Wettability of an epoxy resin-based root canal sealer on dentin treated with different chelating protocols

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ABSTRACT

Aim: Evaluate the wettability of AH Plus in contact with root dentin after different chelating protocols involving etidronic acid (HEBP) and EDTA. **Material and Methods:** Fifty six human polished root dentin slices were used. They were irrigated with 5.25% sodium hypochlorite (NaOCl) or a mixture of 5.25%NaOCl/18%HEBP to simulate irrigation during chemomechanical preparation. The specimens irrigated with NaOCl were divided into 5 groups regarding chelating agents: G1-destiled water (DW); G2-17%EDTA; G3-17%EDTA+2.5%NaOCl; G4-18%HEBP; and G5-18%HEBP+2.5%NaOCl. The specimens irrigated with the mixture NaOCl/HEBP were divided into 2 groups: G6-DW; G7-NaOCl/HEBP+2.5%NaOCl. All protocols received irrigation with DW between irrigants and as final rinse. Rame-Hart goniometer was used to measure the contact angle between the dentin surfaces and the sealer. Kruskal-Wallis and Dunn tests were applied ($p<0.05$). **Results:** Groups in which the smear layer was removed showed a lower contact angle ($p<0.05$), except for G7. The G6 showed the lowest contact angle of AH Plus, but the NaOCl final irrigation (G7) increased the angle. G2 and G4 have similar behaviour and final irrigation with NaOCl (G3 and G5) did not change wettability when these chelators were used. **Conclusions:** The mixture NaOCl/HEBP showed good effect on the wettability of sealer on to the root canal dentine, when used as main irrigant.

Keywords: Root Canal Irrigants. Chelating Agents. Wettability. Edetic Acid. Etidronic Acid.

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Introduction

Endodontic treatment comprises a series of procedures that aims disinfection and sealing of the root canal system. Mechanical instrumentation produces a smear layer composed of dentinal debris and bacteria that adheres weakly to the root canal walls and obliterates dentinal tubules, reducing its permeability.[1] Due to mechanical obliteration of the dentinal tubules, this layer has an adverse effect on dentin bonding, on the penetration of the irrigating solutions and the sealers to the dentinal tubules,[2] increasing microleakage after canal obturation.[3] Removing the smear layer from the root canal using irrigating solutions has been suggested to ensure that the root canal filling can perfectly adapt to the canal[4], as well as to reduce the bacterial load of microorganisms inside root to dentin, increasing the chance of success of the endodontic therapy.[5]

The alternate use of sodium hypochlorite (NaOCl) and 17% EDTA is an efficient method for removing the endodontic smear layer. While NaOCl dissolves the necrotic tissue as well as the organic components of this layer, inactivates endotoxins, disintegrates endodontic biofilms and is the main substance responsible for root canal debridement,[6] EDTA removes calcium ions (Ca^{2+}) from the mineralised portion of the smear layer.[7,8] Ideally, combination of these irrigating agents would prevent formation of the smear layer on the dentin during mechanical instrumentation. However, hypochlorite is very reactive and cannot easily be combined with other chemicals in the same solution and for this reason two steps of irrigation procedures should be made.[9] The 1-hydroxyethylidene-1,1-bisphosphonate (HEBP), which is also known as etidronic acid, has been described as a decalcifying agent which compatible with NaOCl. It is a biocompatible chelator that can be mixed with a NaOCl solution without any short-term loss in antimicrobial properties[9] and causes minimal changes in the ability of sodium hypochlorite to dissolve organic matter.[9] This mixture could be used as a single irrigant during and after instrumentation to remove the produced smear layer.[8]

The use of chemical solutions to irrigate root dentin during such preparation cause alterations in the chemical and structural composition of human dentin.[7,11-13] EDTA is a well-known strong chelating agent, which can not only extract Ca^{2+} ions from the smear layer and dentin, but can also produce severe areas of demineralisation after prolonged contact with surface dentin,[14] decreasing thus, its the calcium/phosphorus (Ca/P) ratio.[11] These changes may also negatively affect the adhesion and sealing ability of dental materials, such as root canal sealers, to dentin.[2] Therefore, a weak or moderate decalcifying agent may represent a good choice for preservation of the peritubular and intertubular dentin. Lottanti et al.[16] suggested that etidronic acid has the potential to replace the conventional treatment with EDTA. Being a weak chelator, HEBP results in less damage to dentin, resulting in adequate removal of smear layer without jeopardizing the quality of adhesion.[17]

