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Topographical effects of Gutta Percha immersed in different concentration of Sodium Hypochlorite disinfection at different time interval: An atomic force microscopy study

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Abstract

Background: Aim of the study to evaluate the topographical effects of more than 5 min immersion of gutta-percha in different concentration of sodium hypochlorite with the help of Atomic Force Microscope.

Materials and Methods: This investigation was carried out on four standardized gutta percha (GP) cones (ISO size 60). The gutta-percha cones were cut 3mm from their tip and then immersed in 0.5%, 2.5%, 5.25% NaOCL and de-ionized water for 2mins, 5mins and 10 mins immersion time interval. De-ionized water served as a control. The topographical analysis in atomic force microscope (AFM) were performed for each cone on twelve different points (n=12) located between 1 and 2 mm from the cone tip after every immersion time period. After the immersion, the samples were thoroughly rinsed with 5 ml of distilled water to remove sodium /chloride crystals, and the region around the cone was dried with filter paper.

Results: The surface roughness of the gutta percha was evaluated by using AFM. The mean values of root mean square (RMS) for different groups were calculated and subjected to statistical analysis. Statistical analysis was done by one way Anova test & multiple comparison with Post–hoc Tukeys test. There was a statistically significant difference in the RMS values between 0.5%, 2.5% and 5.25% NaOCL groups ($p<0.05$) but there was no statistically significant difference between 0.5%NaOCL and control group ($p>0.05$).

Conclusion: Sodium hypochlorite immersion significantly decreased the RMS values of gutta-percha as the concentration of sodium hypochlorite was increased from 0.5% to 5.25%.

5.25% NaOCl immersion till 5 minutes decreased surface roughness but roughness increased significantly at 10 min with 5.25% NaOCl.

Keywords: Disinfection, Gutta percha, Sodium hypochlorite, Surface topography.

Introduction

Gutta-percha is the most widely used root canal filling material. Microbial contamination of Gutta-percha with microbes during packaging and storage can be detrimental for the success of endodontic therapy. Some studies have shown that 5-8% of the cones from sealed packages can be contaminated with bacteria.(1) The gutta percha cones can be contaminated by handling, when exposed to the dental operatory environment and during storage.(2) Given these conditions, and the importance of preventing cross contamination of the root canal during endodontic treatment, it has been recommended that gutta-percha cones be sterilized prior to obturation. The thermoplastic nature of the guttapercha cones, these cones cannot be sterilized by conventional moist or dry heat process which may cause alteration of guttapercha structure. Hence, chemical disinfection an be the choice for disinfection of gutta-percha cones. There are various chemical agents that have been employed for chemical disinfection of gutta-percha cones, one among them is sodium hypochlorite which is widely used.

One of the potential drawback is the changes in the physical properties of gutta-percha cones after disinfection with chemical agents. This was first described by Miller and Orstavik (1985).(3) Valois et al. (2005) reported that a 1 minute treatment with 5.25% NaOCl increased the elasticity of gutta-percha cones in comparison with untreated cones. The authors also found topographic changes after gutta-percha was placed in 5.25% NaOCl for 5 minutes.(6) Since the immersion time of 5 min could not be practically done in a clinical scenario, due to increase popularity of single visit root canal therapy, higher concentration of NaOCl is being used as the irrigating solution. This high concentration of NaOCl can also be used for disinfection of gutta-percha. Moreover, in molar endodontics, obturating multiple canals could take more than 5 minutes and during this time the operator can immerse the gutta-percha cones in hypochlorite at start of obturation for disinfection.

Hence, the aim of this in-vitro study was to analyse the surface characteristics of gutta percha when immersed in different concentrations i.e. 0.5%, 2.5%, 5.25% of sodium hypochlorite for 2mins, 5 mins and 10mins using atomic force microscopy.

Material & Methods

Four standardized gutta percha (GP) cones (ISO size 60, Dentsply, USA) were taken from the same lot. Each GP cones were cut 3mm from their tip and attached to a glass slide with rapid setting cyanoacrylate glue. After which the samples were divided in four groups.

In Group I, the GP cone is immersed in 10ml of de-ionized water for 2 mins, 5mins and 10 mins respectively, which serve as control.

