Chemical analysis of the flocculate formed by the association of sodium hypochlorite and chlorhexidine

Melissa Andréia Marchesan, DDS, MSc, a Braulio Pasternak Júnior, DDS, MSc, a Márcia Maisa de Freitas Afonso, MSc, PhD, b Manoel Damião Sousa-Neto, DDS, MSc, PhD, a Cristina Paschoalato, MSc, PhD, b Sao Paulo, Brazil

UNIVERSITY OF RIBEIRÃO PRETO

Objective. The association of chlorhexidine (CHX) and sodium hypochlorite (NaOCl) to enhance their chemical properties during root canal biomechanical preparation can lead to a brown flocculate being formed. Therefore, this study evaluated the metals present in their association by atomic absorption spectrophotometry, and evaluated a possible reagent that could dissolve the flocculate, allowing its clinical use.

Study design. Five concentrations of CHX and NaOCl were mixed (2.5% NaOCl / 0.2% CHX, 2% CHX / 0.5% NaOCl, 2% CHX / 5% NaOCl, 0.2% CHX / 0.5% NaOCl, and 0.2% CHX / 5% NaOCl). Methanol and hexane, acetic acid, and vinegar were evaluated to dissolve the flocculate.

Results. A flocculate was formed with all proportions and concentrations. Spectrophotometry analysis showed the presence of Ca, Fe, and Mg.

Conclusion. It can be concluded that the association proposed led to the formation of brown flocculate regardless of concentration and proportion. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:e103-e105)

Among the auxiliary products used during the biomechanical preparation of root canals, irrigating solutions are vital for the removal of preexisting debris, dentine chips, bacteria, toxic products, and substrates necessary for bacterial growth.1-4

Sodium hypochlorite at different concentrations is the most widely accepted irrigant because of its effective antibacterial action, dissolution of organic materials, clearing, transformation of amines into chloramines, and deodorizing effects;5,6 however, high concentrations are toxic and irritate periapical tissues.7,8 Therefore, other chemical solutions have also been recommended for irrigation. Among these, chlorhexidine digluconate, at concentrations ranging from 0.1% to 2%, shows a large spectrum antibacterial effect, substantivity, and low toxicity, but does not dissolve organic material, which is an important property for adequate root canal therapy.9-12 The antibacterial effect of chlorhexidine is comparable to sodium hypochlorite and is effective against some resistant bacterial strains that lead to treatment failure.13 Some authors report more favorable results with chlorhexidine for disinfecting the root canal system when compared to sodium hypochlorite.14,15 Others, using similar methods, report better results with sodium hypochlorite.3,16,17 A third group of researchers shows similar results when using either product for root canal irrigation.18,19

In light of this lack of agreement, Kuruvilla and Kamath20 proposed the microbiological evaluation of the root canal system after irrigation with sodium hypochlorite and chlorhexidine digluconate alone or associated. They found not only an increase in the antibacterial activity by the association, but also tissue dissolution due to sodium hypochlorite and lower toxicity due to chlorhexidine. According to the authors, these effects can be attributed to the formation of chlorhexidine chloride, which increased the ionization capacity of the chlorhexidine molecule. However, Cathro9 suggests that this association can form a dense brown flocculate, which is difficult to remove from the root canal and could cause darkening of the dental structures.

Thus, this study associated these substances at different concentrations and proportions, trying not to form the flocculate, and evaluated the metals present by atomic absorption spectrophotometry. A possible reagent that could dissolve the flocculate, allowing its clinical use, was also evaluated.

MATERIAL AND METHODS

Chlorhexidine digluconate was prepared at 0.2% and 2.0% and sodium hypochlorite was titrated at 0.5%, 2.5%, and 5.0% at the Dental Research Laboratory, University of Ribeirão Preto (UNAERP).

Kuruvilla and Kamath20 recommend the association of 0.2% chlorhexidine digluconate and 2.5% sodium hypochlorite.
hypochlorite for root canal irrigation. Thus, in the present laboratory study a mixture of 0.2% chlorhexidine digluconate and 2.5% sodium hypochlorite was made at a 1:1 proportion. The formation of a brownish flocculate occurred at the surface. To remove this flocculate from the solution, quantitative filter paper (JP41; JProlab, Sao Jose do Pinhal, PR, Brazil), 12.5 cm in diameter with 28-μm pore, was used.

Subsequently, atomic absorption spectrophotometry (model Analysty 100, ICP-inductively coupled plasma, PerkinElmer, Wellesley, MA) was performed and colorimetric methods were applied. Detection values for different metals were calculated for 2.5% sodium hypochlorite, 0.2% chlorhexidine digluconate, and also for the brownish flocculate that was retained on the filter paper.

Other concentrations and proportions were also associated with trying not to form the flocculate: 2% chlorhexidine + 0.5% sodium hypochlorite, 2% chlorhexidine + 5% sodium hypochlorite, 0.2% chlorhexidine + 0.5% sodium hypochlorite, and 0.2% chlorhexidine + 5% sodium hypochlorite. However, the brownish flocculate was formed at the contact of the first drop of the solutions regardless of the concentration or proportion.

