

ORIGINAL RESEARCH

Structural Effects of Various Commonly used Disinfectant Solutions on Gutta-Percha: An Atomic Force Microscopic Study

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ABSTRACT

Introduction: Gutta-percha cones can be contaminated by various ways. Literature revealed several methods for rapid decontamination of gutta-percha cones in dentistry. Atomic force microscope (AFM) is a well-established methodology for structural characterization of materials.

Aim: The purpose of the study is to evaluate the effects of 5% sodium hypochlorite 2% chlorhexidine, 2.2% glutaraldehyde, 6% hydrogen peroxide on the surface structure of standardized gutta-percha cones in their respective cold sterilization times 1, 10, 15, 10 minutes respectively as found in many studies.

Materials and methods: Forty standardized gutta-percha cones (ISO standardized size 60) were cut 3 mm from the tip, attached to a glass base and immersed in 5% sodium hypochlorite, 2% chlorhexidine, 2.2% glutaraldehyde, 6% hydrogen peroxide for 1, 10, 15, 10 minutes. After this, the samples were positioned in the atomic force microscope. The analyses were performed between 1 and 2 mm from the tip after each period of immersion in NaOCl. Gutta-percha cone without any NaOCl treatment were used as control. Root mean square (RMS) parameters for contact mode imaging variations were measured.

Results: The differences between RMS values were tested by ANOVA with Fisher's protected LSD test for multiple comparisons ($p < 0.05$). Aggressive deteriorative effects on gutta-percha cone elasticity were observed for 5.25% NaOCl at 1 minute when compared to the control ($p < 0.05$). In addition, 2% chlorhexidine has caused topographic changes after 15 minutes when compared to the control ($p < 0.05$). Conversely, 2.2% glutaraldehyde, 6% hydrogen peroxide solution did cause

minimal alteration on topography or elasticity of gutta-percha cone structure when compared to the control ($p > 0.05$).

Conclusion: Six percent hydrogen peroxide and 2.2% glutaraldehyde are safe alternative for chair side decontamination of gutta-percha cones when compared to 5.25% NaOCl and 2% chlorhexidine.

Keywords: Gutta-percha sterilization, NaOCl, Chlorhexidine, Hydrogen peroxide, Glutaraldehyde, Atomic force microscope.

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INTRODUCTION

Gutta-percha cone is a root canal filling material used in the endodontic therapy over 100 years. During endodontic therapy, an aseptic sequence is one of the professional main concerns and must not be broken.¹ Gutta-percha and Resilon cones are manufactured under aseptic conditions, but they can be contaminated by handling, aerosols and physical sources during the storage process. Because of their thermoplastic characteristics, the conventional heating processes cannot be used to sterilize those.² To keep the asepsis chain, gutta-percha cones require rapid chairside decontamination. Literatures revealed several methods for rapid decontamination of gutta-percha cones in dentistry. Among others, these include the following chemical agents: Polyvinylpyrrolidone iodine, glutaraldehyde, sodium hypochlorite, hydrogen peroxide, chlorhexidine, quaternary of ammonium, alcohol iodine and ethyl alcohol.³⁻⁶

A member of the scanning probe microscopies family, the high-resolution atomic force microscopy (AFM) has opened several applications in studies of surface of different materials, such as elasticity and stiffness. The key principle of the AFM is the probing of a sample surface with a small tip attached to a flexible cantilever. It provides qualitative and quantitative information about the sample structure.⁷

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The purpose of the study is to evaluate the effects of 5% sodium hypochlorite, 2% chlorhexidine, 2.2% glutaraldehyde, 6% hydrogen peroxide on the surface structure of standardized gutta-percha cones in their respective cold sterilization times 1, 10, 15, 10 minutes respectively, as found in many studies.

MATERIALS AND METHODS

Forty standardized gutta-percha cones (ISO standardized size 60) within a same lot were randomly selected from the packs which are used for multiple cases in this study. Gutta-percha cones were cut 3 mm from their tip and attached to a glass base with rapid setting cyanoacrylate glue. Following these procedures, the samples were divided in five groups as following: group I, eight gutta-percha cones left untreated:

Group II, eight gutta-percha cones immersed in 5% NaOCl for 1 minute; group III, eight gutta-percha cone immersed in 6% hydrogen peroxide for 10 minutes, group IV, eight gutta-percha cone immersed in 2.2% glutaraldehyde for 5 minutes and group V eight gutta-percha cone immersed in 2% chlorhexidine for 10 minute.

