



**Subject Area : Conservative dentistry and Endodontics**

## EVALUATION OF SURFACE TOPOGRAPHY OF GUTTA-PERCHA FOLLOWING DISINFECTION WITH 5.25% SODIUM HYPOCHLORITE, CHITOSAN, AND GRAPHENE O

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| ARTICLE INFO                                                                                                                                                                            | ABSTRACT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
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| Received 15 <sup>th</sup> July 2025<br>Received in revised form 27 <sup>th</sup> July, 2025<br>Accepted 18 <sup>th</sup> August, 2026<br>Published online 28 <sup>th</sup> August, 2026 | <b>Aim:</b> This study aims to assess the surface topography of graphene oxide (GO) nanoparticle-disinfected GP and compare it to other disinfectants, such as chitosan nanoparticles (ChNps) and NaOCl. <b>Materials And Methods:</b> 40 gutta-percha cones (ISO size 40, 6% taper) were collected and were cut three millimeters from the tip and split up into four groups according to the respective treatment they underwent. Group A (control group): (n=10), Group B: GP points submerged in 5.25% NAOCL for one minute (n=10), Group C: GP points submerged in 1.5mg/ml chitosan nanoparticles for one minute (n=10), Group D: GP points submerged in 80µg/ml graphene oxide for one minute (n=10). The surface topography of the samples was examined using an atomic force microscope, following treatment with the aforementioned disinfection agents. <b>Statistical Analysis And Results:</b> Surface roughness and root mean square (RMS) metrics were used to examine the surface of GP points. These values were analyzed using post hoc testing and one-way ANOVA in IBM SPSS Statistics 20.0. If $P < 0.05$ , they were deemed statistically significant. <b>Conclusion:</b> Treatments with GO and ChNPs reduced the surface topographical deterioration of GP cones within the study's constraints, whereas NaOCl increased the deterioration. |
| <b>Key words:</b>                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| Graphene oxide, Chitosan, Disinfection, Atomic Force Microscopy, Nanoparticles                                                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
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### INTRODUCTION

The application of aseptic procedures and comprehensive disinfection is essential for the effectiveness of root canal therapy. This also applies to obturating materials. Gutta-percha is a commonly utilized and approved material for root canal fillings (Siqueira et al., 1998). Due to their intimate interaction with peri-radicular tissues during root canal obturation, gutta-percha cones must be free from harmful microbes. [1] Modern theories of infection control state that all instruments and supplies need to be sterilized before being used in a root canal. [2] Because it contains zinc oxide, gutta-percha cones have some antibacterial activity. However, during storage and treatment, they can become contaminated by physical sources, handling, and aerosols. [3]

According to the microbiological assay, GP cones that have just

been opened may be potentially contaminated. It's important to note that contamination of the sterile root canal filling material can lead to reinfection and ultimately cause the treatment to fail. Therefore, the root filling material must be disinfected. [4] GP cones are not easily sterilised by dry or moist heat, as these high-temperature methods have been shown to damage the cones. [5]

Before using these cones for obturation, the clinician must clean them with disinfectant such as ethylene oxide gas to prevent contamination, but this method was found to be time taking and practically unfeasible. [6]

GP cones have been cleaned using a variety of chemical disinfectants, including alcohol, hydrogen peroxide, glutaraldehyde, sodium hypochlorite (NaOCl), and iodine compounds. In dental clinics, the best decontaminating agent should allow us to use it repeatedly and allow disinfection without changing the surface of GP cones. [7]

Despite the large number of disinfectants available, NaOCl at a concentration of 5.25% is most frequently employed because of its wide range of activity against bacteria. [8] According

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to the literature, exposure to higher concentrations of NaOCl alters the topography of gutta-percha cones. [5] The issues brought on by NaOCl have compelled researchers to search for novel antibacterial agents that have negligible negative effects on tissue and material. [8]

The antibacterial qualities of nanoparticles made them useful in this situation. Chitosan nanoparticles (chNPs) and graphene oxide (GO) nanoparticles are two of them. Their broad-spectrum action has made them useful antibacterial agents. [9]

Atomic force microscopy (AFM) is widely utilized today for material characterization because of its scanning probe approach. The AFM's fundamental idea is to use a tiny tip fastened to a flexible cantilever to probe a sample's surface. It offers both quantitative and qualitative details regarding the sample structure. [10]

The goal of this research is to examine the structural changes of gutta-percha following decontamination using graphene oxide (GO), a novel disinfecting substance, and compare it with other disinfecting substances, such as chitosan nanoparticles (chNPs) and NaOCl.