In the context of adhesion, both the substrate and adhesive must come into intimate contact with the substrate to allow either chemical adhesion or micromechanical surface attachment. Wettability is one of the most important physicochemical properties that interfere with the ability of materials to interact with the substrate and, in the case of endodontic sealers, to allow penetration into the main canal and dentinal tubules. It is represented by the contact angle between the drop of liquid and the plane surface of the solid, showing the ability of the liquid to spread on this surface.[18] Root canal sealers with good flow ability and low surface tension spread and interact better with the dentin surface, resulting in low contact angle and thus can be easily placed along the entire root canal and even penetrate slightly into lateral canals and dentinal tubules.[19]

Some studies have evaluated the wettability of endodontic sealers in dentin treated with chelators like EDTA[20] and maleic acid,[21] but there are no available studies in the literature on wettability of sealers into root canal dentin treated with HEBP. Hence, the purpose of this study was to investigate the wettability of one epoxy resin-based sealer on root canal dentin treated with different irrigation protocols using HEBP, with and without a final irrigant. A standard 17% EDTA solution was used as a reference for comparison. The null hypothesis was that the irrigation solutions do not influence epoxy resin based sealer wettability.

Materials and Methods

The study protocol was approved by the ethics Committee on Human Research from the Universidade Federal do Pará (Belém/PA, Brazil), under registration number at CEP 519.036 (CAAE 23520513.2.0000.5174).

BioEstat 5.0 software (Civil Society Mamirauá, AM, Brazil) was used to calculate the sample size using data from a pilot study, which was conducted with 14 single-rooted (28 specimens) human teeth and which followed the same procedures as the present study (each group with 4 specimens). The contact angle between the sealer and treated dentin surfaces was adopted as the main outcome of this study and, in this way, the lower mean difference between groups were used to calculate the sample size, adopting a statistical power of 80%, an alpha error of 5%. The sample calculated for this study consisted of 28 single-rooted (56 samples) human teeth.

Only roots with a minimum length of 11 mm, without caries, cracks, and root dilacerations were used. After extraction debridement of the surrounding soft tissue and debris the teeth were placed in saline solution at 4°C. The crowns were removed at the cement-enamel junction and the remaining roots were split into a buccal-lingual direction using a diamond disk (KG Sorensen Ind. e Com., Barueri, SP, Brazil) at low speed under water cooling, providing 56 specimens which were polished with a series of ascending grades (80, 100, 120, 150 and 180) of silicon carbide abrasive papers (3M do Brasil Ltda., Sumaré, SP, Brazil) under water cooling, during 20 seconds of each abrasive paper, to make the surface flatter and smoother.[20] Then, the samples were thoroughly washed and ultrasonicated in distilled water to remove residual particles.

The specimens were than randomly divide into seven experimental groups, as follows: the solution treatments were carried out in three stages. In stage 1, 40 specimens were irrigated, using a syringe, with 25 mL of irrigating solutions - 5.25% NaOCl (Fórmula&Ação, São Paulo, SP, Brazil) and 16 specimens were irrigated with a mixture of 5.25% NaOCl/18% HEBP (Zschimmer & Schwarz Mohsdorf GmbH & Co KG, Burgstädt, SN, Germany) - to simulate the chemo mechanical preparation. In stage 2, the specimens irrigated with NaOCl on stage 1 were had the inorganic phase of the smear layer removed by immersion on chelating agents - 17% EDTA (Fórmula&Ação, São Paulo, SP, Brazil) for 3 minutes or 18% HEBP for 5 minutes. In step 3, surface final treatment was performed using a 2.5% NaOCl (Fórmula&Ação, São Paulo, SP, Brazil) solution for 1 minute. Between each step and as a final rinse, the specimens were washed with distilled water for 1 minute. The complete protocols of experimental group is shown in Figure 1.

