Interaction between Sodium Hypochlorite and Chlorhexidine Gluconate

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Abstract

The combination of sodium hypochlorite (NaOCl) and chlorhexidine (CHX) results in the formation of a precipitate. The aim of this study was to determine the minimum concentration of NaOCl required to form a precipitate with 2.0% CHX. This was accomplished with a serial dilution technique. X-ray photon spectroscopy (XPS) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) were used to qualify and quantify the precipitate. A color change and precipitate were induced in 2.0% CHX by 0.023 % and 0.19 % NaOCl, respectively. Both XPS and TOF-SIMS showed the presence of para-chloroaniline in an amount directly related to the concentration of NaOCl used. Until this precipitate is studied further, its formation should be avoided by removing the NaOCl before placing CHX into the canal. *(J Endod 2007;33:966–969)*

Key Words

Chlorhexidine, interaction, precipitate, sodium hypochlorite.

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Bacteria in the root canal system can initiate and cause periapical inflammatory lesions (1). The aim of root canal treatment is to eliminate bacteria from the infected root canal and to prevent reinfection. Biomechanical cleaning and shaping of the root canal greatly reduces the number of bacteria (2). Nevertheless, because of the anatomical complexity of the root canal system, organic and inorganic residues and bacteria cannot be completely removed and often persist (3). Various irrigants have been used during the canal preparation to minimize the residual debris, necrotic tissue, and bacteria, as well as to remove smear layer formed by the mechanical preparation of the dentin (2, 4, 5).

The most common irrigant used in root canal treatment is sodium hypochlorite (NaOCl) in a concentration range from 6% to 0.5% (6–8). NaOCl is an effective tissue solvent and antimicrobial agent (6–8). Its germicidal ability is related to the formation of hypochlorous acid when in contact with organic debris. In high concentration NaOCl is toxic and can cause inflammation in the periapical tissues (6, 7, 9, 10), whereas in low concentrations it is ineffective against specific microorganisms (6). NaOCl is not a substantive antimicrobial agent (11); it tends to discolor (9) and corrode surgical instruments; and it has a very unpleasant odor (11).

Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that has been advocated as an effective medication in endodontic treatment (8, 12). When used as a root canal irrigant and intracanal medication, it has an antibacterial efficacy comparable to that of NaOCl (11, 13, 14), while being effective against certain NaOCl resistant bacterial strains (11, 15). Prolonged exposure of the root dentin to CHX may result in residual antimicrobial activity of the dentin surface (11, 14–17). CHX has a low grade of toxicity (18); however, the inability of CHX to dissolve organic matter is a perceived drawback (19).

A combination of NaOCl and CHX has been advocated to enhance their antimicrobial properties. Kuruvilla (7) suggested that the antimicrobial effect of 2.5% NaOCl and 0.2% CHX used in combination was better than that of either component. Zehnder (20) proposed an irrigation regimen in which NaOCl would be used throughout instrumentation followed by EDTA, and CHX would be used as a final irrigant. If hypochlorite was still present in the canal, a precipitate was observed when the medications interacted (20, 21). Therefore, the goals of this study were (1) to determine the minimum concentration of NaOCl that causes color change and precipitate formation when mixed with 2.0% CHX, and (2) to characterize the resulting precipitate.

Materials and Methods

Solutions

Distilled water (dH_20) was filter-sterilized using a Gelman Filter Funnel and Whatman Filters, then autoclaved at 121°C. 10 mL of 20% CHX (Chlorhexidine Digluconate BP Lot 15243, Willer-PCCA London, ON) was diluted with 90 mL dH_20 to prepare 2.0% CHX. In addition, 6.0% NaOCl (Sodium Hypochlorite Lot 050613-9, Fair Lawn, NJ) and para-chloroaniline (PCA) (ACP Chemicals, Cat # C2285-100g, Montreal, PQ) were used.

Color Change and Formation of Precipitate

Ten flat-top 1.5-mL polypropylene microtubes were used. The first microtube contained 1 mL of 6% NaOCl and the others 0.5 mL sterile dH_20. Two-fold serial dilutions of the NaOCl were done by pipetting 0.5 mL from the first microtube and

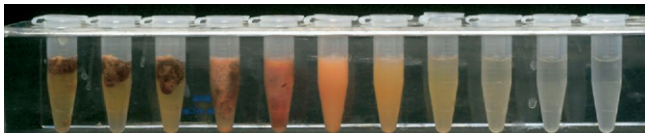

Figure 1. Microtubes containing different concentrations of sodium hypochlorite (NaOCl) mixed with 2% and chlorhexidine (CHX). The concentration of NaOCl decreases from 6.0% (the first tube on left) to 0.023% (ninth tube from left). The tenth and eleventh microtubes contain controls of 6% NaOCl and 2% CHX alone, respectively. Note the color change in the mixed liquids, ranging from dark brown at left to light orange in microtube nine, as well as formation of precipitate in varying amounts.

adding it to the second microtube and repeating the process from each microtube to the next. The last microtube was discarded. In this manner, 9 microtubes with the following concentrations of NaOCl were obtained: 6.0%, 3.0%, 1.5%, 0.75%, 0.38%, 0.19%, 0.094%, 0.047%, and 0.023%. Two more microtubes were added as controls, containing 6.0% NaOCl alone and 2.0% CHX alone, respectively.

