ORIGINAL ARTICLE

Surface Topographic Analysis of Gutta-percha Using Novel Disinfecting Materials by Atomic Force Microscopy: An *In Vitro* Study

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Abstract

Aim: The present *in vitro* study was undertaken to assess the surface topographic changes of Gutta-percha (GP) cones after disinfection with a new disinfecting material and to compare the same with sodium hypochlorite (NaOCl) and chitosan nanoparticles using an atomic force microscope (AFM).

Materials and methods: Forty gutta-percha cones (ISO Size 30, 6% taper, DIADENT) were taken and randomly divided into four groups: Group A – control (untreated gutta-percha cones, $n = 10$), group B – 5.25% NaOCl ($n = 10$), group C – Curcumin Nano-dispersion ($n = 10$), and group D – Chitosan Nano-dispersion ($n = 10$) respectively. After being treated with the respective disinfection materials for 1 minute, the surface topography of the gutta-percha cones was assessed using atomic force microscopy.

Results: Among the experimental groups, the root mean square (RMS) values and surface roughness (Ra) values were highest for sodium hypochlorite and least for the chitosan group. For the curcumin nanoparticle group, the values were between these two groups.

Conclusion: Within the scope of this *in vitro* study, it is evident that the groups treated with chitosan and curcumin nanoparticles experienced less surface deterioration than those treated with NaOCl. Consequently, these nanoparticles could be considered alternative disinfectants for gutta-percha cones.

Clinical significance: The success of root canal treatment depends on the complete removal of microorganisms from the root canals. To maintain aseptic conditions, gutta-percha disinfection is required. Chitosan nanoparticles and curcumin nano-dispersion can be a promising disinfecting agent for gutta-percha cones with less surface topographic changes.

Keywords: Atomic force microscopy, Chitosan nanoparticles, Curcumin nanodispersion, Gutta percha, Original article, Surface topography.

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Introduction

Ensuring meticulous disinfection and employing aseptic techniques are pivotal for the success of root canal therapy. It's imperative that all instruments and materials introduced into the root canal are sterilized. Introducing a potentially contaminated filling material into a sterile root canal can lead to re-infection and compromise the treatment's success. Therefore, disinfecting the obturating material is essential.

Gutta-percha stands as the predominant obturation material in root canal therapy. Despite gutta-percha cones being made under sterile conditions, they can become contaminated through handling, exposure to aerosols, and during storage and treatment. Typically, chemical disinfectants are employed for gutta-percha disinfection. These include sodium hypochlorite (NaOCl), chlorhexidine digluconate, isopropyl alcohol, and hydrogen peroxide. While ethylene oxide gas can also be used to disinfect gutta-percha cones, its usage is time-intensive and not practical for routine clinical applications.[1]

About 5.25% NaOCl is commonly used in clinics to disinfect gutta-percha due to its wide-ranging effectiveness. However, issues associated with NaOCl have prompted us to seek alternative antimicrobial agents with fewer side effects. Nanoparticles, specifically curcumin and chitosan, are being explored for their antimicrobial properties.[2,3] Therefore, this research was undertaken to evaluate the surface topographical modifications of gutta-percha when treated with these innovative substances and to contrast these changes with those induced by 5.25% sodium hypochlorite.[4]