The analyses in atomic force microscope were performed for each cone located between 1 and 2 mm from the cone tip after cumulative immersion time of 1 minute.

Atomic force microscopy images of gutta-percha samples were recorded in the contact mode operation on a XE 70 AFM under ambient condition. Typical AFM probes (curvature radius < 20 nm) mounted on cantilevers (200 μm), with spring constant of 0.032 N/m were used. Scanned areas (4.6 μm^2 speed scan) were perfect squares (2.3 \times 2.3 μm) in which was applied a weak force (1 nN). Contact mode imaging (CMI) was obtained from scanning procedures. AFM images (500 \times 500 lines) were processed with the SPM Lab 4.0 software (TopoMetrix, Santa Clara, CA) and analyzed by WSxM scanning probe microscopy software 2.0 (Nanotec Electronica SL, Madrid, Spain) with only background slopes corrected.

For the purpose of comparison, the root mean square (RMS) was chosen to investigate the structure of the gutta-percha cones. Mean and standard error of the mean values of the RMS parameters achieved from CMI were calculated. The difference among the studies groups were tested by ANOVA with Fisher's protected LSD test of multiple comparisons and were considered significant when $p < 0.05$.

RESULTS

Graph 1 shows control and NaOCl, CHX, hydrogen peroxide; glutaraldehyde treated gutta-percha cones

evaluated by contact mode AFM in a vertical (Z) nano meter scale displaying three-dimensional images at their respective immersion times for sterilization.

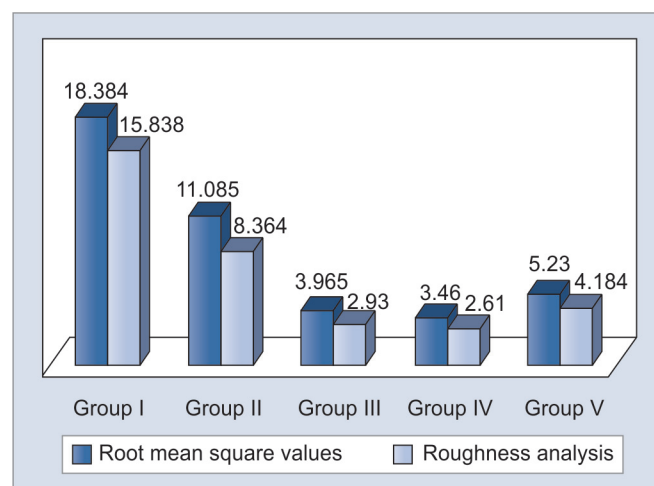
To investigate any quantitative statistically significant difference in vertical topographic amplitude resulting of the disinfectant solutions treatments. The RMS of CMI profile data was evaluated. CMI is the standard topographical imaging of AFM technique.⁹ Mean values of RMS for CMI profiles are shown in Figure 1. The values are expressed in nm (1:1000 μm). However, 5% sodium hypochlorite solution for 1 minute immersion and 2% CHX for 10 minutes presented RMS lower (RMS = 11.085 \pm 4.3 and 5.230 \pm 3.9 nm respectively) compared to control (RMS = 18.384 \pm 6.3 nm) and RA values of 8.36 \pm 2.3 nm, 4.18 \pm 2.1 nm compared to control (15.83 \pm 3.4 nm) ($p < 0.05$). Whereas hydrogen peroxide and glutaraldehyde showed lesser values for RMS (3.96 \pm 2.1 and 3.46 \pm 1.8 nm) and RA values (2.93 \pm 1.8 nm and 2.61 \pm 1.6 nm) compared to control.

DISCUSSION

Gomes et al verified that, even though gutta-percha cones are usually sterile during storage, they can be easily contaminated if incorrectly manipulated.⁸ In their study, 100% of the cones manipulated with gloves showed microbial growth, demonstrating the importance of cone disinfection procedures.