To determine the minimal concentration of NaOCl at which a change of color occurred, and a precipitate was formed, 0.5 mL of 2.0% CHX was added to each of the 9 tubes with NaOCl (Figure 1). The microtubes were observed for color change and precipitate formation every 15 minutes for the first 2 hours, and once more after 1 week. The tests were repeated 10 times and the minimum NaOCl concentration in which color change and precipitate formation occurred were recorded.

Characterization of the Precipitate

To characterize the precipitate, a new set of six flat-top polypropylene microtubes were prepared with diluted concentrations of NaOCl (6.0%, 3.0%, 1.5%, 0.75%, 0.38%, and 0.19%) and 0.5 mL of 2.0% CHX was added, as described above.

The precipitate in these microtubes was washed with sterile dH_2O several times to remove ions that could interfere with the analysis. All microtubes were centrifuged for 2 minutes at a G Force of 5000 using a Vortex Genie (Fisher Scientific, Boston, MA). The supernatant was discarded using a Pasteur pipette (Maple Leaf Brand, Mississauga, ON) and replaced with 1 mL sterile dH_2O. The microtubes were then vortexed until the precipitate was re-suspended. This was repeated six times for each of the concentrations.

After the final wash with sterile dH_2O, a few drops were added to each microtube to remove some of the sample, because the precipitate was so thick that it was stuck to the inside of the microtube. The entire sample from each concentration was then spread onto 2 × 2 cm aluminum foil and left to dry at room temperature. The dried samples were analyzed by X-ray Photoelectron Spectroscopy (XPS) (Leybold /Specs Max 200-Cologne, Germany) and time-of-flight secondary ion mass spectrometry (TOF-SIMS) (ION-TOF GmbH, Munster, Germany).

As controls 2.0% CHX and PCA powder were used. The CHX sample was spread onto 2 × 2 cm aluminum foil and left to dry, and both XPS and TOF-SIMS were run. The PCA powder was pressed onto Indium foil, and then mounted on a heating/cooling stage supplied by the instrument for TOF-SIMS analysis. XPS could not be run on the PCA powder because it could not be cooled, and the sample evaporated in the vacuum system due to a low melting point at reduced pressure. In addition, the heat produced by the X-rays during the analysis could have caused evaporation and consequent false reading.

XPS was used to determine the chemical composition and atomic ratios of the samples. In this technique, the sample is excited by irradiating it with X-rays of known energy, typically K X-ray line. The X-rays cause photo ionization of atoms in the sample. The results are observed by measuring the energy spectrum of the emitted photoelectrons. The spectrum shows a peak for the various electron energy levels, the relative intensity of which can be directly related to the atomic composition of the specimen. A chemical shift, related to the atoms' chemical environment, allows further information of chemical composition to be obtained (22).

TOF-SIMS was used to determine the chemical composition of the samples. The sample is exposed to pulsed primary ion that ionizes its surface. Secondary ions are then accelerated into a mass spectrometer and mass analyzed by measuring the "time of flight" from the sample surface to the detector. The secondary ion image and the mass spectrum analysis are used to determine chemical composition of the sample (23).

Unique peaks were identified and reproducibility was tested 3 times for both XPS and TOF-SIMS for each combination and each control groups.

Results

Color Change and Formation of Precipitate

The color change and precipitate formation after addition of 2.0% CHX to various dilutions of NaOCl is shown in Figure 1. Color change occurred in all nine microtubes where CHX was added, including the one with lowest concentration of 0.023% NaOCl. As the concentration of NaOCl increased, the color range varied from peach to brown. The color change was noted immediately and did not change with time. The lowest concentration of NaOCl to induce a precipitate was 0.19% (sixth dilution in the series). In the first three microtubes, a precipitated brown mass was suspended at the top of the tube. In the fourth microtube, there was brownish-pink, thick, miscible liquid. In microtubes 5 and 6, there was a slight orange precipitate in the liquid. As with the color change, the precipitate occurred immediately and showed no change with time.