## MATERIAL AND METHODS

In this investigation, 40 gutta-percha points were selected randomly from the same group(ISO size 40 6%).

Three millimeters from the tip, samples were sectioned and split up into three treatment groups and one control group as follows:

**Group A:** (control group): No treatment group (n=10)

**Group B:** GP points submerged in 5.25% NaOCl for 1 minute (n=10)

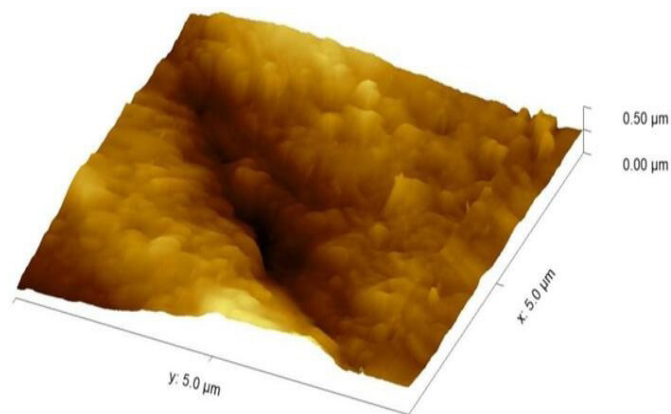
**Group C:** GP points submerged in 1.5 mg/ml chitosan nanoparticles for 1 minute (n=10)

**Group D:** GP points submerged in 80µg/ml graphene oxide nanoparticles for 1 minute(n=10)

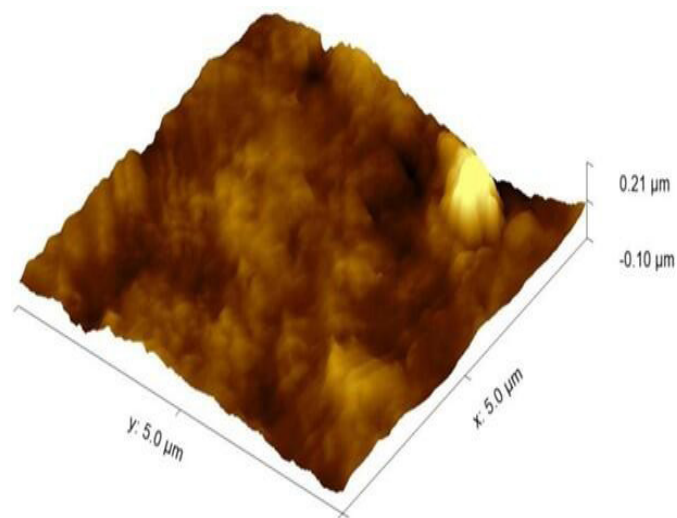
For every immersion, fresh liquid (5ml) each of 5.25% NaOCl, ChNPs, and GO was used. Following the immersion, 5 ml of deionized water was used to completely rinse the samples, and filter paper was used to dry them.

### Atomic force microscopic analysis

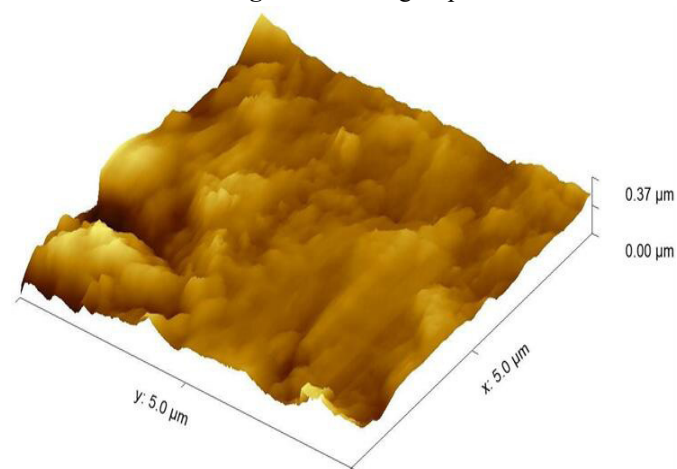
Fast-setting cyanoacrylate glue was used to adhere each sample to a glass slide. Each glass slide was viewed using contact mode imaging on an AFM.[Figures 1-4].



