Effect of Superoxided Water and Sodium Hypochlorite, Associated or Not with EDTA, on Organic and Inorganic Components of Bovine Root Dentin

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Abstract

Introduction: This study aimed to evaluate the effects of Sterilox (Sx), a superoxidized water, 5% and 2% sodium hypochlorite (SNaOCl and 2NaOCl), and 17% EDTA (E) on the organic and inorganic components of bovine dentin. Methods: Eighty bovine incisors were randomly divided into 8 groups (n = 10): 5NaOCl, 5NaOCl + E, 2NaOCl, 2NaOCl + E, Sx, Sx + E, E alone, and distilled water (H2O). Root canal instrumentation was performed by using the corresponding irrigant. The apical 15 mm was longitudinally sectioned into 2 fragments, one for light microscopy analysis in slides stained with picrosirius red (organic component) and the other for scanning electron microscopy analysis (inorganic component). Scores data obtained in the light microscopy analysis were submitted to the Kruskal-Wallis test, followed by multiple comparisons test (P < .05). Scanning electron microscopy images were analyzed descriptively. Results: The chemical solution 5NaOCl had a greater effect on the organic component of dentin in area and depth than 2NaOCl. The chemical solutions 5NaOCl + E, 5NaOCl and 2NaOCl + E caused the greatest change in the collagenous organic matrix near the root canal lumen. The chemical solution 2NaOCl showed similar behavior to Sx, associated or not with E, promoting more superficial disorganization of collagen in a smaller area. Demineralization was detected only when it was combined with NaOCl. Conclusions: Five percent NaOCl promoted the most extensive damage to the organic component of dentin, and when associated to EDTA, dentinal erosion could be seen. Considering these specific aspects, 2% NaOCl and Sx had milder effects on bovine root dentin. (J Endod 2015;41:925–930)

Key Words

Collagen matrix, demineralization, dentin, EDTA, SEM, sodium hypochlorite, Sterilox, super-oxidized water

The use of auxiliary chemical substances is essential for endodontic treatment success. However, these substances alter the dentin compounds, mainly collagen, which may cause negative effects on its mechanical properties (1–5). As a consequence, the union of dental materials, especially adhesive systems, to dentin may be compromised, impairing the durability of cemented intracanal posts and restorations, or even dentin fracture strength may be affected (4).

Sodium hypochlorite (NaOCl), a chemical solution widely used in concentrations ranging from 0.5% to 6%, has high antimicrobial activity and is effective in dissolving organic tissues (5). However, it also has well-known cytotoxic and caustic effects (6). Moreover, in vitro studies have demonstrated that highly concentrated NaOCl leads to the degradation of dentin matrix components, especially collagen (7), which may impair the mechanical properties of teeth (2,4), although no long-term clinical studies are available on this subject.

Because of these limitations of NaOCl, alternative root canal irrigants are still being searched for. Sterilox solution, initially used in the disinfection of endoscopes in health care facilities (8, 9), has been suggested for endodontic use. This solution is obtained by the electrolysis of a saline solution in a process similar to that used in the commercial production of NaOCl. It belongs to the group of so-called electrochemically activated waters or superoxidized waters and shows low toxicity (10). Furthermore, it has been shown that Sterilox presents favorable antimicrobial potential (11, 12). Although the literature reveals a promising future for its use in endodontics, some features, such as its effect on dentin compounds, need to be further investigated.

It is also a known fact that chelating agents such as EDTA are important auxiliary substances in root canal cleaning, particularly for removing the smear layer created by chemomechanical preparation (13, 14). However, their demineralizing effect...
promotes changes in the most superficial inorganic layer of dentin as well as in its physicochemical properties (15).

To investigate the effects of auxiliary chemical substances on the organic components of dentin, picrosirius red staining and light microscopy (LM) have been used (16, 17). This methodology allows a better view of collagenous compounds. On the other hand, changes in the inorganic layer of dentinal walls caused by these agents have been analyzed by scanning electron microscopy (SEM) (17).

Thus, the purpose of this study was to evaluate the effect of Sterilox, 5% and 2% NaOCl, associated or not with 17% EDTA, on the collagen matrix and the topography of the inorganic matrix of bovine root dentin.

