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Effect of Sodium Thiosulfate on Interfacial Adaptation and Penetration of an Epoxy Resin-Based Root Canal Sealer

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ABSTRACT

Introduction: Our study evaluated the impact of sodium thiosulfate (ST) irrigation, subsequent to sodium hypochlorite (NaOCl) and just before root canal filling, on the filling quality (interfacial adaptation and penetration segment) of an epoxy resin-based root canal sealer. **Methods and Materials:** Twenty single-rooted human teeth were prepared with the ProTaper system. The specimens were then divided into the following groups: 5.25% NaOCl irrigation (NaOCl group) and 5.25% NaOCl irrigation+0.5% sodium thiosulfate (NaOCl+ST group). The root canals were filled using single-cone technique with ProTaper F3 cones and AH-Plus sealer, labeled with rhodamine B dye to allow analysis under a confocal laser scanning microscopy (CLSM). All samples were sectioned at 2, 4, and 6 mm from the apex and prepared for CLSM analysis. The percentage of voids, gaps and dentinal sealer penetration segment of the canal were calculated at the apical, middle and coronal thirds. The non-parametric Mann-Whitney statistical test was used at 5% significance level. **Results:** Higher percentage of gaps and voids were observed at all root thirds of the NaOCl group when compared to the NaOCl+ST group ($P<0.05$). There was a significant increase in the penetration segment of NaOCl+ST group at the coronal and middle root third when compared to the NaOCl group ($P<0.05$). **Conclusion:** Our *in vitro* results showed that the use of ST as an antioxidant agent after NaOCl irrigation promoted a better interfacial adaptation and penetration of epoxy resin-based root canal fillings.

Keywords: Confocal Laser Scanning Microscopy; Endodontics; Root Canal Filling; Sodium Hypochlorite; Sodium Thiosulfate

Introduction

Sodium hypochlorite (NaOCl) is the most frequently used irrigant during endodontic therapy, mainly because of its antibacterial effect and its ability to dissolve organic tissues [1]. However, the use of NaOCl can result in significant changes in the mechanical properties of dentine, such as the microhardness, rigidity, and modulus of elasticity [2]. Moreover, NaOCl disrupts the polymerization of epoxy resin-based endodontic sealers due to the presence of oxygen on the dentinal walls and tubules, causing adaptation failures at the dentin–resin interface, affecting the root canal filling [3].

Strategies have been tested to promote better root canal filling adaptations. To overcome these problems, the use of antioxidant agents have been encouraged due to its capacity to reduce the prejudicial effects of oxygen liberation after the use of NaOCl and its harmful effects on dental materials [4]. Antioxidant agents break up free radicals, reacting and neutralizing NaOCl products. Sodium thiosulfate ($Na_2S_2O_3$ - ST) is an antioxidant that has been widely used in medicine [5] and as a neutralizing agent of NaOCl, with promising results when used as reducing agent for NaOCl-treated dentin [4]. Based on the foregoing considerations, it would be interesting to assess the effect of this antioxidant previously to root canal filling.

Therefore, the aim of the present study was to evaluate the influence of using ST after using NaOCl and before root canal filling on the filling quality (interfacial adaptation and penetration segment) of an epoxy-based root canal sealer. Irrigation using NaOCl without applying the antioxidant agent was used as reference for comparison. The null hypothesis tested was that there would be no difference in the interfacial adaptation of epoxy resin-based root canal fillings when ST was used after using NaOCl.

Materials and Methods

Tooth selection

Upon obtaining approval by the Local Research Ethics Committee, single-rooted human teeth were collected. The sample size of 10 specimens per group was determined after a pilot study, with a test power of 80% and significance level of 5%. After visual and radiographic evaluations, 20 teeth that met the following inclusion criteria were obtained: complete root formation and lack of root canal curvatures (<5°), cavities, resorptions, calcifications, or cracks.

