Evaluation of the Interaction between Sodium Hypochlorite and Chlorhexidine Gluconate and its Effect on Root Dentin

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Abstract

The combination of sodium hypochlorite (NaOCl) and chlorhexidine (CHX) forms a precipitate. The aim of this study was to evaluate the effect of irrigating root canals with a combination of NaOCl and CHX on root dentin and dentinal tubules by using the environmental scanning electron microscope (ESEM) and a computer program (Photoshop CS2). Forty-four extracted single-rooted human teeth were instrumented and irrigated with both NaOCl and CHX to produce a precipitate. Root canal surfaces were analyzed with the ESEM. The amount of remaining debris and number of patent tubules were determined. There were no significant differences in remaining debris between the negative control group and the experimental groups. There were significantly fewer patent tubules in the experimental groups when compared with the negative control group. The NaOCl/CHX precipitate tends to occlude the dentinal tubules. Until this precipitate is studied further, caution should be exercised when irrigating with NaOCl and CHX. *(J Endod 2008;34:181–185)*

Key Words

Chlorhexidine, interaction of irrigants, precipitate, sodium hypochlorite

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It has been demonstrated that bacteria are the etiologic agents of pulpal necrosis and apical periodontitis (1). Because of the complexity of the root canal system, mechanical instrumentation cannot adequately remove bacteria and tissue from all root canal surfaces (2). In addition, mechanical instrumentation forms a smear layer on the canal surface (3). Thus, irrigation is required to remove debris, tissue remnants, microbes, and the smear layer.

A combination of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) is an effective combination for removing both tissue and smear layer (3, 4). NaOCl is effective at dissolving organic tissue and is antimicrobial (5, 6). However, there is a safety concern if NaOCl is extruded out of the root canal apex and into the periapical tissues, resulting in destructive tissue damage (7). NaOCl also corrodes and weakens endodontic instruments and has a disagreeable odor (8). Thus, alternative irrigants such as 2% chlorhexidine gluconate (CHX) have been considered (9).

CHX is a broad-spectrum antimicrobial agent that disrupts the membranes of microbes (10). It has been suggested for use as an irrigant and intracanal medicament because of its lower toxicity when compared with NaOCl (11). CHX has comparable antibacterial efficacy to NaOCl (12) and has the advantage of having substantivity (13). However, CHX lacks the tissue dissolution capabilities of NaOCl. Hence, it has been suggested that CHX not be a replacement irrigant of NaOCl but a supplemental final irrigation step after NaOCl and EDTA irrigation (14). A concern about this irrigation regimen is that with the presence of NaOCl in the root canal, irrigation with CHX produces an orange-brown precipitate (9). It has also been demonstrated that mixing EDTA with CHX also creates a precipitate that is pink in color (15). Basrani et al. (16) used x-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (TOF-SIMS) to identify this precipitate. The precipitate contains a significant amount of parachloroaniline (PCA); a hydrolysis product of CHX (16). PCA can further degrade to 1-chloro-4-nitrobenzene (17). Even without the presence of NaOCl, CHX might spontaneously hydrolyze into PCA in the presence of heat and light (18). Parachloroaniline has industrial uses in pesticides and dyes (19) and has been demonstrated to be carcinogenic in animals (18, 20). Its degradation product, 1-chloro-4-nitrobenzene, is also a carcinogen (21). Currently, there are no studies presenting the effects of the precipitate on the root canal surface. The amount of precipitate left behind is unclear. A concern is that this precipitate might attach to the root surface and slowly leach into the periapical tissues. Also, the presence of this precipitate on the root surface might affect the seal of an obturated root canal, especially with resin sealers in which a hybrid layer is required (9). The aim of this study was to evaluate the effect of irrigating root canals with a combination of NaOCl and CHX on root dentin surface and dentinal tubules by using the environmental scanning electron microscope (ESEM) and Photoshop CS2 (Adobe Systems Incorporated, San Jose, CA).

Materials and Methods

Forty-four single-rooted, extracted human teeth were used for this study. Calculus, bone, and residual soft tissue were removed from the teeth. The teeth were stored in refrigerated 0.9% saline and used within 1 month (Baxter Health Care Corp, Round Lake, IL).

All teeth were instrumented as follows. Occlusal access was prepared by using a high-speed #2 round bur. Coronal flaring was achieved by using #2, 3, and 4 Gates

Glidden drills. With the aid of a surgical operating microscope, working length was determined with a #10 K-file introduced into the canal until the tip of the file was visible at the apical foramen. A glide path was established by using #15 and #20 K-files.

