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Antibacterial Efficacy of Surface Modified Gutta Percha with Zinc Oxide Nanoparticle

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ABSTRACT

Background: Endodontic treatment may fail due to secondary or persistent infection. Failure of endodontic treatment may arise from the microorganisms that survived root canal therapy and those in root canal filling materials.

Aim: The aim of this study was to increase the antimicrobial efficacy of commercial Gutta percha cones by modifying the surface with thin deposition of zinc oxide nanoparticles.

Materials and methods: Zinc oxide nanoparticles were synthesized using a chemical precipitation method and incorporated into a polyvinyl acetate (PVA) polymer solution for surface coating. Commercial gutta-percha cones were sterilized and divided into two groups: uncoated (control) and ZnO nanoparticle–coated. The antibacterial activity against *Enterococcus faecalis* was evaluated using a direct contact test, and bacterial growth was assessed by colony-forming unit (CFU) counts.

Result: Zinc oxide nanoparticle–coated gutta-percha cones demonstrated markedly greater antimicrobial efficacy than uncoated cones, showing nearly a ten-fold reduction in *Enterococcus faecalis* colony counts.

Conclusion: Within the limitations of this in vitro study, zinc oxide nanoparticle coating significantly enhanced the antimicrobial activity of gutta-percha cones against *Enterococcus faecalis*, suggesting its potential as an effective modification to reduce bacterial persistence in root canal therapy.

Keywords: *Antimicrobial activity, Dip coating, E. faecalis, Gutta Percha, zinc oxide nanoparticles*

1. INTRODUCTION

Endodontic therapy targets a tooth's infected pulp with the goals of curing the infection and preventing reinfection[1,2]. The preservation of teeth through root canal therapy is far more beneficial than having them extracted[3]. Following the excision of the diseased pulp tissue, the root canal area is prepared, filled, and cleaned with a core root filling substance[2,4,5]. The success rate of root canal therapy has increased as a result of developing technology, a better knowledge of the anatomy of the root canal, and the use of better biocompatible materials[3,6]. Irrigating the root canal system and providing adequate biomechanical preparation are essential to the success of endodontic treatment[7]. The objective of root filling is to preserve the aseptic chain established during the earlier stages of root canal therapy[6]. There is evidence of a minor antibacterial effect for gutta-percha cones. But the impact is too slight for this substance to function as a reliable microbiocide. Despite the fact that GP has a long history of use, biocompatibility, cost effectiveness, ease of removal and many other benefits, one of the challenges in endodontic therapy is its inability to provide a sufficient seal to prevent bacterial percolation[8]. For example, a poor root canal seal around voids in the obturated root canal space can lead to leaks because GP and the dentinal surface do not bond [4,5]**.** When gutta-percha cones were tested for antibacterial qualities, Moorer and Genet discovered that zinc oxide, which is the primary ingredient in gutta-percha, was in charge of some of the cones' antimicrobial qualities. To improve the adherence and sealing capacity of root canal filling materials to the dentin and the sealer, the idea of coating the GP was introduced [3]. But commercially available materials coated GP with glass ionomer-based or methacrylate resin-based materials were unable to stop leaks and hermetically seal the root canal.[9]. The low melting point and tapered shape of GP are among its properties that make coating difficult [10].

Nanotechnology has opened new avenues in dentistry, and zinc oxide nanoparticles (ZnO NPs) are among the most widely explored due to their unique physicochemical properties and potent antimicrobial effects. Zinc, an essential trace element naturally present in hard and soft tissues, forms ZnO NPs that are FDA-recognized as safe and exhibit tunable optical, catalytic, and biological characteristics, which improve as particle size decreases[4]. Their antimicrobial activity is attributed to multiple mechanisms, including the generation of reactive oxygen species (ROS), release of H_2O_2, disruption of microbial enzymes via interaction with –SH groups, displacement of magnesium ions affecting bacterial metabolism, and the "Trojan Horse effect," whereby acidic lysosomal degradation enhances ion release and cellular toxicity[11][4]. These actions collectively impair protein function, damage DNA, and inhibit microbial replication, making ZnO NPs effective against biofilm-forming pathogens[12][13]. Owing to their high surface-to-volume ratio and enhanced reactivity, ZnO NPs have been incorporated into various dental materials, and their application in modifying gutta-percha surfaces offers a promising strategy to improve its antimicrobial efficacy and reduce the risk of persistent root canal infections.