Endodontic treatment

The tooth crowns were sectioned near the enamel–cement junction to obtain a standard root length of 12 mm for each specimen. The working length was established by measuring the penetration of a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until it reached the apical foramen and then subtracting 1 mm. The root canals were prepared with ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) as follows: SX at the coronal part of the canal and S1, S2, F1, F2, and F3 at the working length at a speed of 300 rpm and torque of 3 N. Irrigation of the specimens was performed according to the groups (10 specimens per group):

- NaOCl group-Irrigation with 5.25% NaOCl and final rinse with saline solution
- NaOCl+ST group-Irrigation with 5.25% NaOCl and final rinse with 0.5% ST

At the beginning, and after each file, the root canals were irrigated with 2 mL of 5.25% NaOCl using a 5-mL syringe and a 30-G needle (NaviTip; Ultradent Products Inc., South Jordan, UT, USA). After the F3 file, the canals from NaOCl+ST group were rinsed with 0.5% ST for 10 min. The canals from NaOCl group were rinsed with saline solution for 10 min. The solutions for final rinse were inserted into the canals at 1 mm coronal from the working length. After complete preparation, a final irrigation was performed with 5 mL of saline solution, followed by 17% ethylenediamine tetraacetic acid (EDTA) for 3 min and 5 mL of saline solution again. The canals were then aspirated with capillary tips (Ultradent Products Inc., South Jordan, UT, USA) and dried with paper points.

Root canal filling

AH-Plus (Dentsply Maillefer, Ballaigues, Switzerland) was manipulated according to the manufacturer's instructions. To allow visualization under a confocal laser microscope (IX 81 Olympus, Tokyo, Japan), fluorescent rhodamine B dye (Sigma-Aldrich, St Louis, MO, USA) was incorporated in the sealer mixture at a concentration of 0.1% [6]. The sealer was inserted in each root canal by using a size 30 rotary lentulo (Dentsply Maillefer, Ballaigues, Switzerland), keeping the instrument 2 mm from the apex. The root canals were filled using single-cone (SC) technique, a single gutta-percha cone F3 that matches the tapered size of the final rotary instrument was selected and fitted to the working length with the additional endodontic sealer. The gutta-percha cones excess was removed by using a heated plugger and vertical compaction was performed at the orifice level. The specimens were stored at 100% humidity and 37 °C for 7 days to allow the sealer to set.

Specimens preparation and CLSM analysis

Specimens were included in epoxy resin, and sectioned horizontally at 2, 4, and 6 mm from the apex. In this manner, three slices per root were created, corresponding to the apical, medium, and coronal root thirds. The apical aspect of each slice was polished (Politriz; Arotec, Cotia, Brazil) with 600, 800, and 1200 sandpapers under running water. The specimens were examined by confocal microscope (IX 81, Olympus, Tokyo, Japan). The images were obtained with the absorption and emission wavelengths of 540 and 590 nm, respectively, for rhodamine B. Analyses of all images were performed by a single blinded evaluator with Image J software (NIH, Bethesda, MD, USA).

To calculate the void area percentage, a similar method to the one proposed by Hirai *et al.* [7] was used, with the polygon selections tool of the Image J software (NIH, Bethesda, MD, USA). First, the total area of the canal was delimited for each specimen, and the area values were registered. Then, the area of the gutta-percha cone was measured. The sealer filled area of the canal was obtained by subtracting the gutta-percha area from the total area of the canal. The voids area was obtained as a percentage from the sealer filled area.

The method proposed by Moon *et al.* [8] was used for the analysis of the penetration segment. The segmented line tool was used (Image J software, NIH, Bethesda, MD, USA). For each specimen, first the total perimeter of the root canal was delimited, to obtain the length values. Then, the perimeter within which endodontic sealer penetrates into the dentinal tubules were measured. In this way, the sealer penetration segment was obtained as a percentage from the total perimeter.

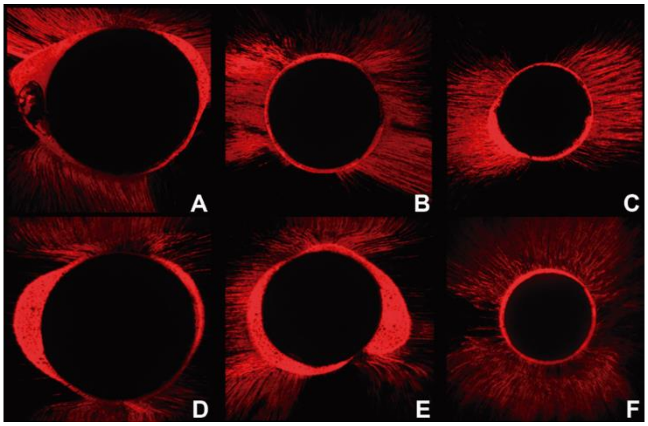

Figure 1. Representative confocal laser scanning microscopic images (magnification 10×) of the root specimens irrigated with 5.25% NaOCl at the *A*) Coronal; *B*) Middle; *C*) Apical root third; and 5.25% NaOCl+0.5% Sodium Thiosulfate at the; *D*) Coronal; *E*) Middle; *F*) Apical root third