A fresh mix of Aquasil vinyl polysiloxane impression material (Dentsply/Caulk, Milford, DE) was used to fill a small glass vial (VWR Scientific, West Chester, PA). The root end of the prepared tooth was inserted into the impression material and allowed to set. This prevented extrusion of irrigant out of the apex and allowed ease of handling during instrumentation. A rotary engine (Aseptico Inc, Dentsply, Woodinville, WA) with a 1:8 gear reduction handpiece was used for instrumentation of the root canals. The handpiece was set at 300 rpm and torque level two, which is within the range recommended by the rotary file manufacturer (Tulsa Dental, Dentsply, Tulsa, OK). Canal enlargement was performed with Profile NiTi rotary files (Tulsa Dental, Dentsply) to a size 40/.06 in a crown-down manner. The apical matrix was further enlarged to a 60/.04 by using Profile NiTi hand files (Tulsa Dental, Dentsply). Needle irrigation 1 mm short of the working length was delivered with a Monojet syringe (Sherwood Medical Co, St Louis, MO) and a 27-gauge needle. Irrigation with a total of 5 mL of 5.25% NaOCl was performed between instrument changes. A #15 K-file was intermittently used to maintain working length. After instrumentation, the teeth were irrigated with 5 mL of buffered (pH 7.4) 15% EDTA (Sigma-Aldrich Inc, St Louis, MO) and then randomly divided into 2 experimental groups (A and B) of 15 teeth and 2 control groups (C and D) of 7 teeth each.

Group A

From our pilot study, to clinically simulate the maximum amount of precipitate formation, 5 mL of 5.25% NaOCl was used to irrigate, and the canal was left filled with NaOCl. Then 5 mL 2% CHX was used as a final irrigant followed by immediately drying of the canal with paper points.

Group B

Similarly from our pilot study, to clinically simulate a minimum amount of precipitate, 5 mL of 5.25% NaOCl was used to irrigate and then aspirated from canal. The canal was immediately dried with paper points to remove excess NaOCl. A final irrigation with 5 mL 2% CHX was followed by immediate drying with paper points. Using additional irrigants to further remove NaOCl was not done to avoid other possible interactions with CHX.

Group C (Negative Control)

A final irrigation with 5 mL 5.25% NaOCl was performed. The canal was aspirated and dried with paper points. From our pilot study, this standard root canal irrigation regimen left the tooth with minimal debris and patent tubules.

Group D (Positive Control)

A final irrigation of 5 mL 5.25% NaOCl was performed. The canal was left flooded and allowed to air dry under cover at room temperature. From our pilot study, crystalline debris was found under the SEM when a canal was flooded with NaOCl and left to dry.

A group with only CHX as an irrigant was not included because it does not have the ability to dissolve tissue remnants.

To prepare the samples for imaging, they were placed in individual Petri dishes and allowed to air dry at room temperature. A small piece of sponge was placed deep into the access opening to avoid introduction of debris into the canal during processing. A #257 high-speed bur was used to decoronate the teeth. A #168-L high-speed bur was used to cut a thin longitudinal slot along the buccal and lingual aspect of the root, avoiding perforating into the canal. By using a wire cutter with gentle pressure, the roots were split longitudinally. One half of the split root was randomly selected for imaging. To keep the imaging procedure blinded, samples were randomized, and a key was constructed to decode the samples for analysis.

TABLE 1. Results of Percentage Debris Remaining

Root location	Mean ± SD			
	Group A (maximum precipitate produced)	Group B (minimum precipitate produced)	Group C (negative control)	Group D (positive control)
Coronal third	7.91 ± 19.34	2.67 ± 4.20	1.02 ± 1.36	53.13 ± 33.84
	└	P < 0.0001		┘
		└	P < 0.0001	┘
			└ P < 0.0001	┘
Middle third	6.10 ± 9.43	2.72 ± 4.12	0.82 ± 0.80	47.41 ± 22.94
	└	P < 0.0001		┘
		└	P < 0.0001	┘
			└ P < 0.0001	┘
Apical third	8.72 ± 14.13	7.81 ± 11.70	2.83 ± 2.95	49.99 ± 32.60
	└	P < 0.0001		┘
		└	P < 0.0001	┘
			└ P < 0.0001	┘

SD, standard deviation.

Statistically significant differences are indicated with *P* values.

