

Effect of the Combination of Sodium Hypochlorite and Chlorhexidine on Dentinal Permeability and Scanning Electron Microscopy Precipitate Observation

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Abstract

Introduction: This study compared the combined use of sodium hypochlorite (NaOCl) and chlorhexidine (CHX) with citric acid and CHX on dentinal permeability and precipitate formation. **Methods:** Thirty-four upper anterior teeth were prepared by rotary instrumentation and NaOCl. The root canal surfaces were conditioned for smear layer removal using 15% citric acid solution under ultrasonic activation and a final wash with distilled water. All teeth were dried, and 30 specimens were randomly divided into three equal groups as follows: positive control group (PC), no irrigation; 15% citric acid + 2% CHX group (CA + CHX); and 1% NaOCl + 2% CHX group (NaOCl + CHX). All roots were immersed in a 0.2% Rhodamine B solution for 24 hours. One-millimeter-thick slices from the cementum-enamel junction were scanned at 400 dpi and analyzed using the software ImageLab (LIDO-USP, Sao Paulo, Brazil) for the assessment of leakage in percentage. For scanning electron microscopy analysis, four teeth, irrigated for NaOCl + CHX samples, were split in half, and each third was evaluated at 1,000 \times and 5,000 \times (at the precipitate). **Results:** Using the analysis of variance test followed by the Bonferroni comparison method, no statistical differences between groups were found when analyzed at the cervical and medium thirds. At the apical third, differences between the PC and NaOCl + CHX ($p < 0.05$) and CA + CHX and NaOCl + CHX could be seen ($p < 0.05$). **Conclusion:** The combination of 1% NaOCl and 2% CHX solutions results in the formation of a flocculate precipitate that acts as a chemical smear layer reducing the dentinal permeability in the apical third. (*J Endod* 2010;36:847–850)

Key Words

Chlorhexidine, dentin permeability, SEM, sodium hypochlorite

The ability to effectively clean the endodontic space is dependent on both instrumentation and irrigation via chemomechanical means (1–3). Irrigants used during cleaning and shaping of the root canal system play an essential role in the successful debridement and disinfection (4–9).

Sodium hypochlorite (NaOCl) in a concentration range from 0.5% to 5.25% possesses many important properties including the ability to be an effective organic solvent and antimicrobial agent (2). However, in low concentrations, it is ineffective against specific microorganisms, and in high concentrations it has low biocompatibility causing periapical inflammation. Its use during chemomechanical debridement causes the formation of a smear layer adhered to the dentinal wall (7, 10, 11). The use of a demineralizing solution is desirable to remove this layer and promote an increase in the dentinal permeability, thus improving the seal of endodontic fillings (4, 5, 7–9).

Chlorhexidine gluconate (CHX) is a cationic bisbiguanide with a broad-spectrum antimicrobial action that acts by absorbing onto microbial cell walls or disrupting them, causing leakage of intracellular components (12). *In vitro* studies have shown CHX to exhibit substantivity in the root canal for some time after being used as an endodontic irrigant solution (2, 12–15). However, it has got no tissue dissolution ability (16).

Both NaOCl and CHX have limitations and, although they have reported good antimicrobial effects, they are limited as to the removal of bacterial LPS (17). A combination of NaOCl and CHX has been recommended to enhance their properties, suggesting that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination is better than that either solution alone (18). An irrigation regimen using NaOCl together with CHX and EDTA has been proposed (9). However, this association forms a dense precipitate (9, 19–24).

The purpose of this study was to evaluate the combined use of NaOCl together with CHX compared with citric acid with CHX on dentinal permeability, testing the hypothesis that the precipitate from the association of NaOCl with CHX could affect their outcome. Additionally, this precipitate formation was observed under scanning electron microscopy (SEM).

Material and Methods

Thirty-four recently extracted upper anterior teeth without caries or extensive restorations were selected and stored in 0.2% thymol solution until use. Roots were accessed, and a size 15 K-type file was inserted until it could be seen at the apical foramen. Then, 1 mm was subtracted, which determined the working length for each specimen.