**Materials and Methods**

Eighty bovine incisors were used in this study. Their crowns and cervical portions were removed, and the root length was standardized at 20 mm. Specimens were stored in distilled water and then divided into 8 groups (n = 10) by using random allocation, with stratification by the initial instrument fitting the canals at full working length (WL): size #40, #50, and #60. The following groups were established, considering the irrigating solutions used during chemomechanical preparation: 5% NaOCl (5NaOCl), 2% NaOCl (2NaOCl), 400 ppm Sterilox (Sx), and 17% EDTA (E) used individually; 5% NaOCl and 17% EDTA (5NaOCl + E); 2% NaOCl and 17% EDTA (2NaOCl + E); 400 ppm Sterilox and 17% EDTA (Sx + E) (groups associated with final irrigation of EDTA for 5 minutes); and distilled water (H₂O) as negative control. NaOCl concentration was determined by iodometric titration, whereas EDTA concentration was established by volumetric analysis. All irrigants were used at room temperature (23°C ± 2°C).

Root canal preparations were performed by a single and calibrated operator. For this purpose, specimens were attached to a fixing device (NBLBC, Neboluz, São Paulo, Brazil). First, 2 ml of the corresponding irrigating solution was used, and the root canal was explored with size #80 K-file (Dentsply Maillefer, Ballaigues, Switzerland). It was inserted until its tip was visible at the apical foramen, and the WL was determined by reducing 1 mm from the total canal length. The root apex was then sealed with utility wax, and the root canal was instrumented by the classic technique (18). The first instrument was the one that fitted the canal walls at full WL (size #40, #50, or #60), and the last instrument was size #80 K-file.

Sterilox solution (Optident Dental, Ilkley, West Yorkshire, UK) was obtained about 15 minutes before use by a double passage of sodium chloride solution into titanium electrodes by using the Sterilox Dental System (Optident Dental). Moreover, NaOCl and EDTA solutions were prepared (CIENTEC-Science and Technology Foundation, Porto Alegre, RS, Brazil) 7 days before use and stored away from light at room temperature.

Irrigating solutions were delivered by 10-ml disposable plastic syringe (BD-Becton Dickinson, São Paulo, SP, Brazil) and a 30-gauge Endo-Eze needle (Ultradent Products Inc, South Jordan, UT). Simultaneously, a no. 20 cannula (Indusbello, Londina, PR, Brazil) was adapted to the root canal entrance for suction. At each instrument change, irrigation/suction was carried out, with 2 ml of the corresponding solution, by using back-and-forth movements. Needle penetration reached up to 3 mm short of the WL to permit irrigating solution reflux. In the combined groups, NaOCl or Sx was suctioned out of the root canal before introducing EDTA to avoid direct mixture of solutions.

The total time for each irrigation protocol was standardized at 30 minutes. In the groups with final irrigation (5NaOCl + E, 2NaOCl + E, and Sx + E), 2 ml EDTA was used and remained for extra 5 minutes in the root canal. At the end, 2 ml NaOCl or Sx was used. All canals were dried with size #80 absorbent paper points (Tanari; Tanariman Industrial Ltda, Manacapuru, AM, Brazil).

After root canal preparation, the apical 15 mm of each sample was sectioned perpendicular to its long axis and then cleaved longitudinally into 2 halves; one was designated for LM analysis (n = 10 per group) and the other for SEM analysis (n = 10 per group).

**LM**

After decalcification, dehydration, and inclusion of the samples, 6-µm-thick slices were obtained and stained with picrosirius red. A representative section of each root canal third was examined under a light microscope ( Olympus BX50; Olympus Optical Co Ltd, Tokyo, Japan) at ×40–×400 magnifications by a calibrated operator (kappa = 0.855) who was blinded to the experimental groups.

The following scores were assigned to the sections, considering the lack of parallel orientation and organization of collagen fibers near the root canal lumen as well as the presence of gaps between them, featuring a discontinuous outline of the root canal perimeter:

1. **Score 0:** Organized collagen fibers, arranged parallel to each other and homogeneous contour of the root canal lumen

2. **Score 1:** Collagen fibers showing an altered pattern with discontinuous and irregular contour of the root canal lumen in up to 25% of the analyzed area

3. **Score 2:** Collagen fibers showing an altered pattern with discontinuous and irregular contour of the root canal lumen in up to 50% of the analyzed area

4. **Score 3:** Collagen fibers showing an altered pattern with discontinuous and irregular contour of the root canal lumen in more than 50% of the analyzed area

5. **Score 4:** Collagen fibers showing an altered pattern with discontinuous and irregular contour of the root canal lumen throughout the analyzed area

The final score of each sample corresponded to the root canal third in which the damage to the organic compound was more expressive. Data were submitted to the Kruskal-Wallis test, followed by multiple comparisons test at 5% significance level.