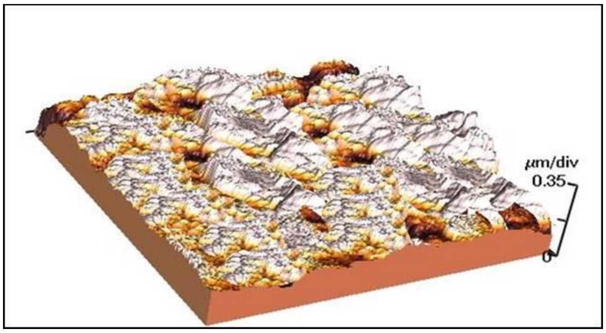
In Group II, the GP cone was immersed in 10ml 0.5% NaOCL for 2mins, 5mins and 10 mins.

In Group III, the GP cone was immersed in 10ml 2.5% NaOCL for 2mins, 5mins and 10 mins.

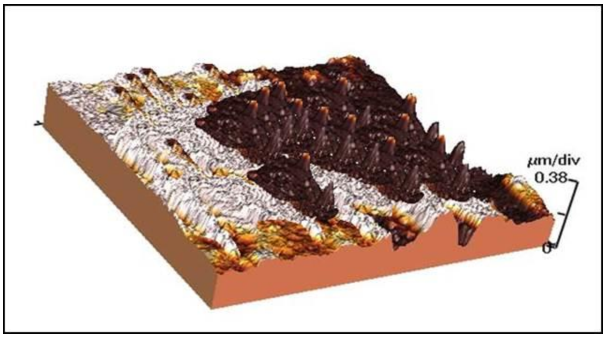
In Group IV the GP cone was immersed in 10ml 5.25% NaOCL for 2mins, 5mins and 10 mins.

The analysis was performed using atomic force microscope (Dimension 5000, Veeco Digital instruments, America) on each cone at twelve different points (n=12) which was located within 1 to 2 mm from the cone tip. The solutions of the experimental group, were freshly used for each time interval. After each immersion of different concentration of NaOCL, the samples were thoroughly rinsed with 5 ml of distilled water to remove sodium or chloride crystals and the region around the cone was dried with filter paper**.**

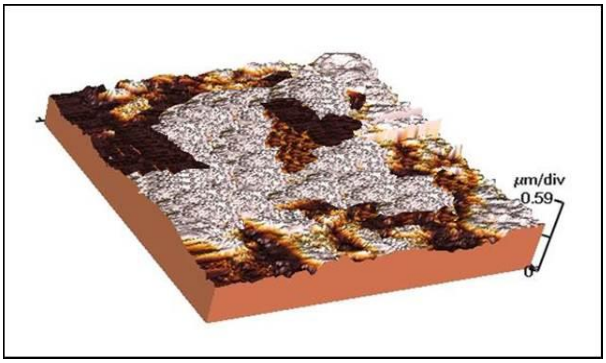
The samples were subjected to AFM images of the samples were recorded in the contact mode operation, as described by Valois et al.[(5)] AFM probe with a curvature radius of less than 20 nm mounted a 3 dimensional cantilever with a spring constant of 0.032N/m was used. The scan speed was 4.6µm/s AFM images were processed by SPM Lab 4.0 software and analyzed 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 and average surface roughness values were determined. Three dimensional AFM images of the samples of different groups are shown in Fig. 1, 2, 3 and 4.