Statistical analysis

Non-normal distribution of the data was observed (Shapiro-Wilk test, $P<0.05$); therefore, the Mann-Whitney test were used for statistical analysis for both void and root canal sealer penetration segment data. Intragroup comparisons between the root thirds were made by the Friedman test. A 5% level of statistical significance was applied to the analyses.

Results

After analyzing the areas of voids and gaps (Table 1), the NaOCl group showed a significantly higher percentage of voids and gaps when compared to the NaOCl+ST group in the coronal, middle, and apical root third ($P<0.05$). The intragroup analysis showed a significant difference only for the NaOCl group, where there was a higher percentage of voids in the cervical third when compared to the apical third ($P<0.05$). Regarding the root canal sealer penetration segment, the NaOCl group showed a lower segment when compared to the NaOCl+ST group in the coronal and middle root third ($P<0.05$). No significant differences in the apical third were observed between the groups ($P>0.05$) (Table 2). Intragroup analysis also demonstrated no significant differences between root thirds ($P>0.05$). Representative images of each group can be observed in Figure 1.

Discussion

Interfacial gaps between sealer and root dentin are clinically relevant since a shrinkage of root canal sealer can lead to gaps that are susceptible to bacteria penetration [9]. In the present study, none of the tested irrigation regimes at neither of the root thirds, provided a void and gap-free root canal filling. This finding is in accordance with previous studies [10-12]. However, the percentages of combined gaps and voids in filled canals were significantly higher for the NaOCl group when compared to the NaOCl+ST group all root thirds. Therefore, the first null hypothesis was rejected.

The adequate fillings, without voids, of the prepared root canal space are associated with more successful outcomes of primary endodontic therapies [13]. However, other studies demonstrated that gap-free filling is a challenge independent of obturation technique used [7, 14]. Voids occur at the dentinal wall, when a gap is present at the interface with the root canal sealer, or inside the filling material, by forming unfilled areas between sealer and gutta-percha cone, or within the sealer itself [15]. These areas of voids and gaps may lead to microleakage, from both apical and coronal direction, adversely affecting the success of root canal therapy [16]. In addition, these unfilled areas may contain bacteria, which can multiply when in contact with substrate from the periapical region or lateral canals.

AH-Plus is an epoxy resin-based sealer, and it was used in this study for being considered the gold standard endodontic sealer [17]. Since a previous study demonstrated that the sealer distribution on the root canal walls is not affected by the sealer placement method [18], the sealer insertion as performed by a rotary lentulo spiral, which provided a better standardized protocol. This study used the SC technique, which has been

Table 1. Mean (SD) values of percentage (%) of voids and gaps in the root canal filled teeth

Groups	Overall	Coronal	Middle	Apical
NaOCl	20.7 (15.6)^{A}	31.3 (18.5)^{Aa}	20.3 (12.4)^{Aab}	10.6 (6.7)^{Ab}
NaOCl+ST	4.9 (2.6)^{B}	5.9 (2.9)^{Ba}	4.4 (2.2)^{Ba}	4.5 (2.6)^{Ba}

Different capital letters in a column indicate statistically significant differences between the groups; different lowercase letters in a row indicate statistically significant differences between root thirds within the groups

Table 2. Mean (SD) values of percentage (%) of penetration segment in the root canal filled teeth

Groups	Overall	Coronal	Middle	Apical
NaOCl	52.5 (23.6)^{A}	55.4 (29.9)^{Aa}	55.0 (10.1)^{Aa}	47.9 (38.1)^{Aa}
NaOCl+ST	67.9 (34.6)^{B}	82.6 (21.1)^{Ba}	83.2 (21.8)^{Ba}	46.2 (40.3)^{Aa}

Different capital letters in a column indicate statistically significant differences between the groups; different lowercase letters in a row indicate statistically significant differences between root thirds within the groups reported to prevent leakage as efficiently as lateral compaction techniques [12, 19]. However, it has been demonstrated that the formation of voids and gaps inside irregularly shaped canals is higher when using the SC technique [20].