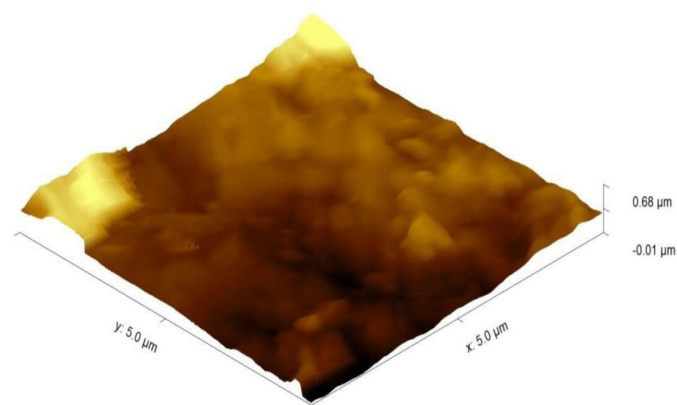
**Figure 1.** Control Group



**Figure 2:** NaCl group



**Figure 3.** Chitosan Nanoparticles Group



**Figure 4.** Graphene Oxide Group

### Statistical Analysis

Statistical analysis of data was done using IBM SPSS software (Version 21.0, Armonk, NY: IBM Corp.). To examine the structure of GP points, Root mean square (RMS) and surface roughness metrics were used. Using one-way ANOVA and post hoc tests, these values were analyzed. P-values below 0.05 were considered to be statistically significant.

## RESULTS

The mean Root mean square (Rq) and Surface roughness (Ra) were statistically significant among the four experimental groups ( $p < 0.05$ ) (Control  $\approx$  Graphene oxide  $\approx$  Chitosan  $<$  NaOCl).

The control group showed the least Rq and Ra values, followed by the Graphene Oxide group, the Chitosan group, and the NaOCl group. [Table 1].

**Table 1.** Mean values of Root mean square (Rq) and Surface roughness (Ra) among the four experimental groups

|    |                   | N  | Mean    | SD<br>Lower<br>Bound | 95% Confidence<br>Interval for Mean |          | Chi<br>square | P<br>Value |
|----|-------------------|----|---------|----------------------|-------------------------------------|----------|---------------|------------|
|    |                   |    |         |                      | Upper<br>Bound                      |          |               |            |
| Rq | Control           | 10 | 20.7920 | 27.70148             | 0.9756                              | 40.6084  | 20.029        | 0.000*     |
|    | Naocl             | 10 | 77.9960 | 47.10196             | 44.3013                             | 111.6907 |               |            |
|    | Chitosan          | 10 | 23.2650 | 7.37640              | 17.9882                             | 28.5418  |               |            |
|    | Graphene<br>Oxide | 10 | 22.9320 | 17.54732             | 10.3794                             | 35.4846  |               |            |
|    | Total             | 40 | 36.2462 | 37.00698             | 24.4108                             | 48.0817  |               |            |
| Ra | Control           | 10 | 13.1490 | 24.06765             | -4.0680                             | 30.3660  | 19.184        | 0.000*     |
|    | Naocl             | 10 | 52.9620 | 21.95538             | 37.2561                             | 68.6679  |               |            |
|    | Chitosan          | 10 | 16.4180 | 12.84918             | 7.2263                              | 25.6097  |               |            |
|    | Graphene<br>Oxide | 10 | 14.4460 | 8.54046              | 8.3365                              | 20.5555  |               |            |
|    | Total             | 40 | 24.2438 | 24.14947             | 16.5204                             | 31.9671  |               |            |

## DISCUSSION

Thorough preparation of the root canal and the application of aseptic methods throughout the procedure are essential for the success of endodontic therapy. The practitioner should be mindful of both external bacterial contamination and endogenous oral microbial flora. [3] Reinfection can occasionally happen even in endodontic procedures that are carried out with the highest level of care. This could be explained by the use of contaminated root filling material. [11] Research carried out by Gomes and colleagues found that the majority of the Cones that had been taken out of their boxes showed signs of contamination. [12]

