Penetration into dentin of sodium hypochlorite associated with acid solutions

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Objective. The objective of this study was to evaluate the penetration of 2.5% NaOCl associated with 17.0% EDTA, 1.0% citric acid, and 1.0% peracetic acid into dentin tubules.

Study design. The roots of 44 bovine incisors were cross-sectioned and 5-mm-long fragments were produced from their middle thirds. The specimens were instrumented with ProTaper hand files, stained in crystal violet, then sectioned mesiodistally. The buccal fragments were divided into 4 groups (n = 9) and subjected to 2 consecutive 10-minute immersion periods in one of the following acid solutions combined with 2.5% NaOCl: 17.0% EDTA (group 1), 1.0% citric acid (group 2), and 1.0% peracetic acid (group 3). Nine fragments were immersed in 2.5% NaOCl (group 4). The analysis of the penetration of NaOCl solutions into dentin was performed by measuring the depth of crystal violet stain that was bleached using a stermicroscope under 50 magnification. Statistical comparisons were carried out by Kruskal-Wallis and Dunn’s tests at the 5% significance level.

Results. Group 1 showed less penetration into dentin than group 4 (P < .05). No statistically significant differences were observed among groups 2, 3, and 4 (P > .05).


The success of endodontic therapy depends on many factors, with decrease or elimination of bacterial infection being one of the most important determinants of treatment success. Root canal preparation, which includes instrumentation and irrigation, is unable to completely eliminate endodontic infection, because bacteria can be found not only in the main canal, but also disseminated throughout the root canal system. According to Ando and Hoshino, bacterial penetration depth into the dentin tubules is highly variable, ranging from 150 μm to half the distance between the main root canal and the cementum dentinal junction.

Therefore, penetration depth of irrigants into the dentin tubules is potentially an important factor affecting their effectiveness, and may influence treatment outcome. Very little is known in terms of endodontic irrigant penetration into dentin tubules. It has been demonstrated that temperature, time, and concentration contribute to the penetration of sodium hypochlorite (NaOCl) into the dentin tubules.

NaOCl is the most commonly used root canal irrigant, mainly because of its antimicrobial activity and its soft tissue dissolution capacity. Several variables may interfere with the efficacy of NaOCl, such as concentration, contact time, temperature, and pH. The NaOCl in aqueous solution gives rise to hypochlorous acid (HOCl), which in turn dissociates into H+ and hypochlorite anion (OCl). The antimicrobial activity of NaOCl is intensified by stabilization of the solution in low pH. This can be explained by the greater concentration of HOCl in relation to OCl, which prevails in high pH. HOCl has antimicrobial properties and oxidative capacity stronger than OCl. However, commercially available NaOCl is kept in alkaline solutions.

The pH of NaOCl solutions may be lowered by its association with acids, such as EDTA, citric acid, or acetic acid. Peracetic acid has demonstrated good antimicrobial properties arising from its decomposition into acetic acid and oxygen and may therefore be an alternative for association with NaOCl. Although the combination of NaOCl with acidic solutions may increase its antimicrobial effect and its oxidative capacity, it is unknown whether the combination of NaOCl with acids increases its ability to penetrate into dentin.
The goal of this study was to evaluate the penetration of 2.5% NaOCl into dentin tubules when used in combination with 17.0% trisodium EDTA, 1.0% citric acid, and 1.0% peracetic acid.

MATERIAL AND METHODS

The methodology used for assessing the penetration of NaOCl solutions into dentin was adapted from the stained dentin block model developed by Zou et al.4 Forty-four freshly extracted bovine permanent mandibular incisors stored in a solution of 1% thymol for 1 month at 4°C were selected. The crowns, as well as the coronal and apical root thirds of all teeth were removed. The remaining middle thirds of the roots were processed into 5-mm-long blocks using a slow-speed saw (Isomet 1000, São Paulo, SP, Brazil) under water cooling. To standardize the size and taper of the canals, they were instrumented with ProTaper SX hand files (Dentsply-Maillefer, Ballaigues, Switzerland) until the D16 instrument fit the coronal edge of the specimen. During instrumentation, each dentin block was irrigated with 1 mL of 2.5% NaOCl (Asfer Ind. Quim. Ltda, São Caetano do Sul, SP, Brazil). After preparation, each specimen was immersed in 5 mL of 2.5% NaOCl and placed on a laboratory shaker (Kline NT 51, Novatécnica Equipamentos Laboratoriais, Piracicaba, SP, Brazil) at 40% power for 5 minutes, followed by immersion in 5 mL of 17.0% EDTA (Merck & Co, Darmstadt, Germany) for another 5 minutes. Thereafter, specimens were washed in distilled water and dried with paper towels.