**SEM**

Samples were fixed in 2.5% glutaraldehyde (Miyako, Guarulhos, SP, Brazil) overnight, mounted on stubs with the root canal portion facing upward, dried, and then sputter coated with 150 A thick gold (BAL-TEC SCD 005; Balzer, Lichtenstein). Topography of the inorganic matrix of the root canal walls was analyzed under a scanning electron microscope (Phillips XL-30, Eindhoven, Netherlands) by using secondary electrons emission. SEM images were evaluated by a single examiner who was blinded to the experimental groups. The areas examined were those corresponding to dentinal tubules of the coronal third, near the root canal lumen, exposed because of the root cleavage, at ×1000 and ×1500 magnification.

The parallelism between dentinal tubules, their obliteration, and the presence of areas of erosion (divergence of tubules orientation near the root canal lumen) were indicated descriptively in each group.

**Results**

The effects caused by irrigating solutions on the organic component of dentin are expressed in Table 1.

In general, higher concentrations of NaOCl had greater effect in area size and depth on the organic component of dentin. Moreover,
its association with EDTA appears to have increased this deleterious effect.

The 5NaOCl + E group caused expressive damage to the collagen layer near the canal lumen, also advancing deep into the root dentin (Fig. 1A and B). This group showed the highest mean rank but did not differ from 5NaOCl and 2NaOCl + E. When 5NaOCl was used (Fig. 1A–C), the damage to collagen was higher than 2NaOCl (Fig. 1D and E), regardless of their association with EDTA.

TABLE 1. Mean Ranks of Effect Caused by Irrigating Solutions on Organic Component of Dentin Close to the Root Canal Lumen

<table>
<thead>
<tr>
<th>Score</th>
<th>5NaOCl + E</th>
<th>5NaOCl</th>
<th>2NaOCl + E</th>
<th>2NaOCl</th>
<th>Sx + E</th>
<th>Sx</th>
<th>E</th>
<th>H2O</th>
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<td>0</td>
<td>0</td>
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<td>2</td>
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<td>6</td>
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<tr>
<td>4</td>
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<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Mean rank</td>
<td>62.1^A</td>
<td>50.4^AB</td>
<td>46.5^ACD</td>
<td>30.9^CE</td>
<td>20.3^EF</td>
<td>34.7^DE</td>
<td>11.0^FG</td>
<td>13.1^FG</td>
</tr>
</tbody>
</table>

Mean ranks followed by distinct uppercase letters differ significantly, according to the nonparametric Kruskal-Wallis test, complemented by multiple comparisons Student-Newman-Keuls test, at 5% significance level.

Figure 1. Histologic sections stained with picrosirius red showing effect of irrigating solutions on organic component of dentin near the root canal lumen. (A) Note distinguished effect of 5NaOCl + E in depth and along the entire root canal perimeter, altering the structural pattern of collagen fibers, including loss of substance. The root canal outline is discontinuous (score 4); (B) magnification of area traced in (A), with disoriented collagen fibers and loss of substance; (C) 5NaOCl with score 3, because damage was detected in more than 50% of the perimeter; (D) 2NaOCl + E, score 3; (E) 2NaOCl, score 3. Note that damage created by 2NaOCl (D and E) was smaller than that caused by 5NaOCl (A–C), regardless of their association with EDTA. Also, in groups where 2NaOCl and Sx (D, E, and G) were used, the damage was more superficial and in a smaller area of the root canal; (F) SX + E, E, and H2O, respectively, score 0 (no damage to collagen fibers and continuous root canal outline). EDTA alone resulted in higher concentration of organic matter close to the canal space (H); (G) Sx with score 1. (A and C–I) Original magnification, ×100; (B) original magnification, ×400.
In the 2NaOCl group, the effect on collagen was statistically similar to that in Sx and Sx + E. Although these substances have caused some alteration, with more superficially disorganized collagen in a small area (Fig. 1E–G), it was significantly lower than that displayed by 5NaOCl, 5NaOCl + E, and 2NaOCl + E.