Therefore, a third substance was added to this association to dissolve the flocculate: methanol (Merck, Darmstadt, Germany) or hexane (Merck) because they are universal solvents (“similar dissolves similar”) for polar and nonpolar substances, respectively. Because only methanol dissolved the flocculate and cannot be used in vivo, pure acetic acid (PA) (Merck), which has low polarity, was used. As expected, it also dissolved the flocculate. Therefore, common vinegar (acetic acid) was used at 0.1 mol/L because of its low toxicity and biocompatibility.

RESULTS
Results of the concentrations of the metals analyzed are reported in Table I.

Pure acetic acid dissolved the flocculate; however, when the lower concentration of 0.1 mol/L was used, the flocculate was dissolved but the brown coloring of the solution continued.

DISCUSSION
Different solutions are associated and mixed during root canal biomechanical preparation to enhance the chemical properties that each solution presents. In endodontics, the possibility of preparing mixtures and associations has been consecrated by many researchers. Although Kuruvilla and Kamath recommend the association of sodium hypochlorite and chlorhexidine for root canal irrigation, the physico-chemical properties involved are still not clear.

Table I. Metals found by atomic absorption spectrophotometry reported in mg/L.*

<table>
<thead>
<tr>
<th>Metals</th>
<th>Chlorhexidine</th>
<th>Sodium hypochlorite</th>
<th>Flocculate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt;0.001</td>
<td>0.42000</td>
<td>0.09900</td>
</tr>
<tr>
<td>Zn</td>
<td>0.00120</td>
<td>0.10600</td>
<td>0.02300</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>K</td>
<td>&lt;0.001</td>
<td>8.56000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fe</td>
<td>0.05800</td>
<td>0.67800</td>
<td>0.10030</td>
</tr>
<tr>
<td>Mn</td>
<td>0.00900</td>
<td>0.08300</td>
<td>0.01900</td>
</tr>
<tr>
<td>Mg</td>
<td>0.05600</td>
<td>10.25300</td>
<td>0.18300</td>
</tr>
<tr>
<td>Ca</td>
<td>0.12300</td>
<td>25.01230</td>
<td>0.42300</td>
</tr>
<tr>
<td>Ag</td>
<td>&lt;0.001</td>
<td>0.02700</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Values <0.001 indicate no significant expression.

In the present study, we examined only the flocculate with spectrophotometry and not the entire solution of the association of 0.2% chlorhexidine and 2.5% sodium hypochlorite because, at a proportion of 1:1, the resulting filtrate flocculated again with the addition of chlorhexidine. This leads us to suggest that the filtrate was sodium hypochlorite.

In other dental specialties, such as periodontology and preventive dentistry, there are many reports on staining of enamel or restorations in patients using chlorhexidine to control dental plaque, because of the interaction with dentifrices or chromogens contained in foods and beverages. These studies showed that because of the cationic nature of chlorhexidine, it cannot be associated to anionic substances, anionic detergents, phosphates, or sulfates and has competitive inhibition with calcium. Therefore, the association of chlorhexidine and sodium hypochlorite is also contraindicated because sodium hypochlorite tends to be an anionic substance and showed a relatively high quantity of calcium by the atomic absorption spectrophotometric analysis.

Also based on the spectrophotometric analysis, 0.678 mg/L iron was found in sodium hypochlorite. These data are in accordance with the Cumulative Exposure Project of the U.S. Environmental Protection Agency of acceptable percentage of mass in solutions of sodium hypochlorite (3.0 mg/L). However, iron leads to the formation of iron salts, which are soluble in water, easily oxidized, and form iron hydroxides, which tend to flocculate, leading to a brown solution. These flocculations were seen in the mixture of sodium hypochlorite and chlorhexidine.

Additionally, Barkvoll et al. reported incompatibility of sodium monofluorophosphate, a constituent of toothpaste, and chlorhexidine through the formation and precipitation of insoluble salts in vitro. This was
also found in the present study when adding salt (NaCl) to chlorhexidine, which formed a white precipitate.

In preventive dentistry and periodontology, many studies have been carried out in an attempt to associate chlorhexidine and toothpastes.26,27 Therefore, despite all the evidence contraindicating this association in endodontics, a substance that could dissolve the flocculation was used because of the benefits of this association.20 Four solutions were added separately attempting to dissolve the flocculate. Methanol was used because it is a universal solution to determine if the product is polar, and hexane was used because it is also a universal solution to determine if the product is non-polar. Because these 2 solutions are toxic in vivo and methanol dissolved the flocculate, pure acetic acid, which has low polarity, was used. It dissolved the flocculate as expected. Thus, common vinegar (acetic acid), which has low toxicity and biocompatibility, was used at 0.1 mol/L. Vinegar dissolved the flocculate; however, the brown color continued in the solution. This could contraindicate its clinical use because the resulting solution could penetrate the canaliculi and could not be dissolved by the nontoxic solvent used in this study, compromising one of the major objectives of modern dentistry: aesthetics.

REFERENCES


Reprint requests:
Manoel D. Sousa-Neto, DDS, MSc, PhD
Rua Célia de Oliveira Meireles 350 Jd Canadá
14024-070, Ribeirão Preto
SP, Brazil
sousanet@unaerp.br