Endodontic therapy may not be successful if the root canal system has a persistent or secondary infection. Specifically, the highly resilient intra-canal pathogen Enterococcus faecalis is frequently isolated in endodontic failure[14]. Additionally, despite the fact that GP cones are made in an aseptic environment, a number of investigations have shown the presence of microorganisms in recently opened boxes and this contamination rises with [14] incorrect handling, storage, and aerosol application. One of the most frequent microbes discovered in stored GP cones following improper handling is Staphylococcus spp. As a result, a number of physicochemical strategies have been documented with the goal of boosting GP cones' antimicrobial efficacy while maintaining its filling requirements. GP cones contain approximately 20% GP (matrix), 66% zinc oxide (filler), 11% heavy metal sulfates (radiopacifier) and 3% waxes and/or resins (plasticizer)[15]. The present work proposes a novel approach to increase the antibacterial efficacy of GP cones, namely by modifying the surface of the GP cones with thin deposition of zinc oxide nanoparticles.

2. MATERIALS AND METHODS

Extraction of zinc oxide nanoparticle

Zinc oxide nanoparticles (ZnO NPs) were synthesized using a chemical precipitation method. Briefly, zinc acetate dihydrate was dissolved in distilled water to prepare a 0.1 M solution, and sodium hydroxide solution (0.2 M) was prepared separately. Under continuous magnetic stirring, the NaOH solution was added dropwise to the zinc acetate solution until the desired pH (8 or 11) was achieved, resulting in the formation of a white precipitate. The suspension was stirred for 30 minutes and allowed to age to ensure complete reaction. The precipitate was collected by centrifugation, washed several times with distilled water and ethanol to remove impurities, and then dried in a hot air oven at 80 °C for 5 hours. The dried product was subsequently calcined in a muffle furnace at 400 °C for 1 hour to obtain pure, crystalline ZnO nanoparticles.

The resultant fine powder was stored in an airtight container until further use.The morphology and surface characteristics of the synthesized ZnO nanoparticles were examined using Scanning Electron Microscopy (SEM). A small amount of the calcined nanoparticle powder was evenly spread on a carbon-coated grid, sputter-coated with a thin layer of gold to enhance conductivity, and imaged under SEM at suitable magnifications.SEM analysis confirmed the successful synthesis of ZnO nanoparticles, revealing predominantly spherical particles with uniform distribution and nanoscale dimensions[Fig 1].

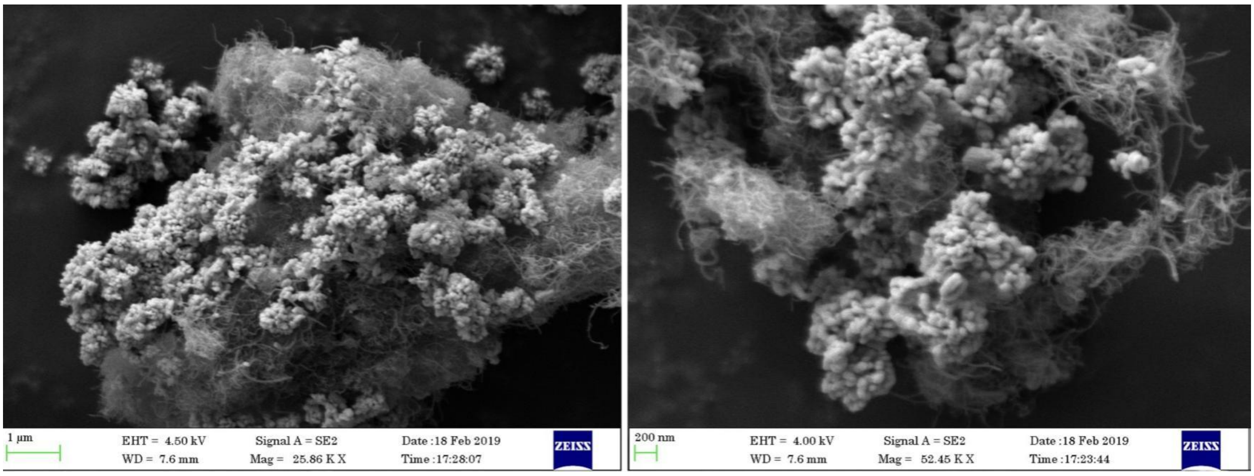

Fig 1: SEM image of pure zinc oxide nanoparticle

Coating of gutta percha

To achieve nanoparticle surface modification, a 1% zinc oxide nanoparticle (ZnO NP) suspension was prepared by incorporating the synthesized ZnO NPs into a polyvinyl acetate (PVA) polymer solution, which served as a binding medium to ensure uniform coating. Freshly opened GP cones (Size 80, Dentsply Maillefer, Switzerland) were sterilized with ethylene oxide and divided into two groups: Group A (uncoated GP, control) and Group B (ZnO NP-coated GP, experimental). For coating, cones were immersed in the ZnO NP–PVA solution using the dip-coating technique, which allows for three-dimensional, conformal coverage of the GP surface with a thin ZnO film. After dipping, the cones were gently air-dried under sterile conditions at room temperature to facilitate solvent evaporation and adhesion of nanoparticles, followed by oven-drying at 37 °C to ensure complete film formation. This process resulted in a uniform, adherent ZnO nanoparticle coating over the GP cones, enhancing their surface properties while maintaining their dimensional stability.

