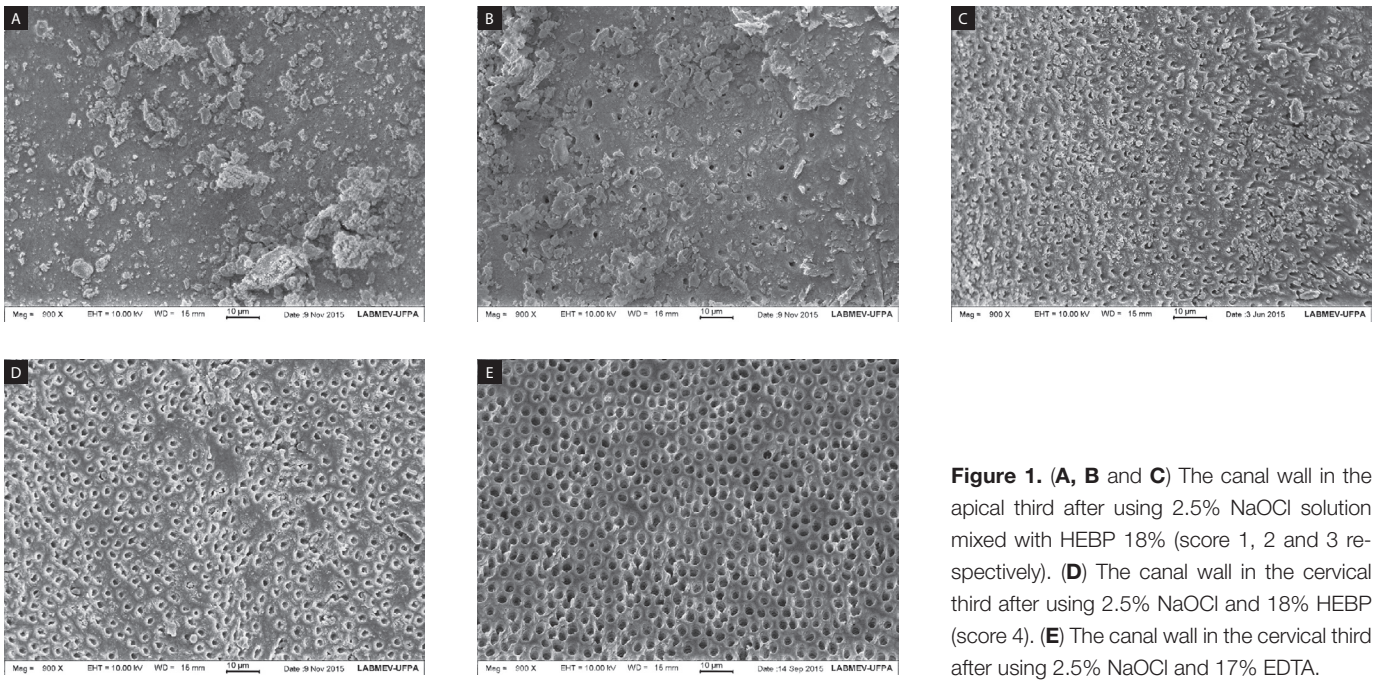

Figure 1. (**A, B** and **C**) The canal wall in the apical third after using 2.5% NaOCl solution mixed with HEBP 18% (score 1, 2 and 3 respectively). (**D**) The canal wall in the cervical third after using 2.5% NaOCl and 18% HEBP (score 4). (**E**) The canal wall in the cervical third after using 2.5% NaOCl and 17% EDTA.

Discussion

The use of HEBP in irrigation protocols may be clinically advantageous as it has been demonstrated that the action of strong chelators such as EDTA can reduce the microhardness of dentin[14] and alter the roughness of this tissue.[15]

The results of this study demonstrated that the irrigation protocol with 2.5% NaOCl followed by 18% HEBP (G2) was able to remove the smear layer of the root canal in all root thirds evaluated, with no statistical difference (p>0,05) when compared to cleaning promoted by 2.5% NaOCl followed by 17% EDTA (G1). The 18% HEBP solution demonstrated efficacy in 3 minutes,[11] although it has been reported that this solution, in concentrations of 9% and 18%, requires 5 minutes to completely remove the smear layer.[16]

The HEBP chelating effect was similar to that of EDTA when used separately from NaOCl (G2), but the mixture of NaOCl and HEBP (G3) was the protocol that had the least effect on the removal of the smear layer (p <0.05). Studies[11,12] have shown that when HEBP is mixed with NaOCl and used as a single solution during the biomechanical preparation of the root canal, there is no formation of a smear layer. In the present study, both formation and persistence of the smear layer were observed in some regions of the dentin walls in the G3 group. This finding may be related to the decrease in HEBP concentration in the mixture or the fact that HEBP was not used alone as a final irrigant, as it was in the previous study.[11]

The effects of decalcification by chelating agents depend on factors such as the type of irrigant used, pH and concentration of the solution, and time of application.[17,18] The mixture of the two substances (HEBP at 18% and NaOCl at 2.5%) may have interfered with the amount of free ions with chelating power and, consequently, reduced the cleansing effect on the channel. On the other hand, it has been shown that NaOCl is capable of creating deproteinization channels which increase the surface area available for chelating agent action.[19] Thus, a decrease in NaOCl concentration, when mixed with HEBP, may have affected the amount of deproteinization channels, reducing the action of the agent as observed in G3.

The removal of the smear layer from the apical third was similar in all studied groups, regardless of the irrigation protocol used. The anatomical complexity of the region, which presents a thinning and reduction of the apical diameter,[19,20] makes access

of irrigated solutions to this area difficult, aside from the presence of sclerotic dentin, which interferes with the dentinal permeability of the apical third;[21] both factors interfere with the irrigation solutions in this region of the root.

The intragroup analysis did not indicate a statistically significant difference in smear layer cleansing between the different root thirds. These findings show that, although the dentin structure is different in the root thirds,[22] the resulting changes when the irrigating solutions are able to come into contact with the surface of the dentin are similar. The use of single-rooted teeth, which have a less complex anatomy and an absence of sharp curves, facilitated the flow of the irrigating solutions inside the root canal, resulting in a similar cleansing of the root thirds.

Future studies on the optimal timing of the application of HEBP solution in irrigation protocols, as well as on the efficacy of its combination with NaOCl, should be performed in order to ensure greater predictability of clinical outcomes.

Conclusion

Based on the results of the present study, it can be concluded that 18% HEBP, used as a final rinse, can be effective in removing the smear layer. Additionally, the cleansing level of the apical root third was similar regardless of the irrigation protocol applied.

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