Effects of 2% chlorhexidine and 5.25% sodium hypochlorite on gutta-percha cones studied by atomic force microscopy

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Abstract

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Aim To compare the effects of 2% chlorhexidine (CHX) and 5.25% sodium hypochlorite (NaOCl) on gutta-percha (GP) cone structure using atomic force microscopy (AFM).

Methodology Two standardized GP cones were sectioned 3 mm from the tip, attached to a glass base and immersed in 2% CHX or 5.25% NaOCl for 1, 5, 10, 20 and 30 min. Untreated GP cones were used as control. Topography and elasticity analyses were performed on 12 different regions located between 1 and 2 mm from the tip. Root mean square (RMS) parameters for contact mode imaging and force modulation microscopy variations were measured. The differences between RMS values were tested by ANOVA with Fisher's protected LSD test for multiple comparisons.

Results There was no deterioration in the topography and physical properties studied when 2% CHX was used in comparison with the control ($P < 0.05$). The RMS parameter for topography increased after 10 min of 5.25% NaOCl exposure in comparison with the control ($P < 0.05$). In addition, 5.25% NaOCl increased the elasticity of the GP cone after an immersion time of 1 min in comparison with the control ($P < 0.05$).

Conclusions Two per cent CHX did not change GP cone structure following up to 30 min exposure. Conversely, 5.25% NaOCl caused elastic changes after 1 min exposure.

Keywords: atomic force microscopy, chlorhexidine, gutta-percha cone, sodium hypochlorite.

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Introduction

Re-infection of the root canal system is one of the crucial factors that influences the outcome treatment. Preventive procedures include rapid decontamination of the gutta-percha (GP) cone with chemical disinfectants (Siqueira *et al.* 1998). A 5.25% sodium hypochlorite (NaOCl) solution is recognized as an effective decontaminant (Senia *et al.* 1975, Siqueira *et al.* 1998). However, it has been demonstrated that NaOCl is a strong oxidizing agent that can cause deterioration of various materials (Arvidsson *et al.* 2002, Yamauti *et al.* 2003, Neagu & Bunia 2004, Yokoyama *et al.* 2004). Therefore, an equally effective, but safer chemical is desirable. Studies have demonstrated that 2% chlorhexidine (CHX) is a valuable alternative disinfectant (Cardoso *et al.* 2000, Vianna *et al.* 2004). However, the effect of 2% CHX on the integrity of GP points has not been fully investigated.

Atomic force microscopy (AFM) is a well-established and documented tool for structural characterization of materials (Jandt 2001, Arvidsson *et al.* 2002). A number of different AFM operation modes, such as contact mode imaging (CMI) and force modulation microscopy (FMM), can be used to optimize the study of surface topography and physical properties. AFM hardware consists of a sharp tip mounted at the end

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of a microscopic cantilever, which systematically probes a surface. The bending of the cantilever is monitored by a laser beam detection system and is used to generate maps of topography and physical properties. The three-dimensional nature of the image allows the measurement of structural features and the generation of structural statistics (Jandt 2001, Bittencourt *et al.* 2003).

The aim of this study was to investigate and compare the effects of 2% CHX and 5.25% NaOCl on the surface topography and physical properties of standardized GP cones using AFM.

Materials and methods

Samples

Two standardized GP points (ISO size 60; Dentsply Indústria e Comércio Ltda, Petrópolis, RJ, Brazil) from the same batch (no. 1861) were randomly selected for study. All samples were used before their expiration date. For experimental controls, the coefficient of variation of the GP points was determined.

Experimental design

Gutta-percha points were sectioned 3 mm from their tip and attached to a glass base with a rapid-setting cyanoacrylate glue. Following these procedures, the samples were divided into two treatment groups: GP point immersed in 2% CHX and GP point immersed in 5.25% NaOCl. Untreated GP points were used as control. After cumulative treatment times of 1, 5, 10, 20 and 30 min for each solution, the samples were positioned in the AFM. The AFM analyses were performed on 12 different regions located between 1 and 2 mm from the specimens tip. Fresh aliquots (10 mL) of 5.25% NaOCl or 2% CHX (Farmogral, Brasília, DF, Brazil) were used for each period of immersion. After the immersion, the samples were thoroughly rinsed with 5 mL of nanopure water, and the region around the point then dried with filter paper.

Analyses with AFM

Atomic force microscopic images of the GP points were recorded in the contact mode on a TopoMetrix Explorer TMX 2000 atomic force microscope (TopoMetrix, Santa Clara, CA, USA) under ambient conditions. Typical AFM probes (curvature radius <20 nm) mounted on cantilevers (200 μm), with a spring constant of 0.032 N m^{-1} were used. Scanned areas (9.2 μm s^{-1} scan) were perfect squares (2.3 μm × 2.3 μm) in which was applied a weak force (<1 nN). CMI and FMM were simultaneously obtained during scanning procedures to investigate topography and elasticity of specimens, respectively. AFM images (500 × 500 lines) were acquired with SPMLab 4.0 software (TopoMetrix, Santa Clara, CA, USA) and analysed by WSxM Scanning Probe Microscopy Software 2.0 (Nanotec Eletronica S.L., Madrid, Spain) with background slopes corrected. For the purpose of comparison, the root mean square (RMS) was used to investigate the structure of the GP points.