The isolated use of EDTA and distilled water had no effect on the organic component of dentin (Fig. 1H and I). In the group where EDTA was used alone, a higher concentration of organic matter was observed near the root canal space (Fig. 1H).

EDTA, alone or associated with other solutions, caused areas of erosion (ie, divergence in dentinal tubules orientation, associated with open tubules, without smear layer) (Fig. 2A, C, E, and G).

In 5NaOCl, 2NaOCl, and Sx groups, where EDTA was not applied as a final irrigant, no changes in the dentin morphology were observed. Dentinal tubules were obliterated by smear layer and showed a parallel arrangement (Fig. 2B, D, and F).

Figure 2. SEM images showing effects caused by irrigating solutions on inorganic component of dentin. (A and C) Original magnification, ×1500; 5NaOCl + E and 2NaOCl + E, respectively. Both indicate extensive areas of erosion associated with open dentinal tubules, with divergent arrangement near the root canal lumen (arrows); (E) original magnification, ×1000; Sx + E showing parallelism between tubules (absence of demineralization); (G) original magnification, ×1000; EDTA showing less intense demineralization while maintaining parallelism between tubules; (B, D, F, and H) original magnification, ×1000; 5NaOCl, 2NaOCl, Sx, and distilled water, respectively. No demineralized areas and parallel tubules, obliteration by smear layer (*).
Samples treated with distilled water showed no effect on the inorganic component of dentin (Fig. 2H). However, a massive presence of pulpal debris obliterating the dentinal tubules was observed.

**Discussion**

Although several studies address the properties of chemical substances used during chemomechanical preparation, information about the influence of these substances on the organic component of dentin is scarce (17). This aspect is important because collagen is essential for resin-dentin bonds, considering either restorative materials or endodontic sealers. Bonding efficiency of contemporary adhesive systems to the dentin substrate depends on several factors such as the integrity of dentin collagen. Furthermore, the implications for dental fracture strength need to be further elucidated because the literature reports structural changes caused by NaOCl, which may compromise tooth resistance, depending on certain factors related to its use, especially the concentration (19).

Another implication related to NaOCl use is a decrease in the rigidity of root-treated teeth, which provides a greater tendency to fracture. This probability is clinically relevant because it can lead to tooth loss. However, the most important factors that predispose a tooth to fracture are dental structure loss produced by carious lesions or endodontic access cavities. Probably the interaction of all these factors, cumulatively, influences the occurrence of fracture (2). The harmful effects of NaOCl, such as toxicity (6) and collagen degradation (7), are well-recognized, but some beneficial properties of this substance are still surpassing such as the ability to dissolve organic matter (20) and the antimicrobial potential (12). Although Sx and NaOCl are chlorine-containing solutions, they have different concentrations and pH. The pH is reflective of the chlorine forms available in solution, with higher values containing mostly hypochlorite ion and lower hypochlorous acid (21). The hypochlorite ion is associated with higher proteolytic activity and subsequent increased effect on the dentin matrix (22).

In this context, the present study compared the effects of NaOCl with superoxidized water (Sterlinox), a not yet common irrigating solution. The product was prepared just before use to avoid changes in its properties because the period that it remains stable if stored under ideal conditions is 14 days (23). Similar caution was taken with NaOCl and EDTA solutions.

Bovine incisors were used in this study because of their availability and the similarities between human and bovine dentin, especially regarding the number and diameter of dental tubules (24, 25). Yet bovine teeth, derived from animals of similar genetic lineage and dietary environment, might show higher homogeneity of mineral composition than different human teeth, which are collected from various donators with diverse dietary or fluoride supplementation (26, 27).

The images obtained under LM allowed analyzing the effect of different irrigating solutions on the organic component of dentin because picrosirius red is a specific stain for collagen visualization (17). Major damage on collagen was noticed when 5NaOCl was used (Fig. 1 A–C), regardless of their association with EDTA, if compared with a less concentrated solution, 2NaOCl (Fig. 1D and F). It has been shown that NaOCl dissolves the collagen by breaking down bonds between carbon atoms and disorganizing the protein primary structure (28).