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TABLE 1. Dye Penetration Means (%) in the Sliced Dentin

Treatment	Mean dye penetration (%)		
	Apical third	Medium third	Cervical third
PC	40.18 ± 18.75	66.56 ± 14.10	80.98 ± 8.24
CA + CHX	38.83 ± 24.20	68.95 ± 10.61	79.63 ± 13.05
NaOCl + CHX	16.59 ± 10.14	54.74 ± 20.44	72.90 ± 13.42

All teeth were prepared by rotary instrumentation with K3 taper 0.06 instruments (SybronEndo, Orange, CA) until an apical stop corresponding to an ISO size 45 file was obtained. Irrigation was performed using 5 mL of 1% sodium hypochlorite solution (Phytogalen GMA Ltda, Sao Paulo, SP) between each instrument. The syringe with the solution was added a 25-mm 29Ga NaviTip (Ultradent Prod Inc, South Jordan, UT) positioned 1 mm short of the working length. The solution was delivered with in-out motion and concomitant aspiration using vacuum and a Withe Mac Tip (Ultradent Prod Inc) positioned at the pulp chamber. Apical patency with the use of an ISO size 25 K-file (SybronEndo) was performed to standardize the apical foramen diameter.

The root canal surfaces were conditioned for smear layer removal using 15% citric acid solution under ultrasonic activation during 5 minutes in a BioSonic UC50 unit (Coltène/Whaledent Inc, Cuyahoga Falls, OH) followed by a final flush with distilled water to remove any trace of the demineralizing solution. All teeth were dried, externally coated with fast polymerizing epoxy resin (Brascola Ltda, Sao Bernardo do Campo, SP). For dentin permeability analysis, 30 specimens were randomly divided into three equal groups according to the irrigation sequence and using the previously mentioned solution delivery

protocol: (1) positive control group (PC), no irrigation regimen was performed; (2) citric acid and chlorhexidine group (CA + CHX), irrigation with 10 mL of 15% citric acid solution followed by 10 mL of 2% chlorhexidine gluconate solution; and (3) sodium hypochlorite and chlorhexidine group (NaOCl + CHX), irrigation with 10 mL of 1% NaOCl solution followed by 10 mL of 2% chlorhexidine gluconate solution.

After irrigation, all roots were immersed in 0.2% Rhodamine B solution for 24 hours. Afterwards, specimens were rinsed continuously under tap water during 24 hours.

The epoxy resin coatings were removed with a sharp blade, and the teeth were embedded in a polyester resin (Redelease Ltda, Sao Paulo, Brazil). This allowed 1-mm thick horizontal sectioning of teeth starting from the cementum-enamel junction using a low-speed water-cooled circular saw (Labcut 1010; Extac Corp, Enfield, CT). The cuts were polished (Politriz; Struers A/S, Ballerup, Denmark) using #600 and #1000 grit wet silicon carbide papers to obtain a flat surface.

One slice from each third was randomly selected, preferably from the third's middle portion, and scanned using an Epson Perfection 1240U scanner (Epson Corp, Nagano, Japan) with resolution at 400 dpi and analyzed using the software ImageLab (LIDO-USP, Sao Paulo, Brazil), which allowed a standard quantitative assessment of mean leakage in percentage.

Statistical analysis was performed with a one-way analysis of variance test followed by the Bonferroni comparison method at $\alpha = 0.05$. Four teeth, irrigated as for the NaOCl + CHX group, were dried and split in halves using a chisel to expose the root canal. After gold sputtering, SEM analysis was performed. In each hemisection, along to the medium line, three points (3, 6, and 9 mm from the apex) of each third were evaluated at 1,000 \times , and, when precipitate was observed, a 5,000 \times magnification was made. Only two specimens were evaluated at 25,000 \times magnification to enhance this characterization.

