

# Antibiofilm Efficacy of Surface Modified Gutta Percha with Zinc Oxide Nanoparticle

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# Introduction

The gold standard of care for treating infected pulp tissue in teeth and preventing further infection is root canal therapy. Microorganisms have frequently been the cause of infections in the periapical tissue and dental pulp. The infected pulp tissue is removed, and the root canal space is then prepared, cleaned, and filled with a core root filling material [1]. While the microbiota in the infected root canal is considerably reduced by irrigation and instrumentation during biomechanical preparation to a level that is conducive to healing, total eradication is not achievable. Gutta percha (GP) is biocompatible, inert, and malleable, it is commonly used as the filler material. Because the therapeutic result of endodontic therapy depends on sealing the root canal space throughout the root canal, the final obturation step is essential [2]. The last element of the endodontic triad is the full, three-dimensional, fluid-tight seal of the root canal system. Gutta-percha (GP) is the most established and closest material that satisfies this requirement.

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 insufficient seal to stop bacterial percolation. A persistent or secondary root canal system infection may be the cause of endodontic treatment failure. Specifically, the highly resistant intra-canal pathogen Enterococcus faecalis is frequently isolated in endodontic failure [3]. The importance of aseptic (sterile) conditions during endodontic procedures is to prevent the introduction of bacteria into the root canal system. If gutta-percha is improperly handled or contaminated during the procedure, it could potentially introduce bacteria into the filled canal, leading to infection and treatment failure [4]. Concerning the issue, it is the need of the hour to look for alternative coating materials for gutta-percha cones to increase their

antimicrobial efficacy for a successful outcome of the root canal therapies [5]. This study is a novel approach to increase antimicrobial and antibiofilm activity of gutta percha by modifying the surface of gutta percha with thin deposition of zinc oxide nanoparticle.

# Materials and Method

#### Coating Gutta Percha with Zinc Oxide Nanoparticle

Pure zinc oxide nanoparticles are extracted for coating Gutta percha cones. Zinc acetate and sodium hydroxide which has pH of 8,11 are mixed and stirred for 30 minutes. The mixture was dried in the hot air oven at 80 degree celsius for 5 hours followed by calcination at 400 degree celsius for 1 hour. The resultant product is the pure zinc oxide nanoparticle.

Pure zinc oxide nanoparticles were incorporated in PVA-polyvinyl acetate polymer which acted as a medium to coat gutta percha surface with zinc oxide nanoparticle. GP cones (Size 80, Dentsply Maillefer, Switzerland) from freshly opened boxes were organized into two groups-(A-Uncoated GP, B-Coated GP) (Fig ure1). Gutta percha cones are coated with zinc oxide by dipping in PVA polymer which is incorporated with zinc oxide nanoparticles. Dip coating helps to get 3-dimensional coating of Gutta percha cones with thin zinc oxide film.

#### Antibiofilm Activity

A 96-microtitre well plate was used to conduct a quantitative investigation on biofilm development Freshly grown bacteria were added to Brain Heart Infusion (BHI) broth and the mixture was then incubated for 72 h at 37°C. The cell suspensions were diluted at a ratio of 1:100 in the freshly made BHI broth medium after 24 h. Bacterial cells that were not exposed to ZnO NPs were regarded as the positive con-



trol. ZnO NPs were also added to the treated bacterial cultures at a concentration ranging from 25, 50, 75 to  $100\mu g/mL$ . The sterile BHI broth medium remained empty. Then,  $200\mu L$  culture suspensions with and without ZnO NPs treatment were added to the sterilised 96-well microplates, which were then incubated for a further 24h at 37°C with-

out shaking. Three replicates of each bacterial suspension were stored. By rotating the plates over, all of the treated and untreated cells in the microtiter wells were discarded. Free-floating cells and undesirable material were then removed by washing the plates three times in phosphate buffered saline (PBS, pH 7.2) (Figure 2).