Antimicrobial activity

The antibacterial activity of the coated and uncoated gutta-percha cones was evaluated against *Enterococcus faecalis*. Standardized GP samples were incubated with *E. faecalis* cultures in nutrient broth at 37 °C for 24 hours to allow bacterial adherence and colonization [Fig. 2]. Following incubation, the bacterial suspension was serially diluted at a 1:9 ratio using sterile saline to obtain a workable inoculum concentration. From each dilution, 0.1 mL was aseptically pipetted and dispensed onto the center of freshly prepared nutrient agar plates. The inoculum was evenly spread across the agar surface using a sterile glass spreader, while gently rotating the Petri dish to ensure uniform distribution of bacterial cells. The plates were then incubated aerobically at 37 °C for 24 hours. After the incubation period, visible colonies were observed and manually enumerated. The number of colonies formed on each plate corresponded to the viable bacterial load, expressed as colony-forming units (CFU/mL), which was subsequently compared between experimental (ZnO NP-coated GP) and control (uncoated GP) groups to determine antibacterial efficacy[Fig 2].

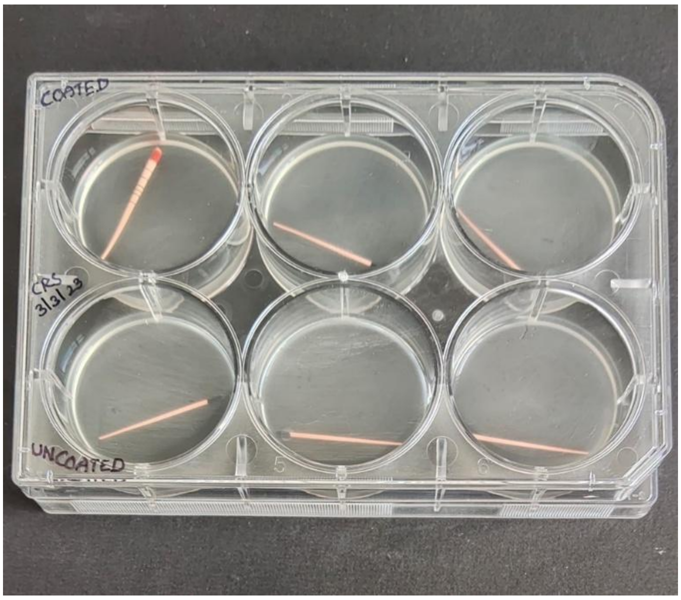

Fig 2 : Direct contact assay showing ZnO nanoparticle-coated and uncoated gutta-percha cones in a 6-well plate.

3. RESULTS

The antibacterial evaluation revealed that gutta-percha cones coated with ZnO nanoparticles demonstrated markedly enhanced antibacterial activity against *E. faecalis* when compared with uncoated control cones. Plates inoculated with suspensions from the uncoated cones (control) exhibited dense bacterial growth with innumerable colonies, whereas those associated with ZnO-coated cones showed a sparse distribution of colonies, indicating nearly a ten-fold reduction in viable bacterial counts [Fig 3]. This pronounced decrease highlights the efficacy of ZnO nanoparticles in inhibiting bacterial adhesion and proliferation on the GP surface.

The improved antibacterial effect can be attributed to multiple mechanisms inherent to ZnO nanoparticles, including their ability to generate reactive oxygen species (ROS), disrupt bacterial membrane integrity, release Zn^{2+} ions, and interfere with essential enzymatic and metabolic pathways. The nanoparticle coating thus creates a bioactive surface that is unfavorable for bacterial colonization and survival.

Furthermore, the findings suggest that the antibacterial activity observed is primarily mediated through interfacial reactions at the bacteria-surface interface, which is consistent with previously reported mechanisms of ZnO. By preventing the initial adhesion and colonization of *E. faecalis*, ZnO-coated GP cones may play a critical role in minimizing reinfection risks during root canal therapy. These results validate the potential of ZnO nanoparticle coatings as an innovative surface modification strategy to enhance the antibacterial performance of endodontic obturating materials.