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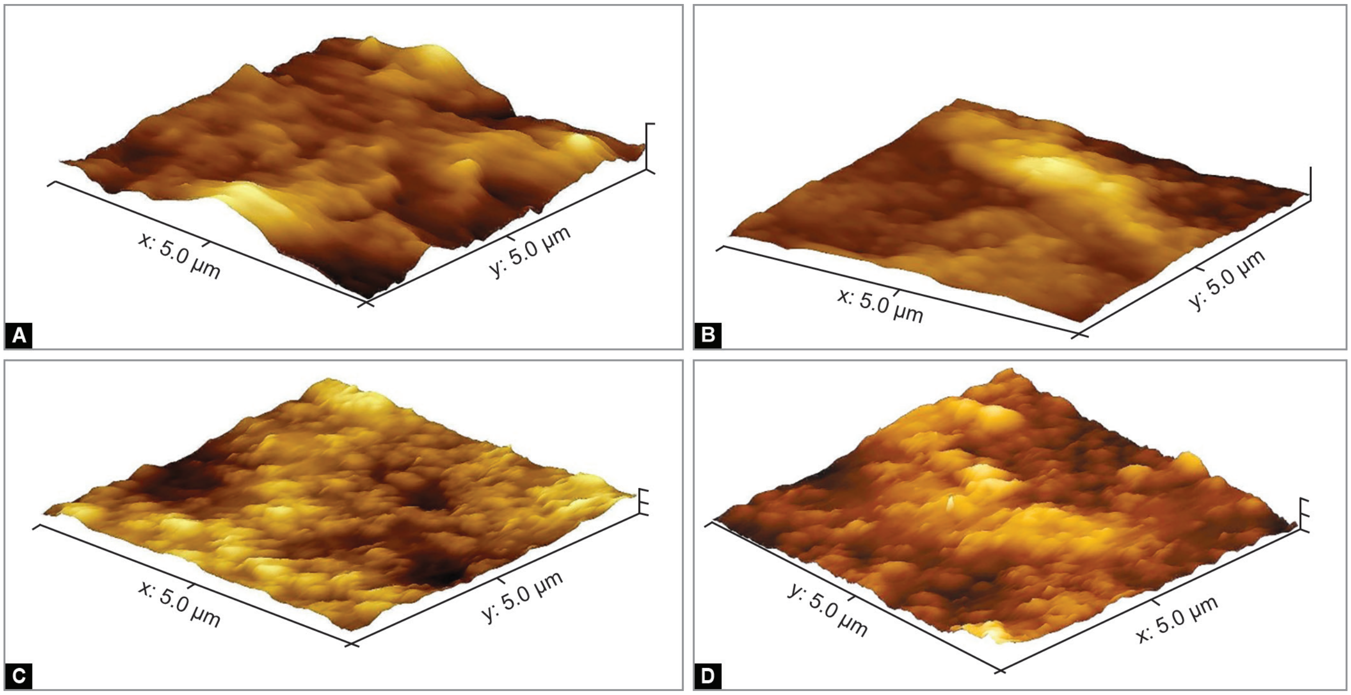
Materials and Methods

The sample size was determined using G-power software version 3.1. Forty gutta-percha cones (ISO size 30, 0.06 taper, DIADENTDia-Pro) were chosen randomly from the identical batch.[5]

Sample Preparation

The selected samples were cut 3 mm from their apex. Samples were grouped into one control and three experimental groups with a sample size of 10 per group: ($n = 10$)



Figs 1A to D: Atomic force microscopic images of gutta-percha. (A) Control group; (B) Sodium hypochlorite group; (C) Chitosan nanoparticles group; (D) Curcumin nanoparticles group

- Group A: Control group (gutta-percha cones left untreated).
- Group B: Gutta-percha cones immersed in 5.25% sodium hypochlorite (Prime Dental Pvt. Ltd, Thane, Maharashtra, India) for 1 minute.
- Group C: Gutta-percha cones immersed in 1.5 mg/mL of chitosan nanoparticles (Nano Research Lab Pvt. Ltd., Jamshedpur, Jharkhand, India) for 1 minute.
- Group D: Gutta-percha cones immersed in 0.5 mg/mL of curcumin nanoparticles (Nano Research Lab Pvt. Ltd., Jamshedpur, Jharkhand, India) for 1 minute.

The samples were grouped and then immersed in 5mL of each of the following solutions for their respective immersion periods: 5.25% NaOCl, 1.5 mg/mL chitosan, and 0.5 mg/mL curcumin nanoparticles.[6] Following immersion, the samples were meticulously rinsed with deionized water and dried using filter paper.

Atomic Force Microscopic Analysis

The study samples were fixed to a glass slab using glue. Subsequently, each sample was examined under an atomic force microscope (AFM) (JPK Nano Wizard) with contact mode imaging. The study employed standard AFM probes of the PPP-CONTSCR variety, characterized by a resonance frequency of 25 kHz. Surface parameters like average roughness (Ra) and root mean square roughness (RMS) were chosen to assess the surface topographic changes of gutta-percha cones. Atomic force microscope images of the sample are shown in Figure 1.