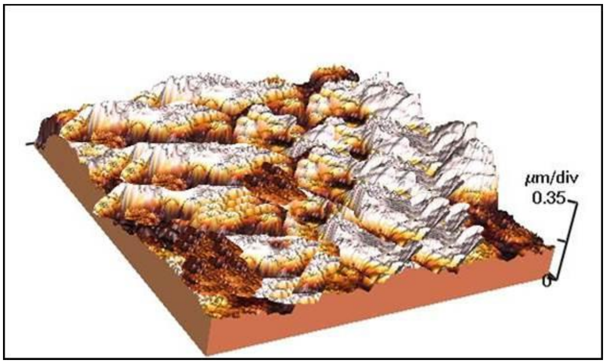
Control Group



0.5% NaOCi-2 Mins

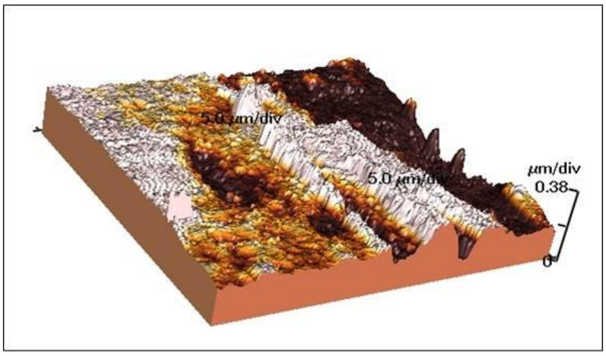


0.5% NaOCI- 5mins



0.5% NaOCI- 10mins

Fig. 1: AFM- dimensional change with 0.5% NaOCI with different time intervals



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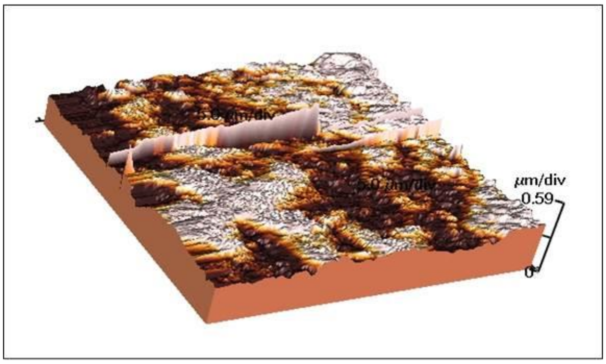
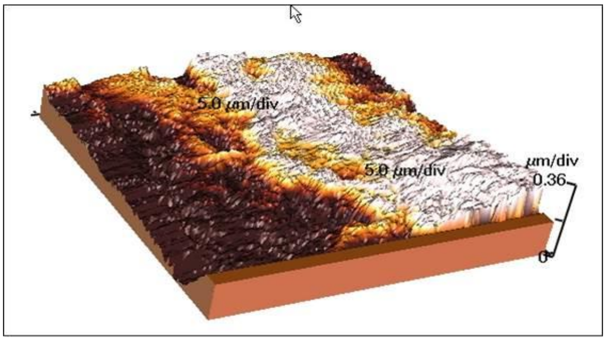
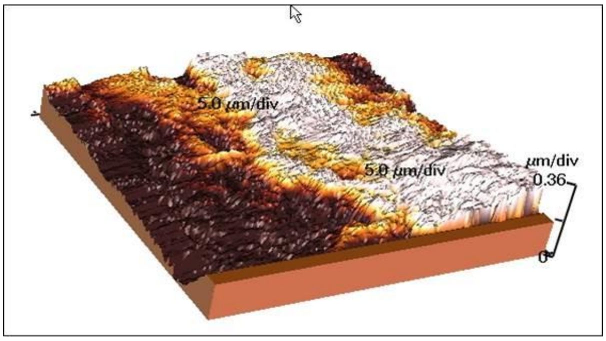