TABLE 2. Results of Number of Patent Tubules per 4843 μm^2 (SEM image area at 4000×)

Root location	Mean ± SD			
	Group A (maximum precipitate produced)	Group B (minimum precipitate produced)	Group C (negative control)	Group D (positive control)
Coronal third	102.33 ± 41.40	87.93 ± 54.14	151.00 ± 29.16	44.29 ± 45.58
		P < 0.05		
			P < 0.05	
			P < 0.05	
		P < 0.05		P < 0.05
Middle third	15.93 ± 19.40	39.60 ± 38.83	65.71 ± 17.71	24.57 ± 20.85
	P < 0.05			
		P < 0.05		
		P < 0.05		P < 0.05
Apical third	16.80 ± 21.82	11.20 ± 16.47	21.29 ± 16.79	16.57 ± 15.99

SD, standard deviation.

Statistically significant differences are indicated with *P* values.

Root samples were imaged at the coronal, middle, and apical root third levels by using an ESEM (FEI Quanta 200, Hillsboro, OR) at a stage height approximately 10 mm, 15 kV, and magnification of 4000×. The images were saved in TIFF format and were used for subsequent analysis. The ESEM does not require the samples to be sputter-coated. This reduces the possibility of artifacts. At 4000× magnification, representative areas for each third of the root canal were chosen for analysis.

To study effects of the treatments on the root canal surface, we used the TIFF format images to quantify gross surface debris by using a computer program (Adobe Photoshop CS2; Adobe Systems) to calculate the number of debris pixels in each image. The magnetic lasso tool was used to outline and select areas of debris. A pixel count of the outlined debris was determined and was divided by the total pixel count for each image to determine the percentage of debris remaining. For the analysis of patent dentinal tubules, the total number of visually patent tubules in each image was manually counted. The total area of the image was 4843 μm^2.

Statistical analysis was performed with analysis of variance (ANOVA) followed by Fisher probable least-squares difference test (PLSD) at a significance level of $P = .05$.

Results

Table 1 summarizes the results of the percentage debris remaining, and Table 2 summarizes the results of the number of patent tubules per 4843 μm^2. Fig. 1 presents representative SEM images of the experiment.

The positive control group had more remaining debris in all thirds of root canals when compared with the negative control group, and this was statistically significant. There was no significant difference between the experimental groups and the negative control group at all thirds of the root canal. Furthermore, the experimental groups had statistically less debris when compared with the positive control group. When experimental groups A and B were compared with each other, no statistically significant differences were found.

The number of patent tubules per 4843 μm^2 was lower in the positive control group when compared with the negative control group at each root third level. This was statistically significant except at the apical level. At the coronal third level, experimental groups were not statistically different. At this same level, experimental groups had significantly more patent tubules than the positive control group and significantly less patent tubules than the negative control group. At the middle third, the negative control group had significantly more patent tubules when compared with both experimental groups. At this same level, there were no significant differences between the experimental groups and the positive control group. However, group B had statistically more patent tubules when compared with group A. At the apical third level, more patent tubules were found in the negative control group; however, no groups were statistically different.

Discussion

From this study, we found that when NaOCl was aspirated and dried with paper points, and even when it was left flooded in the root canal, its interaction with CHX did not leave behind a significant amount of gross precipitate on the root canal surface. There were no significant differences in the percentage of remaining debris between the groups. However, the interaction between CHX and NaOCl significantly affected the patency of the dentinal tubules. There was a statistically significant reduction in the number of patent dentinal tubules in the 2 experimental groups when compared with the negative control group. Removing NaOCl by aspiration and paper points showed no significant reduction to this affect. Apparently, the dentin and its tubules harbor enough residual NaOCl that it reacts with the CHX in the canal. This indicates that a small amount of the precipitate is left behind and raises potential concerns with respect to leaching of the precipitate into the surrounding tissues and the seal of the root canal.

The obliteration of dentinal tubules was not found to be significant at the apical third. There were no significant differences between all experimental and control groups. This might be due to the fact that the apical third is more difficult to irrigate (5). Results at the apex might have been different if irrigation was supplemented with sonic, ultrasonic, or negative pressure irrigation. Because the coronal and middle thirds are significantly affected, these results remain a concern.