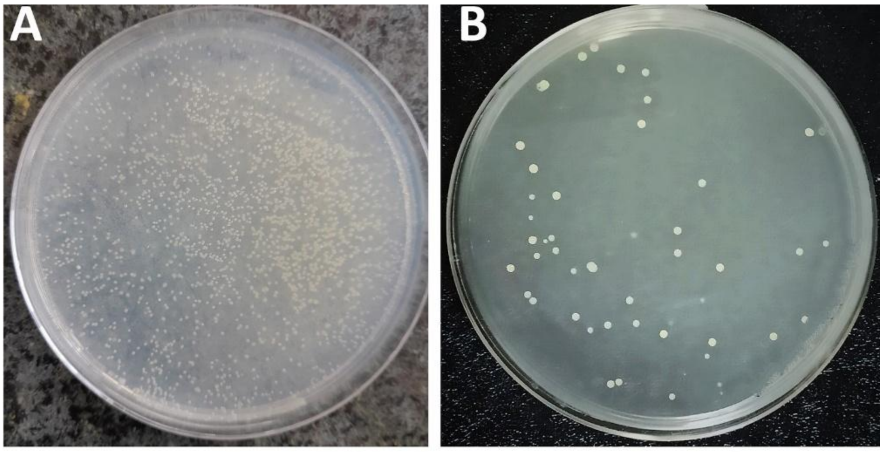

Fig 3 : Antibacterial activity of Uncoated(A) and Coated GP(B)

4. DISCUSSION

Ensuring a three-dimensional seal of the root canal system while effectively preventing both coronal and apical leakage is essential for the success of root canal treatment[7,16]. Endodontic treatment failure may occur due to microorganisms that withstand chemical and mechanical debridement of the root canal or persist within the filling materials[17,18]. A previous study suggested an innovative strategy to enhance the antibiofilm activity of GP by first modifying its surface with Argon (Ar) plasma treatment (PT), followed by the application of a ZnO thin film[6]. Plasma treatment (PT) is widely used for surface modification and functionalization in a controlled and reproducible way without altering the bulk properties of the material[19]. However, the outcomes depend heavily on factors such as energy input, pressure, gas composition, and substrate type, influencing processes like cleaning, activation, etching, or thin-film deposition[20]. In contrast, our study employed the dip-coating method, which offers a simpler, cost-effective, and scalable approach to achieve uniform ZnO nanoparticle coatings on gutta-percha surfaces.

Several modifications of gutta-percha (GP) have been explored to enhance its antibacterial properties, with promising outcomes. Studies have reported that coating GP with agents such as silver-curcumin nanoparticles, chitosan, povidone-iodine, nanocurcumin, and tetracycline significantly improves its efficacy against resistant endodontic pathogens, primarily *E. faecalis[21][22][22,23]*. The enhanced activity has been attributed to mechanisms such as oxidative stress induction, biofilm inhibition, release of antibacterial ions, and interference with microbial membrane integrity and protein synthesis. In line with these findings, our study demonstrates that ZnO nanoparticle-coated GP exhibits superior antibacterial activity compared to conventional GP. This supports the growing body of evidence that nanoparticle modification of GP can substantially improve its antimicrobial performance, thereby reducing the risk of endodontic treatment failure caused by persistent microbial infection.

In recent years, ZnO nanoparticles (ZnO-NPs) have been extensively investigated for their applications in endodontic materials. Studies have shown that ZnO-NPs enhance antibacterial activity against *Enterococcus faecalis*, a major pathogen linked to endodontic treatment failure[24] . Additionally, ZnO-NPs support the immune response by helping to reduce inflammation associated with *E. faecalis* infections [25]. In light of these findings, it is important to note that gutta-percha itself possesses inherent antibacterial properties, including activity against *E. faecalis*. This observation is consistent with Moorer et al. , who attributed such effects to zinc oxide, the major component of gutta-percha cones. This study further supports this concept, as the supplementation of gutta-percha with an external ZnO nanoparticle coating significantly amplified its antibacterial efficacy[26]. The enhanced reduction in *E. faecalis* colonies observed in the coated group underscores the role of zinc oxide not only as a structural component but also as a potent antimicrobial agent when introduced in nanoparticulate form, thereby improving the overall performance of gutta-percha in endodontic applications.

5. CONCLUSION

Within the limitations of this study, zinc oxide nanoparticle coating was found to significantly enhance the antimicrobial activity of gutta-percha cones against *Enterococcus faecalis*. The coated cones demonstrated nearly a ten-fold reduction in bacterial colonies compared to uncoated controls, indicating that ZnO nanoparticle surface modification is an effective approach to improve the antibacterial properties of gutta-percha.

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