Statistical Analysis

The statistical analysis was conducted using IBM SPSS Version 21.0 from Armonk, New York. Root mean square and surface roughness parameters served as metrics for evaluating the surface characteristics of gutta-percha. To assess the differences among the groups, a one-way ANOVA was performed, followed by *post hoc* Tukey's honestly significant difference tests.

Results

The RMS values and Ra values exhibit maximum surface alterations for sodium hypochlorite (Ra: 52.64 nm; RMS: 66.40 nm) followed by curcumin nanoparticles (Ra: 44.26 nm; RMS: 54.54 nm), chitosan nanoparticles (Ra: 24.94 nm; RMS: 35.45 nm) and control group (Ra: 15.69 nm; RMS: 23.38 nm). These results were statistically significant (Table 1). Among the study groups chitosan nanoparticle group showed the least surface alterations, and the curcumin nanoparticle group showed less surface alterations when compared to the sodium hypochlorite group.[7]

Comparison of Ra and RMS roughness between the study groups using one-way ANOVA and multiple pairwise comparisons of Ra and RMS between the study groups using *post-hoc* test showed the statistical significance of the data (Tables 2 and 3).[8]

Discussion

Improper disinfection of root canals and obturation materials can lead to endodontic failure and the recurrence of periapical pathosis. A study by Gomes et al. found that 5.5% of gutta-percha cones removed from their packaging were contaminated.[9] Therefore, there is a necessity for disinfecting gutta-percha cones.

As the gutta-percha cones are thermoplastic in nature, a quick chair side chemical disinfection or ethylene oxide sterilization is needed. In day-to-day endodontic practice, NaOCl is the most commonly used disinfectant. Apart from NaOCl, other disinfecting agents like 2% chlorhexidine, 2% glutaraldehyde, 3% hydrogen peroxide, MTAD, etc., are also used as gutta-percha disinfectants.

Table 1: Descriptive statistics of Ra and RMS

						95% confidence interval for mean			
Parameter	*Group*	*N*	*Mean*	*Std. deviation*	*Std. error*	*Lower bound*	*Upper bound*	*Minimum*	*Maximum*
Ra	Control	10	15.6900	1.80028	0.56930	14.4022	16.9778	13.70	19.70
	NaOCl	10	52.6400	6.73122	2.12860	47.8248	57.4552	44.40	63.40
	Chitosan Nano	10	24.9400	3.43356	1.08579	22.4838	27.3962	20.70	29.40
	Curcumin Nano	10	44.2600	6.63747	2.09895	39.5118	49.0082	34.50	53.20
RMS	Control	10	23.3800	1.76748	0.55893	22.1156	24.6444	20.00	25.30
	NaOCl	10	66.4000	7.77889	2.45990	60.8353	71.9647	56.10	79.60
	Chitosan Nano	10	35.4500	4.79404	1.51601	32.0206	38.8794	28.40	42.00
	Curcumin Nano	10	54.5400	8.32843	2.63368	48.5822	60.4978	40.60	62.40

Table 2: Comparison of Ra between the study groups

					95% confidence interval for mean			
Group	*N*	*Mean*	*Std. deviation*	*Std. error*	*Lower bound*	*Upper bound*	*f-value*	*p-value*
Control	10	15.69	1.80028	0.56930	14.4022	16.9778	111.04	<0.001*
NaOCl	10	52.64	6.73122	2.12860	47.8248	57.4552		
Chitosan Nano	10	24.94	3.43356	1.08579	22.4838	27.3962		
Curcumin Nano	10	44.26	6.63747	2.09895	39.5118	49.0082		

One-way analysis of variance; $p \leq 0.05$ considered statistically significant; * denotes significance

Table 3: Comparison of RMS between the study groups

					95% confidence interval for mean			
Group	*N*	*Mean*	*Std. deviation*	*Std. error*	*Lower bound*	*Upper bound*	*f-value*	*p-value*
Control	10	23.38	1.76748	0.55893	22.1156	24.6444	94.67	<0.001*
NaOCl	10	66.4	7.77889	2.45990	60.8353	71.9647		
Chitosan Nano	10	35.45	4.79404	1.51601	32.0206	38.8794		
Curcumin Nano	10	54.54	8.32843	2.63368	48.5822	60.4978		

One-way analysis of variance; $p \leq 0.05$ considered statistically significant; * denotes significance