Examination of the ESEM micrographs revealed a subjective change in the morphology of the root surface (Fig. 1). The use of NaOCl and CHX appears to coat the root surface. The substance coating the root surface and obliterating the dentinal tubules was not identified. We

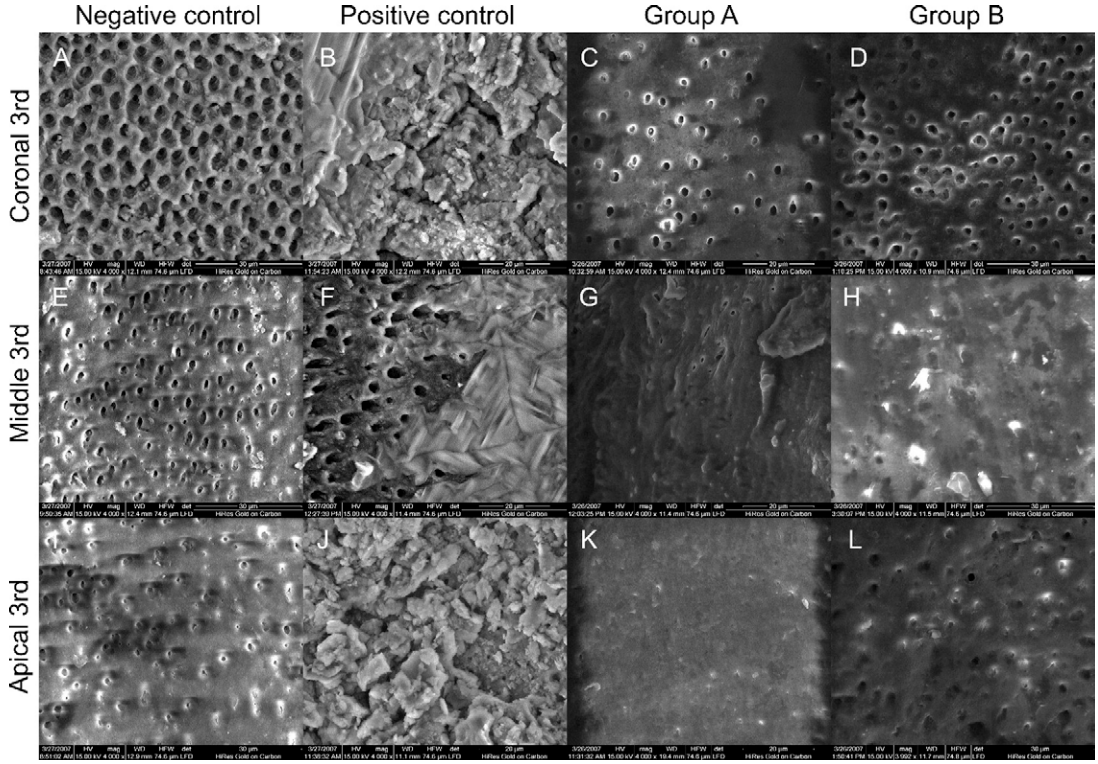

Figure 1. Representative SEM micrographs of root surfaces at 4000×. Negative control group shows no obvious debris and complete removal of smear layer. Presence and diameter of patent dentinal tubules decline in number from the coronal to the apical third. Positive control shows gross amounts of debris that obscure the dentinal tubules in all root thirds. Experimental groups do not show any obvious debris. However, the dentinal tubules appear obliterated especially in the middle third. Subjectively, the experimental groups' root surfaces appear to be coated with unidentified material.

speculate that it was likely the precipitate because the root surface was faintly stained on visual inspection. A future study would be to characterize the substance on the root surface by using a back-scatter sensor on the ESEM. These findings correlate to another study in which a visible color change on the root surface was found along with increased dye leakage results (9). This might affect obturation systems such as Resilon (Pentron Clinical Technologies, Wallingford, CT) in which a dentin hybrid layer is required for proper sealing. However, one Resilon study demonstrated no significant differences in fluid filtration leakage when CHX was used as an irrigant (22).

It is known that PCA and its degradation product are toxic and carcinogenic (18, 21). It has been demonstrated that PCA is produced when NaOCl interacts with CHX (16). Our study demonstrated that such a precipitate on the root canal surfaces and in the dentinal tubules is likely PCA. This raises concerns about potential carcinogens leaching into surrounding tissues.

A final irrigation with water or alcohol to remove residual NaOCl could be used. Using EDTA alone would be convenient; however, it also produces a precipitate in the presence of CHX (15). A future study would be to characterize the interaction between EDTA and CHX. A lower concentration of NaOCl produces proportionately less precipitate, but this strategy is of concern because it reduces its therapeutic effectiveness (16).

In summary, the presence of NaOCl in the root canal before CHX irrigation does not leave behind statistically significant precipitate on the canal walls. However, this reaction coats the canal surface and significantly occludes the dentinal tubules in the coronal and middle thirds of the canal. Because the reaction between NaOCl and CHX produces a carcinogenic product, potential leaching of PCA into the surrounding tissues is a concern. An additional interface between the sealer and the dentin can also affect the seal of the root canal. Further research is needed to determine the nature of the substance occluding the dentinal tubules.

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