All blocks were immersed in crystal violet (F. Maia Ind Com, Ltda, Cotia, SP, Brazil) at room temperature for 72 hours to stain the dentin. Following that, the specimens were placed in distilled water for 30 minutes. The blocks were then grooved on the mesiodistal surfaces along their entire lengths and split into halves using a surgical chisel. Only the buccal fragments were used, because they were larger than their lingual counterparts. Specimens that showed poor or no penetration of the stain were excluded and replaced.

The fragments were randomly divided into 4 groups (n = 9). Each specimen was placed in a 1.5-mL Eppendorf tube, with care to prevent the root canal side from touching the tube walls. In group 1, the fragments were immersed in 0.5 mL of 17.0% EDTA (Inodon, Porto Alegre, RS, Brazil) immediately combined with 0.5 mL of 2.5% NaOCl, and the tube was sealed for 10 minutes. These procedures were repeated for a total 20 minutes of immersion. The solutions were not agitated. The same procedures were carried out for groups 2 and 3, combining 1.0% citric acid (Arte e Ciência, Araquara, SP, Brazil) and 1.0% peracetic acid (Dinâmica Química Contemporânea, Diadema, SP, Brazil) with 2.5% NaOCl, respectively. In group 4 (control), the fragments were subjected to 2 consecutive 10-minute immersion periods in 2.5% NaOCl. Two stained fragments received no treatment, and 6 fragments were treated in duplicate with the acid solutions alone, serving as controls. All tubes were maintained at 37°C throughout the experimental phase.

After exposure to the experimental solutions, the specimens were washed in distilled water for 1 minute, and the dentin surface of each specimen was sanded to remove a layer of approximately 100 μm by using 1000-grit abrasive paper (3M ESPE, St. Paul, MN) under irrigation with distilled water in a lathe (Panambra, São Paulo, SP, Brazil), aiming to expose the dentin areas affected only by solutions that had penetrated the tubules through the root canal surface. This amount of removed dentin was controlled by measuring the specimens with a digital caliper (Mitutoyo, Tokyo, Japan).

The quantitative analysis of the penetration of the NaOCl solution into dentin was performed by measuring the depth of crystal violet stain that was bleached. Measurements were conducted using the LAS EZ software (Leica Application Suite, Leica Imaging Systems, Ltd., Cambridge, UK) in conjunction with a stereomicroscope (Leica M80; Leica Microsystems Wetzlar, GmbH, Wetzlar, Germany) under ×50 magnification, a digital camera (Leica EC3; Leica Microsystems AG, Heerbrugg, Switzerland), and a computer. For each section, the extension of NaOCl penetration, in micrometers, was outlined and measured in at least 3 different areas, on both sides of the root canal. The mean of the measurements was considered as the final value for each specimen. A skilled observer, blinded to the treatment groups, performed the evaluations.

Statistical analyses were carried out using the GraphPad Prism statistics program (GraphPad Software, Inc., San Diego, CA). Statistical comparisons were performed using Kruskal-Wallis followed by Dunn’s test at a 5% significance level.

RESULTS

The penetration depth values of the solutions in the experimental groups are shown in Table I. The solution containing 17.0% EDTA + 2.5% NaOCl (group 1) penetrated less into dentin than 2.5% NaOCl alone (P < .05). No statistically significant differences (P > .05) were detected between 2.5% NaOCl (group 4) when it was compared with the associations 1.0% citric acid + 2.5% NaOCl (group 2) and 1.0% peracetic acid + 2.5% NaOCl (group 3). Group 3 showed greater penetration depth (P < .05)
bility demonstrated great similarities between bovine within 1 minute. This procedure was performed to lead to total or near-total consumption of chlorine combining NaOCl with acids in certain concentrations may before insertion, because it has been reported that combining NaOCl with acids directly inside the tubes, as opposed to acid solutions was investigated using bovine dentin. The depth of dentin penetration of NaOCl associated with acid solutions did not increase its penetration depth into root dentin.