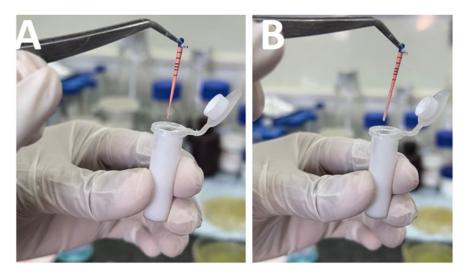


Figure1: A -Uncoated GP, B - Coated GP

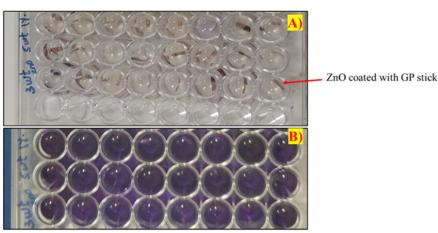


Figure 2: A) Pre crystal violet staining applied to S. mutans biofilm & B) Post crystal violet staining applied to S. mutans biofilm

# **Results and Discussion**

Microbes that endure chemical and mechanical root canal debridement and those that live in filling materials may be the cause of endodontic treatment failure. Biofilms are described as bacterial populations adherent to surfaces or interfaces, each other, and/or a polysaccharide matrix [6]. Biofilm formation is initiated by bacterial deposition on a surface and irreversible adhesion to the substratum [7]. In the initial stages of biofilm formation, the adhesive property of bacterial cells play a major role for irreversible attachment to surfaces and is also influenced by the formation of a conditioning film on the surface as a result of interactions between the substratum and the surrounding environment [6,8]. To address this challenge, this work explored a novel approach to enhance the antimicrobial efficacy of commercial GP cones. This procedure involved deposition of a ZnO thin film by dip coating. The resulting surfaces were evaluated for the antibiofilm efficacy ("Bacterial Leakage in Coronally Unsealed Root Canals Obturated with 3 Different Techniques" 2000). Chairside disinfection of gutta percha with sodium hypochlorite results in significant surface changes. It will affect the physical and mechanical properties of GP points and results in poor obturation sealing and it will increase the susceptibility of biofilm formation [9,10]. Zinc oxide coated Gutta percha showed higher antibacterial activity than uncoated GP. This proves that the surface bacteria interaction is the major reason behind antibacterial activity of coated GP.

Both chemical and physical interfacial mechanisms, as well as the electrostatic interaction between positively charged ZnO and negatively charged bacterial cell surface, account for the antibacterial activity [11,12]. Nanoparticles of zinc oxide are crucial in the destruction of microbes. When it comes to different gram positive and gram-negative bacteria, zinc oxide is selectively toxic. It is anticipated that zinc oxide's nanostructured surface will improve surface-bacteria interactions and thereby boost antibacterial activity [5]. The electrostatic interaction between positively charged ZnO and negatively charged cell surface accounts for the antibacterial activity through both chemical and physical mechanisms. Reactive oxygen species are produced when zinc oxide and bacterial cell wall physically interact, and zinc + ions are released when hydrogen peroxide is used [7,13,14].

In this study antibiofilm activity is assessed for gutta percha coated with 1%,3%,5% zinc oxide nanoparticle. All gutta percha coated with zinc oxide nanoparticles showed antibiofilm activity. On comparison, 1% zinc oxide nanoparticle coated gutta percha showed highest antibiofilm activity (Figure 3).

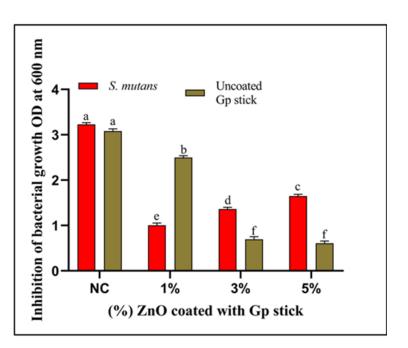


Figure 3: Antibiofilm activity of ZnO coated with GP and Uncoated GP.

## Conclusion

Zinc oxide nanoparticles can prevent the growth of bacteria and protect from dental plaque formation by disrupting the structural integrity of biofilm created by most oral pathogens such as E. faecalis, S. mutans. The eradication of biofilms and resident bacteria using nanoparticle holds a great promise. The clinical translation of nanoparticles targeting microbial and biofilm features is best done with materials that are nontoxic. Based on results and within limitation of the study it is concluded that 1%, 3%, 5% of ZnO nanoparticle coated Gutta percha shows reduced biofilm formation against S. mutans, 1% shows more reduced biofilm formation. Although further studies are needed to evaluate the physical and mechanical properties of the modified GP cones, this novel approach involving surface modification by ZnO film deposition, appears promising in preventing the ingress of microorganisms and the formation of biofilm.

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