Characterization of the Precipitate

The ratio of nitrogen to chlorine (N/Cl) shown by XPS analysis of the precipitate for 6%, 3%, 1.5%, 0.75%, and 0.375% NaOCl are 1.96, 2.05, 2.26, 2.74, and 2.69 respectively. The N/Cl ratio was inversely proportional to the concentration of NaOCl. The results of the TOF-SIMS analysis of CHX showed mass spectrum peaks at 127, 153, 170, and 195 amu. The graphs for PCA and the precipitate in the second microtube (2.0% CHX + 3% NaOCl) are shown in Figures 2 and 3 respectively. A consistent peak at a 127 amu was noted in all the samples. In the precipitate, there was also an additional peak at a 111 amu; this peak was not present in the CHX or PCA alone. The intensity of the 111 peak was positively correlated with the concentration of NaOCl within the range 0.3–6% (correlation coefficient 0.973). The atomic mass represented the molecule C_6H_4Cl.

Discussion

It is well established that biomechanical cleaning and shaping of the root canal system, using files and antibacterial irrigants, reduces the bacteria load (2, 3). However no irrigant can completely eliminate all organic and inorganic matter and at the same time impart a substantive residual antibacterial property to the canal wall dentin (2, 3). A suggested clinical protocol for treating the dentin before root filling consists of irrigation with NaOCl to dissolve the organic components, irrigation with EDTA to eliminate the smear layer and irrigation with CHX to impart substantive antibacterial activity (20). Although such combination of irrigants may enhance their antimicrobial properties (7), possible chemical interactions among the irrigants have to be considered.

Recent studies (20, 21) have reported on the occurrence of color change and precipitation, when NaOCl and CHX are combined. Further-

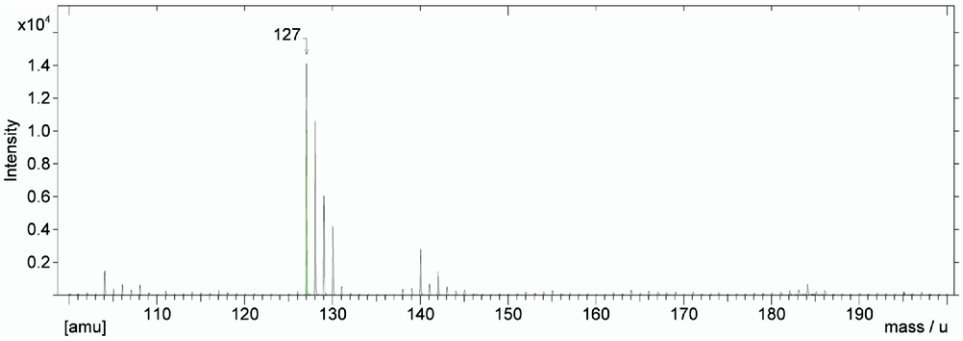

Figure 2. The mass spectrum of para-chloroaniline (PCA) shows a peak at 127 amu. This is the only characteristic fragment of PCA.

more concern has been raised that the color change might have some clinical relevance (21) because of staining, and that the precipitate might interfere with the seal of the root filling (21). Although the occurrence of the interaction has been reported, it has not been characterized in terms of threshold concentrations of the irrigants and the composition of the resulting precipitate.

In the present study qualitative and quantitative data was obtained to highlight the chemical nature of the precipitate and the reason of the color change. The results showed an immediate reaction when 2.0% CHX was combined with NaOCl, even at the low concentration (0.023%). Increase of the concentration of NaOCl to 0.19% (sixth dilution in the series) resulted in the formation of a precipitate. As the concentration increased the color darkened and the precipitate thickened.

It has been reported that, when placed in an aqueous solution, CHX slowly hydrolyzes and forms para-chloroaniline (PCA) (24). This occurs through a substitution of the guanidine group in the CHX molecule (25). Our findings indicated that when mixed with NaOCl, the CHX molecules become hydrolyzed into smaller fragments, each forming a by-product. It is theorized that the first bonds to be broken in this reaction are between carbon and nitrogen because of the low bond dissociation energy that is between the 2 atoms. Molecules with low bond disassociation energies are more prone to breaking (26). This disassociation results in the formation of PCA, among other fragments. The identities of these resulting fragments can be characterized by determining the atomic mass. TOF-SIMS of CHX showed characteristic peaks at 127, 153, 170, and 195 amu. The fragmentation pattern could be explained by the initial breaking of the carbon to nitrogen bonds. This could result in peak 153 amu representing the molecule Cl (C_6H_4) CH_2N_2, the peak 170 representing the molecule Cl (C_6H_4) CH_5N_3, and the 195 peak representing the molecule Cl (C_6H_4)$C_2H_4N_4$. The 127 peak is characteristic of PCA.