**DISCUSSION**

Measurement of hypochlorite penetration in dentin cannot be done in an in vivo study because of obvious ethical and practical limitations. Moreover, from the extracted teeth, dentin can be processed into specimens with standardized size, even the root canals can often be standardized better than what is possible to achieve in vivo.

The depth of dentin penetration of NaOCl associated with acid solutions was investigated using bovine dentin, as previous studies focusing on its chemical composition, structure, number of tubuli, and permeability demonstrated great similarities between bovine and human dentin.

According to Zou et al., greater penetration of NaOCl into the tubes is achieved when the solution remains in the root canal for 20 minutes. For this reason, in the present study the total period of contact of the irrigants with the dentin in the root canal was 20 minutes.

The 2.5% NaOCl solution was combined with the acid solutions directly inside the tubes, as opposed to before insertion, because it has been reported that combining NaOCl with acids in certain concentrations may lead to total or near-total consumption of chlorine within 1 minute. This procedure was performed twice because the dissolving capacity of NaOCl solutions is reduced by contact with organic material and because the chlorine is readily consumed, requiring continuous replenishment of fresh solution.

The methodology in this work involves the use of crystal violet to evaluate the penetration depth of NaOCl solutions into dentin. As a powerful oxidizing agent, NaOCl bleached the violet, revealing the normal light color of dentin. The depth of the bleached area can be easily detected. As the NaOCl has to penetrate the dentin to bleach it, consequently the bleached area can be correlated with the depth of sodium hypochlorite activity.

When NaOCl is added to water, it undergoes the following reaction: NaOCl + H₂O → NaOH + HOCl. In aqueous solution, HOCl partially dissociates into the anion OCl⁻:HOCl → H⁺OCl⁻. When the pH of NaOCl solution is reduced by the addition of acidic solutions, there is greater concentration of HOCl. The “available” chlorine is the sum of the HOCl and OCl concentrations in the solution. Available chlorine might be defined as a measurement of oxidizing capacity. HOCl is considered to be a stronger oxidant than OCI.

In the present study, the association of NaOCl with acid solutions did not increase its penetration depth into root dentin.

**Table 1. Extension of the penetration depth (in micrometers) of the solutions into dentin**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median</th>
<th>Q1-Q3</th>
<th>Min-max</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.0% EDTA + 2.5% NaOCl (group 1)</td>
<td>39⁹</td>
<td>36-41</td>
<td>34-52</td>
</tr>
<tr>
<td>1.0% Citric acid + 2.5% NaOCl (group 2)</td>
<td>98bc</td>
<td>67-109</td>
<td>56-143</td>
</tr>
<tr>
<td>1.0% peracetic acid + 2.5% NaOCl (group 3)</td>
<td>222a</td>
<td>198-252</td>
<td>153-345</td>
</tr>
<tr>
<td>2.5% NaOCl (group 4)</td>
<td>107ab</td>
<td>94-120</td>
<td>87-125</td>
</tr>
</tbody>
</table>

Medians with differing superscript letters indicate statistically significant differences.

Q1, first quartile; Q3, third quartile; Min, minimum; max, maximum.
capable of adjusting the solution pH to 5.0 had no effect on the rates of available chlorine. This could explain the lack of increase in the penetration of 1.0% peracetic acid + 2.5% NaOCl in comparison with 2.5% NaOCl alone, and also its greater dentin penetration when compared with 17.0% EDTA + 2.5% NaOCl and 1.0% citric acid + 2.5% NaOCl.

Under the conditions of this study, it was concluded that association with acid solutions did not promote increase in the dentin penetration depth of NaOCl. Further studies should be undertaken using acid solutions in different concentrations, as well as different acid solutions, to evaluate their effect on the penetration of NaOCl into root dentin.

REFERENCES


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