Senia et al.,[2] proposed a technique for disinfection of gutta-percha cones using 5.25% NaOCl. However, some studies reported that deterioration and chloride-crystal formation was seen on gutta-percha after sterilization with sodium hypochlorite.[10]

The deterioration of gutta-percha cones leads to the formation of deep irregularities, resulting in gaps between the gutta-percha cones and the walls of the root canal. While these irregularities are filled with resin sealers during the coating process in root canals, the shrinkage of the set sealers can leave empty spaces behind. This could potentially elevate the risk of microleakage.[11] Hence, in this study, a novel disinfectant material, curcumin nano dispersion and chitosan nano dispersion were used, and surface topographic changes were analyzed using atomic force microscopy.

Due to their small size, nanoparticles have a significant surface area relative to their volume. They display antimicrobial properties even at low concentrations. In this research, a concentration of 1.5 mg/mL of Chitosan nanoparticles (ChNPs) was used due to its demonstrated strong antimicrobial activity.[12] The antibacterial effectiveness of chitosan stems from its polycationic characteristics, which interact with the negatively charged components of bacteria, changing their cellular permeability. Additionally, it hinders bacterial enzymatic breakdown, decreasing the chance of bacterial infiltration. Chitosan-impregnated gutta-percha points show a high antimicrobial activity which increases with the concentration of chitosan used for impregnation.[13,14]

In the present study, a novel disinfectant material, 0.5 mg/mL curcumin nano-dispersion is used. Although curcumin exhibits promising antibacterial activity, a major drawback of curcumin, when used alone, is its low bioavailability, which is due to its poor absorption, quick metabolism, and rapid elimination.

Nano-curcumin is suggested to enhance the bioavailability of curcumin. Curcumin nanoparticles ranging from 2 to 40 nm demonstrate notable antibacterial effects against *Staphylococcus aureus*, *E. coli*, and *pseudomonas aeruginosa*. Various antimicrobial mechanisms are proposed for curcumin, including disrupting bacterial cell membranes, inhibiting bacterial DNA replication, reducing bacterial motility, and modifying bacterial gene expression. Curcumin-coated GP showed a greater antibacterial activity as did tetracycline integrated gutta-percha and gutta-percha coated with antibiotics. In addition to its antimicrobial activity, curcumin exhibits anti-inflammatory, antiviral, and antifungal activity.[15] Also, in a study by Ammayappan and Moses curcumin shows better antibacterial activity than chitosan.[16,17]

In this study, AFM is employed because of its ability to view molecular surfaces with a nanometer-scale resolution. Atomic force microscope has gained widespread recognition as a reliable technique for visualizing three-dimensional surfaces and interfaces of diverse materials.[18–21] Unlike scanning electron microscopy (SEM), AFM allows for both qualitative and quantitative analysis of materials. Scanning electron microscopy often damages delicate samples due to its aggressive sample preparation methods like sputtering, dehydration, and vacuum exposure. Such damage can be circumvented when using AFM. Moreover, AFM does not necessitate special treatments like metal coating, as required by SEM, and can function effectively in various environments, including air and liquid.[22–28]

In this research, gutta-percha treated with 5.25% NaOCl exhibited the most significant surface alterations, consistent with studies conducted by Valois et al. and Tilakchand et al. In comparison, both chitosan nanoparticles and curcumin nanoparticles demonstrated fewer surface changes. Therefore, these nanoparticles could serve as viable alternatives for disinfecting gutta-percha cones.[29–34]

Limitations

Although these nanoparticles demonstrate reduced surface topographic changes, further studies are required to examine their surface energy and wetting characteristics. This will enhance our understanding of their compatibility with sealers employed as irrigating solutions.

Conclusion

Within the scope of this *in vitro* study, it is evident that the groups treated with chitosan and curcumin nanoparticles experience less surface deterioration than those treated with NaOCl. Consequently, these nanoparticles could be considered as alternative disinfectants for gutta-percha cones.

Clinical Significance

Chitosan nanoparticles and Curcumin nano-dispersion are promising antimicrobial agents for disinfecting gutta-percha cones used in root canal treatment. They offer a strong antimicrobial action, cause minimal cone structure changes, and are biocompatible, thereby enhancing treatment success by ensuring effective disinfection while maintaining cone integrity.

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