The formation of the precipitate could be explained by the acid–base reaction that occurs when NaOCl and CHX are mixed. CHX, a dicationic acid (pH 5.5–6.0) has the ability to donate protons. NaOCl is alkaline and can accept protons from the dicationic CHX. This proton exchange results in the formation of a neutral and insoluble substance, referred to as the "precipitate." It is assumed that this precipitate contains PCA. The samples containing the precipitate were analyzed by TOF-SIMS. The spectra of the mixtures of NaOCl/CHX displayed the same fragmentation pattern as CHX alone, indicating that CHX was still present in the precipitate. The precipitate showed an additional peak at 111 amu that was not seen in the CHX or PCA alone. The intensity of this peak correlated with the concentration of NaOCl. It suggested the formation of another by-product of the NaOCl/CHX, in the form of the molecule NaC_6H_4Cl.

TOF-SIMS was useful for characterizing CHX and PCA; however, it was limited in determining the amount of PCA among the different mixtures of NaOCl/CHX. To quantify the amount of PCA in each sample, XPS was used. Elemental ratios were used to quantify the amount of species in question. In the previous study by Sodhi et al (27), a correlation was established between the amount of PCA and the ratio of N/Cl. The researchers had washed the CHX samples and compared the N/Cl in the washed samples to that in the unwashed samples. The washed samples had a higher ratio; therefore, a higher ratio meant a lower PCA level. Theoretically the N/Cl ratio of PCA alone was 1.0; hence the closer the ratio was to 1.0, the more PCA was present. In our study, CHX alone showed a ratio of 4.9 and the combination with 6% NaOCl showed a ratio of 1.96. Therefore the highest PCA formation correlates with the highest concentration of NaOCl.

In summary, we demonstrated that NaOCl mixed with CHX formed PCA, and the amount of PCA directly increased with the increment in the concentration of NaOCl. Although the precipitate being insoluble raises questions about leaching of PCA, the study findings maybe clinically

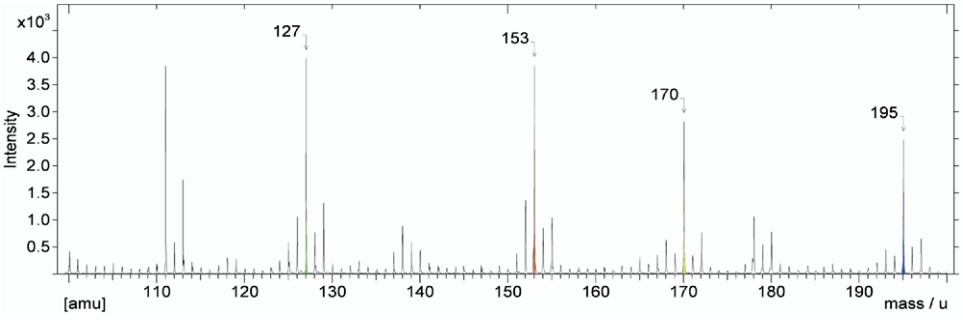

Figure 3. The mass spectrum of the precipitate (2.0% chlorhexidine [CHX] + 3% sodium hypochlorite [NaOCl]) shows peaks at 111, 127, 153, 170, and 195 amu.

relevant because PCA has been shown to be toxic (28, 29). As an aromatic amine, the primary toxic effect is methemoglobin formation (28). Short-term exposure of humans to PCA results in cyanosis, which is a manifestation of methemoglobin formation (28). Toxicological studies in rats and mice have shown that the hemopoietc system is the major target for PCA (28). In 1990, Chhabra et al (28) conducted a 90-day study and found that methaemoglobin formation and accompanying haemolytic anaemia, extra-medullary haematopoiesis, and splenomegaly were indicative of erythrocyte toxicity and regenerative anemia. In 1991, they reported PCA to be carcinogenic in rats due to increased sarcomas in the spleen. In male mice, there was an increase in hepatocellular carcinomas and haemangiosarcomas of the spleen (28). In zebra fish, researchers found that hatching was retarded and fish displayed increases rates of abnormal development and pigmentation (29). Also there have been reports of severe methemoglobinemia in human neonates who were exposed to PCA produced as a breakdown product of CHX resulting from incubator heat (30).

Further investigations of the NaOCl/CHX precipitate in endodontic situations should address the bioavailability of PCA leaching out and cytotoxicity. In the meantime, it would appear prudent to minimize its formation by washing away the remaining NaOCl with alcohol or EDTA, before using CHX.

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