All statistical analyses were performed with StatView for Windows 5.0 Software (SAS Institute, Cary, NC, USA). Mean and standard error of the mean values of the RMS parameters achieved from CMI and FMM measurements were calculated. The differences among the groups were tested by ANOVA with Fisher's protected LSD test of multiple comparisons and were considered significant when $P < 0.05$.

Results

Figure 1 shows the untreated and progressive AFM three-dimensional images for immersion time intervals of 1–30 min with 2% CHX and 5.25% NaOCl. The topographical effects caused by 5.25% NaOCl at time intervals of 20 and 30 min were above the detection limit of the particular AFM scanner used (top limit of ~800 nm). Thus, CMI and FMM parameters could not be detected.

Mean values of RMS for CMI profiles are shown in Fig. 2. The values are expressed in nm (1 : 1000 μm). The 2% CHX produced higher RMS at 10 min (RMS = 141.2 ± 40.8) compared with the 5 and 20 min sample (RMS = 83.7 ± 24.2 and 85.8 ± 24.8, respectively) ($P < 0.05$). The 5.25% NaOCl group produced a higher RMS at 10 min (RMS = 153.2 ± 44.2) compared with the untreated and 5 min exposure sample (RMS = 100.0 ± 28.9 and 98.0 ± 28.3, respectively) ($P < 0.05$).

The mean values of RMS for the FMM profiles are shown in Fig. 3. The values are expressed in nA and are linearly dependent on the applied force (nN). Similar RMS values were observed within the 2% CHX treatment ($P < 0.05$). The 5.25% NaOCl treatment showed higher RMS at 1 min (RMS = 10.3 ± 3.0) compared with the untreated, 5 and 10 min treatments (RMS = 6.4 ± 1.8, 6.2 ± 1.8 and 6.6 ± 1.9, respectively) ($P < 0.05$).

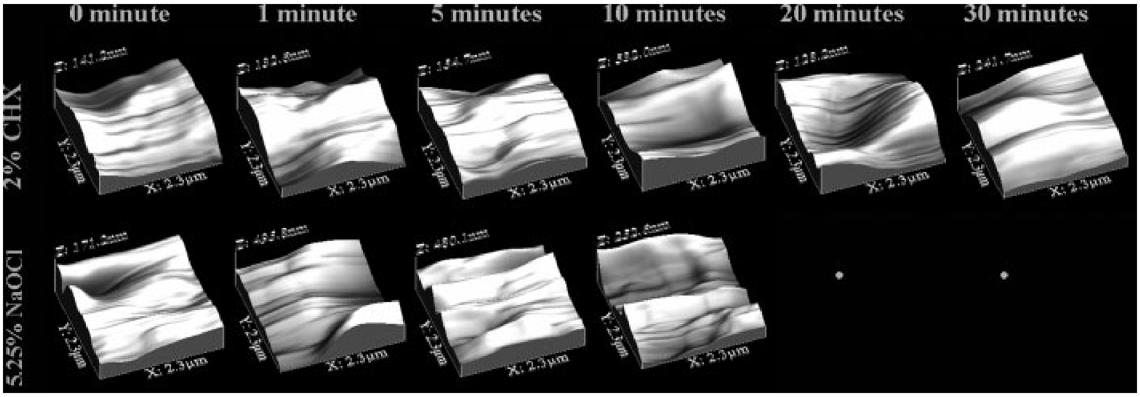

Figure 1 Contact mode AFM three-dimensional images of untreated and treated gutta-percha cone samples according to time of immersion for each group.

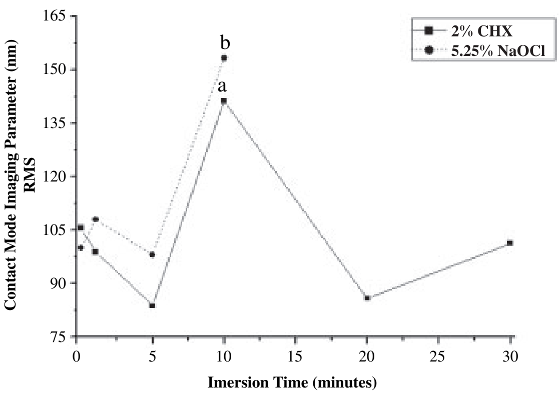

Figure 2 Comparison of RMS mean (±SEM) values for contact mode imaging among the different treatments as a function of time. [a]Statistically different from 5 and 20 min in the same sample; [b]statistically different from 5 min in the same sample ($P < 0.05$).