The 18% HEBP solution was prepared using the pure chemical dissolved in distilled water. The mixture 5.25% NaOCl + 18% HEBP was prepared using equal parts (1:1) of these two solutions mixed togeth-

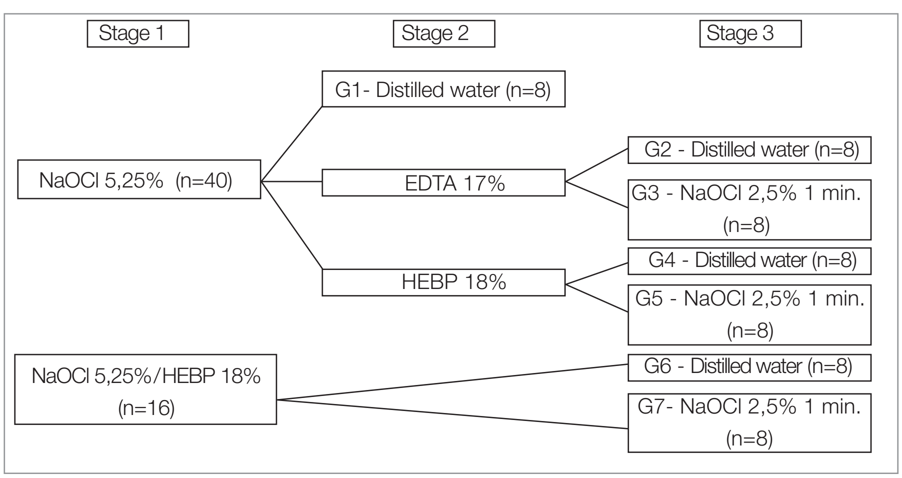

Figure 1. Irrigation regimes conducted for the dentin samples.

er, immediately before the experiments. All solutions were stored at 5°C in dark containers after experiments. However, during the experiment, the solutions were kept at room temperature.

After treatment, the specimens were dried with paper points (Dentsply Ind & Com. LTDA, Petrópolis, RJ, Brazil) and the contact angle was measured.

Contact Angle measurement

After completion of the treatments, the dentin specimens were positioned on microscopic glass slide in a Rame-Hart goniometer (Rame-Hart Instrument Co, Netcong, NJ). This equipment has a flexible video system for measuring the static and dynamic contact angles and surface free energy using the sessile drop technique, and was used to measure the contact angle between AH Plus (Dentsply, Petropolis, RJ, Brazil) and treated dentin specimens. This sealer was mixed according to the manufacturer's instructions. The goniometer was aligned and focused on the dentin-sealer interface.

At this point, a controlled-volume droplet (0.1 mL) of sealer was placed over the internal side of the root canal surface (intraradicular dentin) of a specimen from each group. The volume of the sealer was controlled by means of BD ultra-fine syringe of 0.5 mL/cc (Becton Dickinson, Franklin Lakes, NJ, USA). Two drops were placed on each specimen. The spreading process was recorded and three images/second of each drop were analysed to provide the values of contact angles with the help of the Rame-Hart goniometer software (Rame-Hart Instrument Co, Netcong, NJ). Images of the final three seconds of each drop were analysed to provide the values of contact angles. All measurements were carried out by one calibrated operator.

All experiments were performed under standard environmental conditions.

Statistical Analysis

The data were computed with BioEstat 5.0. Shapiro-Wilk test was used to check normality distribution. The sample exhibited non parametric distribution. Data were analysed with Kruskal-Wallis analysis of variance and Dunn tests ($p < 0.05$) to compare the contact angle between groups.

Results

Table 1 lists the median (Med) and interquartile range (IQR) values of the contact angles between the sealer and treated dentin surfaces. Better spreading was observed for G6 ($p<0.05$). The comparison of measured contact angle values before and after the application of chelating agents shows that when the smear layer was removed, the values of contact angle were lower ($p<0.05$), except for G7 ($p>0.05$). The comparison between the use of chelating agents with and without NaOCl final irrigation showed that final irrigation did not significantly change the contact angle ($p>0.05$), except for when comparing G6 and G7 ($p<0.05$), where this final irrigation increased the contact angle. Representative static contact angles obtained for AH Plus on root canal dentine treated with different irrigant protocols are shown in Figure 2.

Table 1. Median ± interquartile range values of contact angle (Aº) between treated dentin surfaces and AH Plus.