The present study evaluated the structural effects after disinfection procedures by using 5% NaOCl for 1 minute, 9. 2% CHX for 10 minutes, 10. 2% glutaraldehyde for 5 minutes, 11 and 6% hydrogen peroxide for 10 minutes, 12% at their respective sterilization times as provided by the literature.

The results of the present study showed that according to the CMI parameter, 5% sodium hypochlorite solution for 1 minute immersion and 2% CHX for 10 minutes produced decrease in the dimensions of gutta-percha



Graph 1: Contact mode imaging of gutta-percha under AFM

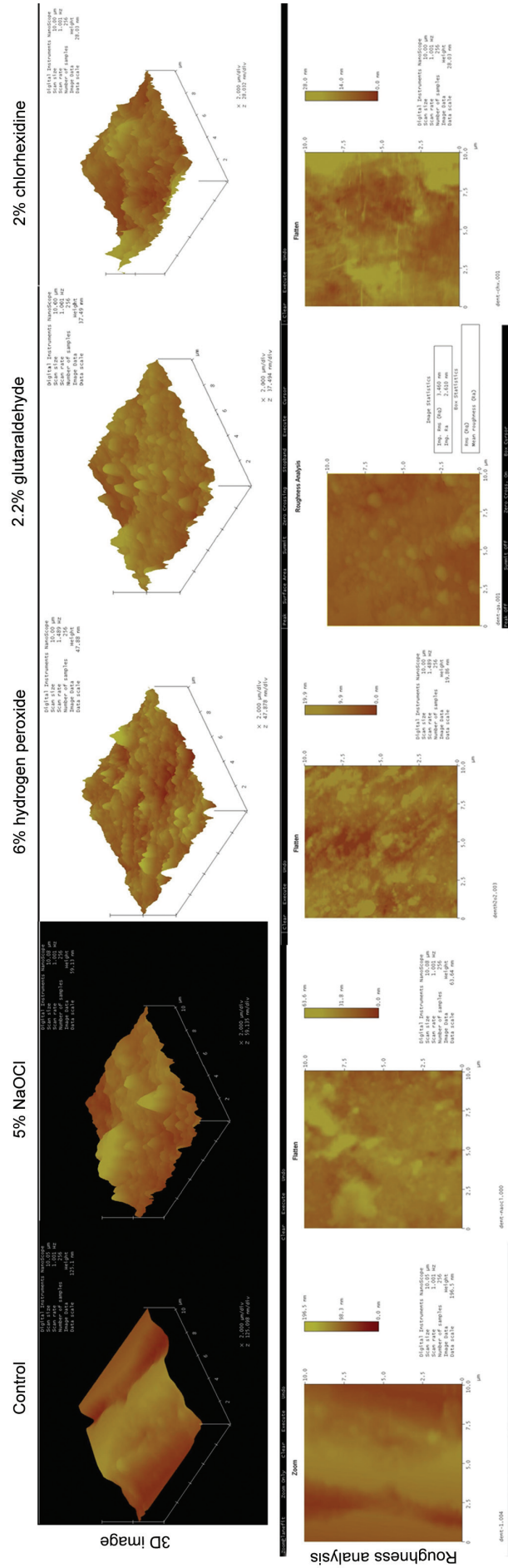


Fig. 1: Intergroup roughness analysis and root mean square values

cone, i.e. vertical amplitude in their respective times of immersion for sterilization compared to control.

These results indicate a deteriorative tendency of these solutions on gutta-percha cone topography. Although the nature of this phenomenon is not quite clear, it appears that changes in topography is because of loss of gutta-percha cone components, resulting in dimensional changes that may represent a great potential for failure of endodontic obturation in agreement with other studies.

However, it has been related that the amount of available chlorine may be responsible for the deteriorative effects of NaOCl solution.

This also causes increased elasticity and can lead to difficulties during the obturation procedure, especially in curved canals. Therefore, the result for elasticity of gutta-percha cone treated with 5% NaOCl and 2% chlorhexidine may be clinically relevant.

On the other hand, it is interesting to note that 6% hydrogen peroxide, 2.2% glutaraldehyde concentration presented similar behavior for CMI parameter compared to the control suggesting that these solutions are less prejudicial to gutta-percha cone structure.

The limitations of the present study are the sample number, environmental conditions that may differ and manufacturer variations in gutta-percha. This may change the results achieved above.

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