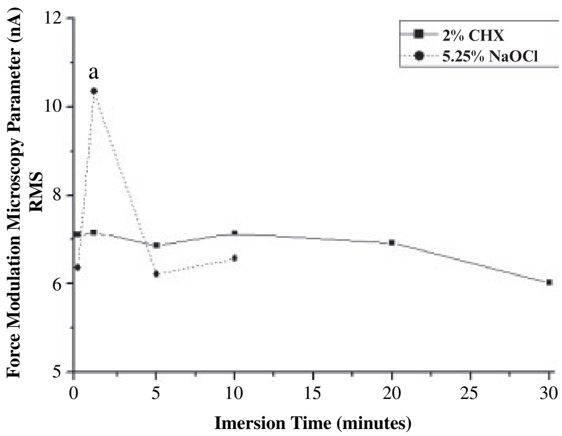

Figure 3 Comparison of RMS mean (±SEM) values for force modulation microscopy parameter among the different treatments as a function of time. [a]Statistically different from 5 and 10 min in the same sample ($P < 0.05$).

Discussion

Deterioration of GP points include increased depth of surface irregularities and elasticity. Deep irregularities can create large interfacial gaps between the GP cone and the root canal wall, increasing the risk of leakage (Goldberg *et al.* 1991). Increased elasticity can lead to difficulties during the obturation procedure, especially in curved canals (Moller & Ørstavik 1985). In contrast, decreased elasticity could be associated with an increased tendency of GP points to fracture. In the present study, the effects of 2% CHX and 5.25% NaOCl on topography and physical properties of the GP cone were investigated and compared by AFM. The AFM preparation prerequisites allowed the evaluation of the same sample before and after the different treatments.

The antimicrobial activity of 2% CHX has been demonstrated (Gomes *et al.* 2001, Vianna *et al.* 2004). These studies revealed that 2% CHX and 5.25% NaOCl have similar antimicrobial performance. However, no general agreement exists regarding the optimal time of action for decontamination of GP points by 2% CHX, which usually ranges from 1 min to more than 10 min (Siqueira *et al.* 1998, Cardoso *et al.* 2000). In the present study, the effects of 2% CHX on the GP cone structure were investigated at immersion times of 1–30 min. According to the CMI parameter, 2% CHX produced increased GP cone vertical amplitude at 10 min compared with 5 and 20 min of exposure. No reports were found in the available literature testing protocols that might allow a direct comparison. However, it is interesting to note that this structural damage was not observed at any other immersion time interval and in untreated specimens. Therefore, it is possible that 2% CHX produces minimal superficial alteration on GP cones. Furthermore, the small scale of these changes (in nm) is unlikely to influence the outcome of endodontic therapy.

In addition, the FMM parameter for 2% CHX showed a similar average of elasticity for all evaluated immersion times and to untreated specimens. The elasticity results obtained for GP points suggest that 2% CHX is a safe procedure during decontamination up to 30 min of exposure. To further clarify the effects of 2% CHX as a GP cone chemical disinfectant, a detailed investigation of the timing required to eliminate all microorganisms on the GP cone would be valuable in future studies.

A 5.25% NaOCl solution has been routinely used as an effective chemical for rapid decontamination of GP points before use, killing microorganisms in only 1 min of exposure (Senia *et al.* 1975, Siqueira *et al.* 1998). The results of the present study indicate that after 10 min, 5.25% NaOCl had resulted in deterioration of GP point topography compared with untreated specimens. After this period, 5.25% NaOCl caused topographical disruption on a level above the detection limit. Such a considerable increase in vertical amplitude indicated the presence of surface irregularities at a scale significantly higher (in nm) than those observed on untreated specimens. Although the nature of these phenomena is unclear, it appears that changes in topography are due to loss of GP cone components, resulting in a modification of the GP cone surface. This is in agreement with other studies that reported surface deterioration of dental materials after NaOCl exposure (Arvidsson *et al.* 2002, Yamauti *et al.* 2003, Neagu & Bunia 2004, Yokoyama *et al.* 2004).

In addition, the FMM parameter revealed that 5.25% NaOCl increased the elasticity of the standardized GP cone at 1 min compared with the untreated sample and other evaluated time intervals. It has been reported that changes in GP cone elasticity may occur due to alterations in the polymer chain (Kolokuris *et al.* 1992). The results for elasticity of GP cones treated with 5.25% NaOCl may be clinically relevant and could influence the success of endodontic therapy (Moller & Ørstavik 1985).

The extreme changes in AFM topographic features after 10 min of exposure to 5.25% NaOCl solution indicated aggressive deterioration of GP cone when compared with 2% CHX treatment, suggesting that 2% CHX is less prejudicial to GP structure. However, the clinical application of 5.25% NaOCl is usually for a much shorter time (1 min). Nevertheless, the rapid changes in the elastic properties of GP brought about by 5.25% NaOCl treatment implies that the use of 2% CHX may be a safer alternative during root canal treatment.

Conclusions

The structural effects of 2% CHX and 5.25% NaOCl solutions on GP points were examined by AFM. A 2% CHX solution did not cause deterioration of GP cone structure, whereas 5.25% NaOCl caused structural changes in GP cone elasticity after 1 min of exposure.

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