A thinner layer of sealer provides a lesser probability of the formation of voids, due to the reduction of polymerization shrinkage and solubility of the sealer by fluid infiltration [21]. Thus, the aim of the root canal filling should be to have a high volume of gutta-percha and minimal layer of sealer within the body of the root canal space. However, the use of SC technique is strongly associated with a higher percentage of sealer and lower of gutta-percha, especially in coronal third of oval shaped canals [22], so it may be a limitation of the present study. Therefore, the thicker layer of epoxy resin-based sealers provided by this technique could be more susceptible to the effects of NaOCl on its polymerization [3]. The oxidizing effect of NaOCl irrigation can be neutralized with an antioxidant agent. Since ST is a potent antioxidant, the lesser formation of voids and gaps observed at NaOCl+ST group, in coronal, middle and apical root thirds, compared to the NaOCl group, can be justified by the neutralization of NaOCl on the surface of root dentin promoted by the final rinse with ST. The NaOCl neutralization allows the complete polymerization of resin bonding materials [4].

The NaOCl+ST group provided a higher penetration segment on coronal and middle root thirds, compared to the NaOCl group. Since oxygen molecules interferes with resin infiltration into the tubules and inter-tubular dentin [23], the oxidizing effect of sodium hypochlorite is likely to be responsible for the lack of sealer penetration on dentinal tubules of NaOCl group. The apical root third did not differ between groups, which can be explained by the smaller number of dentinal tubules, their smaller diameter, and tubular obliterations at this region [24]. Furthermore, the higher efficacy of smear layer removal in the coronal and middle regions, and the lower effectiveness on delivery of irrigants to the apical area of the might also have some influence [25].

De-Deus *et al.* [26] reported that there is no correlation between depth of intratubular sealer penetration and sealing ability, and accordingly, this study did not measure the depth of sealer penetration. Instead of this, the percentage of penetration segment was performed as it provides more reliable data. It has been suggested that a high penetration segment combined with antimicrobial sealer properties allows the isolation of remaining microorganisms inside the dentinal tubules and thus prevents microleakage regardless of the depth of penetration [27, 28]. In addition, the mechanical interlocking provided by a high percentage of tubular penetration of the sealer improves the root canal filling retention [29]. The results of the present study demonstrated that the percentage of penetration segment was higher when $Na_2S_2O_3$ was used. Therefore, the second null hypothesis was also rejected.

In the present study, the filling quality was assessed by using CLSM. CLSM presented several advantages over scanning electron microscopy (SEM), such as being a simpler and nondestructive specimen processing, as well as lower potential to produce artefacts [26]. However, identification of artificial gaps created after traditional high-vacuum SEM might be a challenge, unlike images obtained by CLSM [26]. Moreover, the images obtained by CLSM allows a quantitative analysis, unlike the qualitative evaluation by scores commonly used in SEM images, which may provide less reliability [30]. Rhodamine B dye is the standard fluorophore used, and it has been previously demonstrated that your use of in low concentrations such as 0.1% did not affect the sealer properties, especially with epoxy resin-based sealers [6, 31].

Although the lack of a three-dimensional evaluation of the root canal filling might be a limitation of the analysis by CLSM, and micro-computed tomography (micro-CT) evaluations have been indicated as superior to the analysis of sectioned roots by digital imaging software [11], a recent research [32] obtained a higher incidence of voids after evaluation trough stereomicroscopy, when compared to micro-CT. Therefore, it was speculated that micro-CT observations might be less sensitive to voids detection, when compared with microscopic evaluation of sectioned roots. Moreover, Viapiana *et al.* [33] found a weak correlation between the assessment of sealing ability by micro-CT and CLSM, corroborating with the idea that the methods are complementary for the evaluation of root canal filling quality. Thus, this study evaluated the incidence of voids and gaps on the root canal filling trough CLSM, as similar researches previously assessed [6, 10].

Conclusion

Our *in vitro study* showed the benefits of ST after irrigation with NaOCl in endodontic treatment with epoxy resin-based sealers using single-cone technique, by creating less gap and void formation. We advocate further studies in this field.

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Conflict of Interest: 'None declared'.

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