Since the insertion of GP is the last stage of obliterating the canal of the root, its sterility is crucial to avoiding root canal space contamination. [13] Because these GP cones are thermoplastic, they require immediate chairside disinfection. High concentrations, such as 5.25% NaOCl, were considered superior for the quick decontamination of GP cones. [14] By using AFM, Valois, Silva, and Azevedo (2005) examined the impact of various concentrations of sodium hypochlorite solutions on the surface topography of Gutta percha cones. [15] They observed that topographic alterations were brought on by 2.5% and 5.25% NaOCl. 5.25% NaOCl causes the cones to undergo aggressive topographic changes, which deteriorate them, leading to crystal formation on GP, according to earlier findings. These crystal forms were more noticeable, according to certain investigations, following quick sterilization with

NaOCl. [5] 5.25% NaOCl, which is considered a strong oxidising agent, caused GP cone components to be lost. [16]

Deep abnormalities brought on by GP cone degeneration can lead to gaps forming between the obturating material and the dentin, thereby increasing the risk of fluid seepage. [17] When resin sealers are used in root canals, they effectively fill the gaps that appear after the GP cones are cleaned using a 5.25% NaOCl solution. However, once the sealers set, they shrink, leaving behind empty areas that could raise the risk of leaking.

[8] Similar outcomes that impact the postoperative prognosis were also demonstrated by the current study.

The nanoparticles have antibacterial action even at low concentrations because of their higher surface area and smaller size. [2] 1.5 mg/ml ChNPs is used in this investigation since prior research has demonstrated superior antibacterial efficacy. [18] The antibacterial activity of GP points impregnated with chitosan was higher than that of commercial ones, and it increased with increasing chitosan percentage. Chitosan's polycationic properties interact with bacteria's negatively charged surfaces to change cellular permeability, giving it its antibacterial properties. These particles lessen the chance of bacterial penetration by preventing bacterial enzymatic breakdown. An excellent substitute for obtaining GP points with enhanced antibacterial qualities could be to impregnate them with chitosan. [9]

GO, a novel disinfecting material used in this study. Graphene, a two-dimensional carbonaceous substance that is a crucial structural component of graphite, is one of the most promising nanomaterials.

Since graphene was synthesized, other derivatives, including GO and reduced GO, have been investigated. The main advantages of GO are its affordability, simple manufacturing process, and ability to target multiple platforms for killing.

Graphene oxide possesses outstanding antibacterial qualities and have been used as antimicrobial nanomaterials because of their

capacity to generate reactive oxygen species and damage the integrity of bacterial cell membranes.

The following three primary antibacterial actions have been documented:

1. GO is capable of causing oxidative stress.
2. The sharp edges of GO sheets can physically disrupt microorganisms by severing their membranes.
3. Capable of enclosing and separating environmental microorganisms to prevent them from finding nourishment, hence halting their growth.

GO is a considerable material for many dental applications, such as in the areas of endodontics and restorations.[19]

The lowest concentration that killed bacteria was 40 µg/mL, and higher concentrations, such as 80 µg/mL, made the bacterial biofilms no longer viable. The GO film demonstrated good adhesion to root dentin while maintaining its structural integrity, and it also helped prevent the growth of bacterial films. [13]

In this study, an atomic force microscope was used because it provides improved 3D imaging of molecular surfaces with nanoscale clarity and avoids the necessity for additional treatments (like metal coating), minimizing the risk of damage.

Although the SEM requires a vacuum atmosphere to work, the AFM can function well in both liquid and air. [10]

In this study, it was found that NaOCl-treated GP exhibited the most significant surface alterations, aligning with the findings of the previous studies. [20] In comparison to NaOCl, both GO and ChNPs showed fewer surface changes. Thus, these nanoparticles could be a great option for sterilizing GP cones.

### Limitations

While the mentioned nanoparticles show minimal surface degradation, more research is needed to explore their surface energy and wetting properties. This will help determine if they are appropriate for use as irrigation solutions alongside sealers.

### CONCLUSION

Given the current drawbacks of this in vitro study, it appears that GO and ChNPs showed least surface deterioration in comparison to NaOCl. As a result, these novel disinfecting agents could serve as a great option for gutta-percha disinfection.

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