Also, EDTA associated with NaOCl appears to have increased the degradation of collagen. Although no statistically significant difference was observed, average ranks of 5NaOCl + E (Fig. 1A and B) and 2NaOCl + E (Fig. 1D) were higher than 5NaOCl (Fig. 1C) and 2NaOCl (Fig. 1D), respectively. In addition, EDTA combined with NaOCl seems to have caused increased damage in depth. This effect results from the alternating action of NaOCl, which dissolves the organic components, and EDTA, which demineralizes the inorganic components of dentin (29).

On the other hand, when the samples were individually treated with EDTA, no morphologic changes were observed in the organic matrix of dentin. These findings are in accordance with those of Moreira et al (17); however, they are conflicting with other studies (13, 30). These controversial results may be explained by differences in methodology. In addition, the single use of EDTA resulted in greater concentrations of organic matter near the root canal lumen (Fig. 1H), indicated by the higher concentration of stain in that area, demonstrating their inability to degrade organic materials (17). Thus, once again it reinforced the importance of irrigation regimes where different solutions are associated to optimize their properties, so that one potentiates the beneficial effect of the other, with no interference between them, which could have a deleterious effect on the endodontic treatment outcome.

Sx showed a slight effect on the organic component of dentin in some samples (Fig. 1G), whereas in others it demonstrated a normal aspect with parallel collagen fibers and integrity of the root canal perimeter (Fig. 1F). In this regard, Sx proves to be an alternative irrigating solution, especially if the aim is to improve bonding ability of resins materials, because the literature has shown that NaOCl irrigants may impair bonding strength to dentin, depending on some factors such as adhesive system type, concentration, and time of exposure to the proteolytic agent (4, 31).

Regarding SEM analysis, only the coronal third of the root canals was evaluated because it shows higher number and diameter of dentinal tubules compared with the other segments. Moreover, the use of adhesive systems for the cementation of intracanal posts at that location has been a routine procedure in prosthetic rehabilitation of endodontically treated teeth. Photomicrographs were to illustrate morphologic changes in the inorganic matrix of the dentin wall caused by the auxiliary chemical substances.

SEM results revealed that a combination of EDTA and NaOCl causes areas of erosion near the root canal lumen, associated with wide-open tubules (Fig. 2A and B), in agreement with the reports of Baumgartner and Mader (29) and Moreira et al (17). The first investigation suggests that EDTA associated with NaOCl causes a progressive dissolution of dentin at the expense of the peritubular and intertubular dentin. This is due to the alternating action of these substances as mentioned above (29). However, the isolated use of NaOCl and Sx did not cause significant changes in the inorganic matrix, showing the absence of demineralizing action.

According to the results of this study, 5NaOCl acts intensively in the organic component of dentin, not only superficially but also in depth; thus, the pros and cons of its indication as a root canal irrigant should be carefully considered. This action can have a detrimental effect on tooth strength (19) as well as compromise the adhesion of restorative materials (31). One may ponder that the use of a 2% solution would be preferable for clinical use because it aggregates the properties of a relatively concentrated solution with less harmful effects to the periapical tissues and the organic component of dentin. Nevertheless, before condemning the use of 5NaOCl, these in vitro results must be confirmed by long-term clinical evidence.

Finally, the search for safe and efficient irrigation protocols with alternative substances to NaOCl should be encouraged. This oxidizing agent has been used for many decades with vast history of clinical success, and it is uncertain whether we will find a more cost-effective substance. The present results indicate Sterlinox as a potential irrigating solution, but its limitations as to the ineffectiveness in dissolving organic
tissue should be considered (20). It is noteworthy that this finding was observed with the 200-ppm solution, which is less concentrated than the one used herein (400 ppm). Additional research is needed to find the optimal concentration of chlorine and the stability of their properties.

On the basis of the present methodology and results, it is possible to conclude that 5% NaOCl, whether associated or not with 17% EDTA, causes significant changes in dentin collagen. On the other hand, 2% NaOCl has a behavior similar to 400 ppm Sterilox, combined or not with 17% EDTA, promoting more superficial disorganization of collagen in a smaller area when compared with 5% NaOCl. Regarding modifications of inorganic compounds, demineralization areas were detected in all groups in which EDTA was used. However, areas of erosion and open tubules were observed only when it was combined with NaOCl.

Acknowledgments

The authors deny any conflicts of interest related to this study.

References