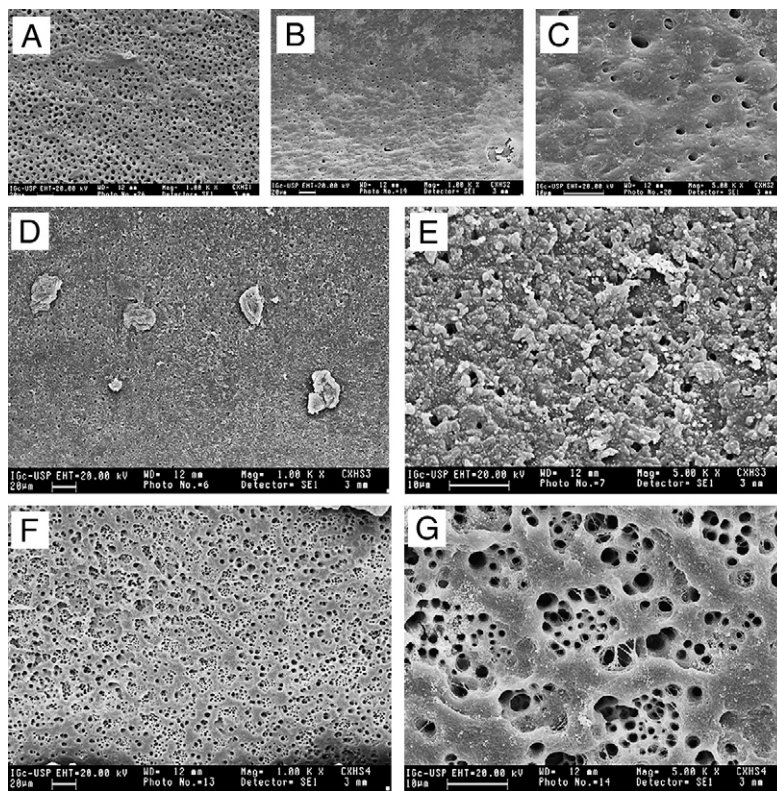


Figure 1. SEM images of the cervical third of samples irrigated with NaOCl and CHX; A and E were magnified at 1,000 \times ; B, C, D, and F at 5,000 \times ; and G at 25,000 \times .

formation of a neutral and insoluble substance referred as the precipitate (24).

When mixing 2% CHX with various dilutions of NaOCl, it was shown that the solution color changes directly according with the concentration of sodium hypochlorite, ranging from peach to dark brown as the concentration increases from 0.023% to 6%. The lowest concentration of NaOCl to induce a precipitate was 0.19% (24). Our study showed precipitate formation immediately when mixing 2% CHX with 1% NaOCl, characterized as a brown mass suspended in the liquid, similarly to previous studies (20, 23, 24).

Sodium hypochlorite at 1% concentration is a commonly used substance because it is stable and imparts little tissue response under clinical conditions. The minimum inhibitory concentration is 0.1%, but stability and speed of action are issues that interfere for the choice of higher concentrations. The more concentrated solution results in more fragile dentin (26). The debate as to the best concentration for root canal cleaning is still vivid and far from being finished. The SEM images were similar to recent studies that showed a precipitate coating the root surface and obliterating the dentinal tubules when NaOCl associated with CHX was used (23).

This precipitate acts as a chemical smear layer and could compromise dentin permeability, the intracanal medication diffusion, and the obturation sealing (21). Our study, in accordance with a previous study (24), showed it is prudent to minimize its formation by washing away the remaining NaOCl with a demineralizing solution. The use of the 15% citric acid considered its demineralizing capacity (5, 6, 8, 27) and adequate biocompatibility (27). However, when mixing citric acid with CHX, a white “milky” solution forms immediately, but this solution returns to a colorless aspect and is easily removed from the root canal and during irrigation with CHX. When analyzing the dentinal permeability, comparing irrigation of 15% citric acid solution followed by 2% CHX solution against the positive control group with opened dentinal tubules, we could not find significant statistical differences ($p > 0.05$) at all thirds. The combination of 1% NaOCl and 2% CHX solutions resulted in the formation of a flocculate precipitate that acted as a chemical smear layer reducing the dentinal permeability in the apical third ($p < 0.05$). Notwithstanding the results reported here, further studies should be conducted to evaluate the influence of precipitate formation in the permeability and leakage when used with other irrigating solutions.

Conclusion

The combination of 1% NaOCl and 2% CHX solutions resulted in the formation of a flocculate precipitate acting as a chemical smear layer that reduced dentinal permeability in the apical third.

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