Groups	A Med ± IQR	
G1 - NaOCl	55,45 ± 3,825 ^{A}	A
G2 - EDTA 17%	44,55 ± 1,425 $^{B\,a\,1}$	B
G3 - EDTA 17% + NaOCl	42,7 ± 1,875 $^{B\,1}$	B
G4 - HEBP 18%	46,55 ± 5,125 $^{B\,a\,2}$	B
G5 - HEBP 18% +NaOCl	44,65 ± 4,225 $^{B\,2}$	B
G6 - HEBP/NaOCl	31,75 ± 2,55 $^{B\,b\,3}$	B
G7 - HEBP/NaOCl + NaOCl	50,25 ± 3,125 $^{A\,3*}$	A

Superscript uppercase letters (A,B) indicate statistically significant values between groups with (G1) and without smear layer. Superscript lowercase letters (a,b) indicate statistically significant values between the chelating agents (G2, G4, G6). The presence of * next to a number indicates statistically significant values after final treatment between each chelating solution (G2/G3-1, G4/G5-2, G6/G7-3).

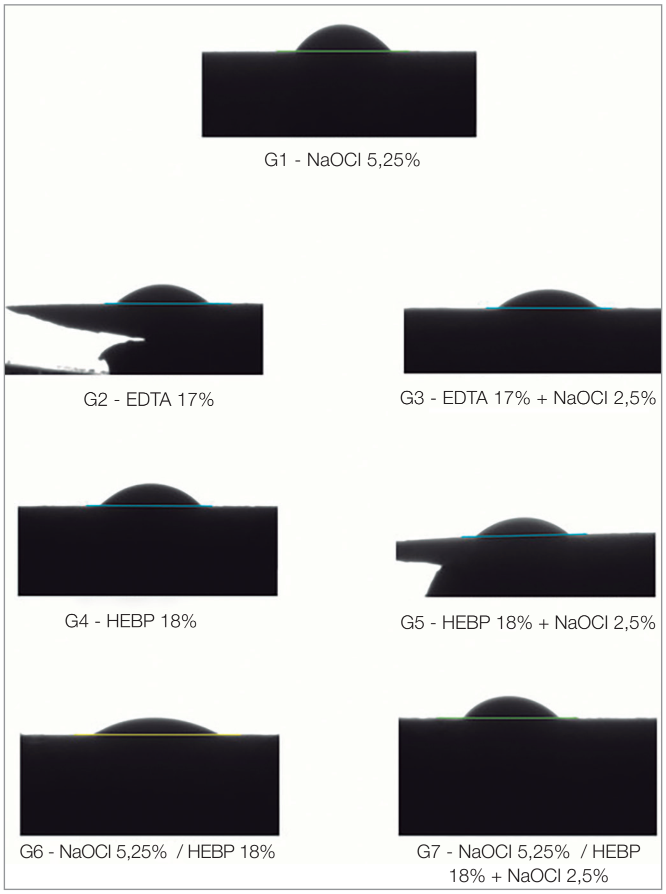

Figure 2. Representative images of sessile drops of AH Plus applied to root canal dentine treated with different irrigating solutions. Different colors indicate statistically significant values between groups.

Discussion

The physicochemical properties of a root canal sealer influences its clinical behavior during and after obturation of the root canal system. One of these properties is the optimal wetting, represented by the formation of a contact angle. The contact angle has an inverse relationship with wettability; meaning that the lower is angle, higher is the wettability of the liquid and hence, the better are the adhesion propriety.[21] In the present study, bonding to root canal dentine was differently affected by the endodontic irrigant schemes. In general, improvements in wettability values were found in the following order: G6 > G3 > G2 > G5 > G4 > G7 > G1 as shown in Table 1.

The experiment was accomplished under standard environmental conditions and with a controlled volume of each of drop sealer. This was performed because the pH and temperature variations caused alterations in the surface tension levels of solutions,[22] and changes in the drop size could affect the value of the contact angle.[23] Only two drops were placed because the root canal surface has a very limited area on which to place more droplets.