2.5% NaOCi-2 Mins

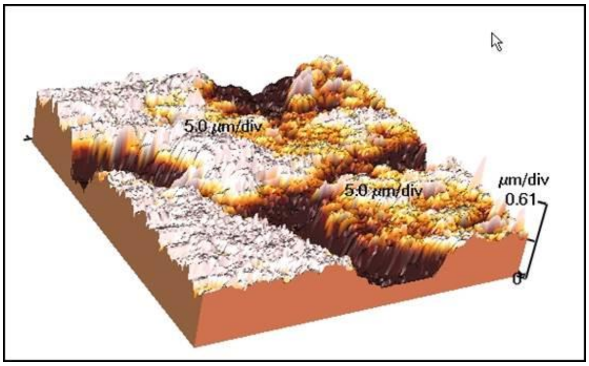


2.5% NaOCI- 5mins

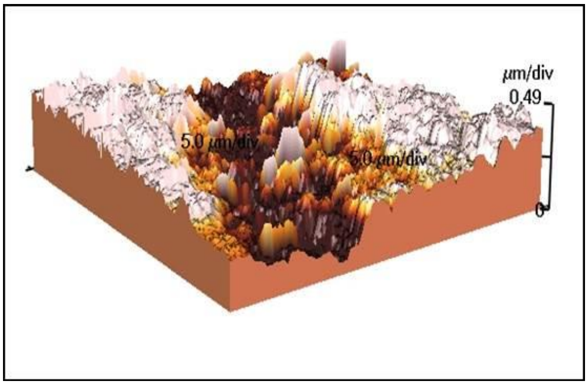


2.5% NaOCI- 10mins

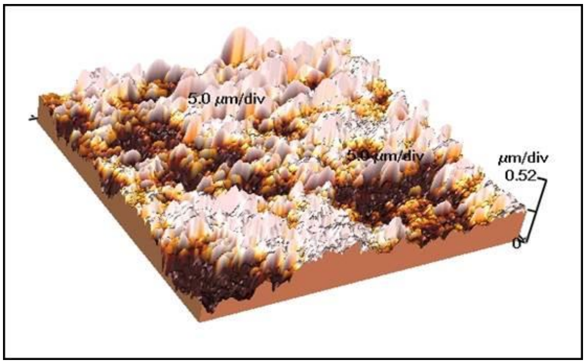
Fig. 2: AFM- images dimensional changes in 2.5% NaOCI at different time intervals



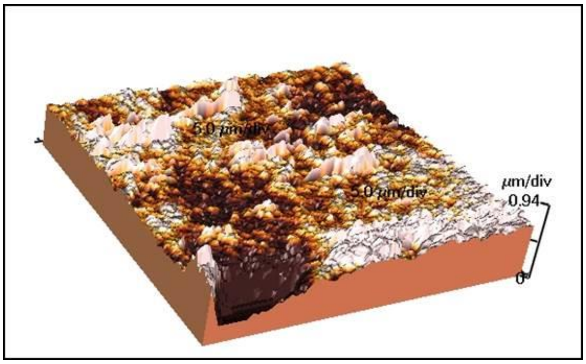
Control Group



5.25% NaOCI-2 Mins



5.25% NaOCI- 5mins



5.25% NaOCI- 10mins

Fig. 3: AFM- images dimensional changes in 5.25% NaOCI at different time intervals

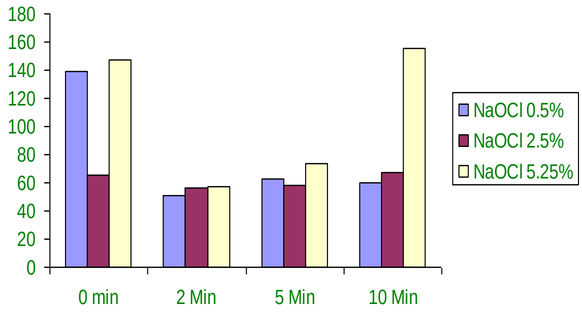


Fig. 4: Shows bar diagram of the RMS at different concentration of NaOCI & different time intervals

Table 1: Comparison of surface roughness in (µm) at different time intervals & concentration

Naocl	Time assessment			
	0 mins	**2mins**	**5 mins**	**10 mins**
Group 1	139.2	51.3	63.1	69.1
Group 2	65.5	56.7	58.2	67.1
Group 3	147	57.4	73.6	155.6

Mean and standard error of the mean of the RMS parameter values achieved from contact mode imaging (CMI) measurements were calculated. The difference among the groups were tested by One way Anova test & multiple comparison with Post –hoc Tukeys test.

Results

The surface roughness of the gutta percha was evaluated by using AFM. The mean RMS values of the experimental Groups are shown in Table 1. Statistical analysis was done by One way Anova test & multiple comparison with Post –hoc Tukeys test. The analysis shows statistical significant difference in the RMS values between the experimental groups i.e. 0.5%, 2.5% and 5.25% NaOCL groups where p value is < 0.05, but there was no significant difference between group I i.e.0.5% NaOCL and control group ($p>0.05$). In group II i.e. 2.5% NaOCl there was surface roughness initially which became significantly smoother at 2, 5, 10 min .However in group III i.e. 5.25% NaOCl group there was Initially surface roughness which became smooth at 2 mins and 5 min but became rougher at 10 mins.