For correct measurement of the contact angle, the surface must be clean, flat and smooth,[24] although the root canal dentin surfaces were not smooth after cleaning and shaping. Therefore, for this study, the fine polishing was a substitute for sandpaper, to obtain completely feat surfaces that were somewhat similar to clinical conditions before instrumentation, as reported in other studies.[20,25]

Comparing groups with (G1) and without (G2, G3, G4, G5, G6) a smear layer, groups in which the smear layer was removed showed a lower contact angle, except for G7, that presents similarity statistic to G1. This has also been shown in other studies[20,25] and is probably related to the significant contribution that roughness has on the wetting behaviour of a surface.[26] In fact, this can be explained by the action of chelating solutions with regard to removing the smear layer and exposing the dentinal tubules[16] thus increasing the roughness of the dentin surface.[13] According to the Wenzel equation, this action increases the wettability of the sealer.[20] The increase in surface roughness favours the micromechanical retention of materials; moreover, this facilitates bacterial adhesion.[27]

Dentin is composed of two different substrates: hydroxyapatite, which has a high surface energy, and collagen, which has a low surface energy. AH Plus is able to bond to the organic phase of root dentin, most likely in the collagen fibres.[28] Knowing that EDTA is a more powerful agent for removing the smear layer and hydroxyapatite than HEBP,[8,9] exposing more of the collagen network, which is better for the adhesive sealer. Although EDTA and HEBP result in structurally different surfaces, in the present study this does not significantly influenced AH Plus wettability. The time of action of HEBP (5 minutes) compared to EDTA (3 minutes) may have influenced the final wettability. In the present study, these different times of action for solutions was determined based on smear layer removal and opening dentin tubules.[8]

Best spreading of the sealer in G6 is probably related to the higher dentin roughness produced for this irrigation protocol, when compared to other substances, as shown by Tartari et al.[13] Another study found that the use of a mixture of NaOCl/HEBP during irrigation had a significant impact on the bond strength of an epoxy-based sealer to root dentin[17]. This can be explained by three factors: (i) the ability of NaOCl to deproteinate and create channels in dentin,[29] increasing the contact area for the action of HEBP and the roughness dentin; (ii) the weak chelating but continued action of HEBP probably removed a greater amount of Ca^{+} (compared to G2 and G4),[11] exposing more collagen fibres, making the surface favourable for this sealer; and (iii) the fact that each sample of this group was irrigated with 25 ml of the solution while, in the other groups, the samples were immersed in 40 ml of chelator, which may have increased the time of action and the substance effect.[30]

In general, after debridement and disinfection, negative bacterial cultures only occur in 40-60% of cases; microorganisms can partially survive chemo-mechanical preparation inside dentinal tubules and irregularities of the root canal. Therefore, after removal of the smear layer, it is recommended to complete irrigation with another disinfectant to address the remaining bacteria. A low concentration of NaOCl is used with this aim.[31] The final irrigation with 2.5% NaOCl, after the addition of 17% EDTA (G3) and 18% HEBP (G5), did not cause any changes in the wettability of the sealer, which is in disagreement with the results of Assis et al[2], that find significant alterations in EDTA group. This contradiction can probably be explained because the samples were dried with nitrogen gas by Assis,[20] while in the present study they were dried with paper points to simulate the clinical environment. This might have caused alterations on the surface free energy, during contact angle measurements. In the HEBP group, the probably explanation is that HEBP was not able to expose a significant amount of the collagen network for NaOCl deproteinization action. However, the wettability was decreased with final 2.5% NaOCl irrigation after HEBP/NaOCl treatment (G7). This probably occurred because the organic-dissolving properties of NaOCl on the collagen components of dentin after removal of the smear layer[29] resulted in dentin surfaces similar to etched enamel and led to a high energy surface,[32] which was unfavourable for AH Plus.

Knowing that different irrigant solutions affect directly dentin morphology and wettability to endodontic sealers; and the first step of the interaction between adhesive and substrate is the wetting of the dentin,[21] this effect should be taken into account. The present study highlights the good effect of the mixture NaOCl/HEBP on the wettability of epoxy-resin based sealer on to the root canal dentine, especially when used as main irrigant, which is required for obtaining good adhesion and obturation seal. The improvement in wettability and adhesion of AH Plus when a mixture 5.25% NaOCl/18% HEBP is used is especially relevant in single cone obturation techniques, in which the sealer must play a more incisive role on the filling of spaces between the cone and dentinal walls. However, further studies are required to evaluate the wettability of HEBP with treated dentin surface as well as the interaction between HEBP-treated dentin and endodontic materials.

Conclusion

The mixture NaOCl/HEBP showed good effect on the wettability of AH Plus on to the root canal dentine, when used as main irrigant.

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