Discussion

In endodontics major efforts are made during instrumentation and debridement of root canals to eliminate bacteria from the infected pulp space. This is achieved by cleaning and shaping where we try to attain complete disinfection and prevent bacterial contamination. After cleaning and shaping the canal is sealed with gutta percha and sealer to provide a fluid tight seal. Moorer *et al*. showed that gutta-percha cones exhibit antimicrobial activity due to their zinc oxide content,(6) however, this activity may be too weak to be effective clinically. Based on recent advances in infection control protocols, the instruments and materials used during endodontic treatment (including gutta-percha cones) should be free of microorganisms. Gutta percha being an inert obturating material has other advantages which includes, non staining in nature, radiopacity, easy retreavibility and antibacterial activity.(7) Gutta percha can also be softened by heat or chemicals, however this property impedes its sterilization by moist or dry heat**.** Given these conditions, and the importance of preventing cross contamination of the root canal system during endodontic treatment, it has been recommended that gutta-percha cones be sterilized prior to obturation. Therefore disinfection of the gutta perha points before use have been recommended.(1)

Different chemicals have been suggested for use in decontamination of gutta percha cones. The following agents have been recommended: Zephirin chloride, untinted tincture of Metaphen, thimerosal, povidone-iodine, alcohol, formaldehyde gas, sodium hypochlorite and glutaraldehyde.(1,8) In recent years, other agents such as chlorhexidine and MTAD have also been suggested.(9) Sodium hypochlorite, the most widely used agent for irrigation, has become the material of choice for chairside chemical disinfection of gutta-percha. In terms of its antimicrobial activity, sodium hypochlorite is a broadspectrum antibacterial agent that is effective against both Gram-positive and Gram-negative bacteria, yeast, fungi and viruses.(10) It is mostly used in concentrations that vary from 0.5% to 5.25%.(11) The most efficient and reliable technique for disinfecting gutta-percha cones was first proposed by Senia *et al.*(8) They suggested disinfecting gutta-percha cones by placing them into 5.25% NaOCl for at least one minute.

Decontamination of gutta-percha has been shown to have potential, unintended drawbacks. Short *et al* showed in a scanning electron microscopic study, that after decontamination of gutta-percha in 5.25% NaOCl, cuboidal chloride crystals are formed on the cones that could impede proper obturation seal.(12) They recommended that rinsing the disinfected gutta-percha cones with 96% ethyl alcohol or 70% isopropyl alcohol, or distilled water enables to remove the chloride crystals. Another drawback is the changes in the physical properties of gutta-percha cones after disinfection with chemical agents. This was first described by Moller and Orstavik (1985).(13) Atomic Force Microscope is similar to a scanning probe microscope has been used to evaluate the surface changes of gutta percha after disinfection. AFM being a sophisticated and sensitive machine shows the surface topographical changes occurring at micron level. Atomic force microscopes (AFMs) have a probe attached to a soft cantilever in a TV-raster-like pattern over a surface with constant force and record deflections of the tip that correspond to the surface topography and these findings are recorded as 3-D image formations on computer. It can show detailed magnification of objects as small as carbon atom and as large as human hair In an atomic force microscopy study, Valois *et al*. (2005) reported that a 1 minute treatment with 5.25% NaOCl increased the elasticity of gutta-percha cones in comparison with untreated cones.(5) The authors also found some topographic changes after gutta-percha was placed in 5.25% NaOCl for 5 minutes. In this study representative time of 10 min was used to evaluate long term effect of NaOCL on gutta percha. In the present study there was a reduction in RMS values of gutta percha after immersion in NaOCL. There was a significant reduction in RMS values of gutta-percha in 2.5 and 5.25% NaOCL, however 0.5% NaOCL and control showed no significant difference in RMS values. Valois, Silva & Azevedo showed similar results.[14] The results of this study shows deleterious effect of GP cone when immersed in 5.25% NaOCL concentration at 10mins. Such topographical changes can be due to selective loss of the components present in the gutta-percha cone, mainly the polymer component. The changes in surface characteristics of GP cone is minimal when the cones were immersed in 5 minute, this can lead to better adaptability of the GP cone to the sealer and canal wall. Also increased elasticity (Valois et al, 2005) could also aid in Cold Lateral Compaction during obturation. However using higher concentration of 5.25% cause initial smooth surface upto 5 min but at 10 min cause increase in roughness due to substantial breakdown of gutta percha components. The study can be further investigated in certain aspects regarding the nature of the component lost, effect on physical properties and how it affects the outcome in endodontic treatment.

Conclusion

1. Immersion of Gutta Percha in 0.5% & 2.5% NaOCl resulted in smooth surface topography even at 10 min.
2. 5.25% NaOCl immersion till 5 minutes decreased surface roughness but roughness increased significantly at 10 min with 5.25% NaOCl
3. At 5.25% NaOCl concentration used for GP disinfection the immersion time should not exceed more than 5 min whereas 0.5% & 2.5% NaOCl can be used at 10 min immersion time.

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