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Evaluation of the Antimicrobial Activity of Selenium nanoparticles Coated Gutta-Percha Against Enterococcus faecalis and Staphylococcus aureus: An In Vitro Study

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ABSTRACT

Aim: The aim of this study is to enhance the antimicrobial effectiveness of conventional Gutta percha(GP) by modifying its surface with selenium nanoparticles(SeNPs).

Materials And Methods: SeNPs were synthesized using a chemical reduction method by dissolving 0.2 g of selenous acid in 100 mL distilled water, followed by mixing with 0.5 g sodium borohydride dissolved in 100 mL water. The resulting SeNPs sol was stirred and stored in a brown glass container. Sterilized GP cones (size 40, Dentsply Maillefer, Switzerland) were immersed in the SeNPs solution for 24 hours to allow uniform nanoparticle deposition. This in vitro study compared the antimicrobial efficacy of SeNPs coated GP (Group 1) and conventional GP (Group 2) against *Enterococcus faecalis* and *Staphylococcus aureus*. Using the Direct Contact Test on Mueller-Hinton agar, zones of inhibition (ZOI) were measured after 24-hour incubation at 37°C. Standard bacterial strains were cultured and standardized to 0.5 McFarland before lawn inoculation. GP cones (10 mm) were placed on the plates, and antimicrobial activity was assessed by measuring ZOI diameters in triplicate.

Results: The antimicrobial efficacy of SeNPs-coated GP was assessed against *S. aureus* and *E. faecalis* by measuring ZOI on agar plates, with uncoated GP as a control. Against *S. aureus*, uncoated GP produced a mean ZOI of 27.22 mm, while SeNPs coated samples measured 28.40 mm (20 µL) and 29.19 mm (30 µL). For *E. faecalis*, the corresponding values were 23.37 mm, 24.37 mm, and 25.14 mm. These results demonstrate a clear dose-dependent increase in antimicrobial activity with higher SeNPs volumes. The SeNPs coating's redox activity and ROS generation likely underpin this enhanced bactericidal effect. Thus, SeNPs coated GP shows significantly greater antibacterial properties compared to conventional GP, suggesting potential benefits for endodontic disinfection.

Conclusion: Within the limitations of the study, surface modified GP with SeNPs exhibited excellent antimicrobial properties, effectively inhibiting key microorganisms associated with root canal failure such as *E.faecalis* and *S.aureus*.

Keywords: *Schiff base, Aromatic amines, Ketones, Benzoxazipine.*

1. INTRODUCTION

Endodontic treatment targets the infected pulp of a tooth, with the goal of eliminating the infection and preventing its recurrence. Once the infected pulp tissue is removed, the root canal system is thoroughly cleaned, shaped, and filled with a core root filling material.[1] The success of endodontic treatment relies heavily on meticulous biomechanical preparation and effective irrigation of the root canal system. The purpose of root canal filling is to preserve the aseptic conditions established during the earlier stages of treatment. Gutta-percha (GP) cones are the most widely used core root filling materials, valued for their biocompatibility, cost-effectiveness, long-standing clinical use, and potential antimicrobial

properties primarily attributed to their zinc oxide (ZnO) content.[2] Disinfectants may not effectively reach bacteria located in complex areas of the root canal system, such as isthmuses, dentinal tubules, and canal ramifications.[3] Endodontic infections involve a diverse range of microorganisms. Therefore, after thorough chemo-mechanical preparation, it is essential to achieve proper three-dimensional obturation to create a fluid-tight seal and block the entry of any microorganisms.[4]

Endodontic treatment can fail due to persistent or secondary infections within the root canal system. Among the various microorganisms implicated, *Enterococcus faecalis (E. faecalis)* is particularly noteworthy for its resistance to antimicrobial measures and its frequent presence in cases of endodontic failure.[5] Although GP cones are manufactured under aseptic conditions, several studies have reported microbial contamination in freshly opened boxes. This contamination risk significantly increases with improper storage, exposure to aerosols during dental procedures, and repeated handling without appropriate aseptic techniques. *Staphylococcus* species are among the most commonly identified contaminants in GP cones subjected to inadequate handling practices. Moreover, contaminated GP cones can serve as a vehicle for introducing microorganisms directly into the root canal system, potentially compromising the aseptic chain established during chemo-mechanical preparation.[6] Various physicochemical strategies have been explored to enhance the antimicrobial properties of GP cones while preserving their essential filling characteristics.[7, 8] These approaches include incorporating antimicrobial agents such as chlorhexidine, calcium hydroxide, and bioactive phosphate glasses. Additionally, advanced nano-scale techniques, such as the development of nanodiamond-reinforced GP composites, have been investigated to further improve their antimicrobial effectiveness.[9]

Selenium is an essential micronutrient in biological systems. Owing to its antimicrobial, anticancer, and antioxidant properties, Selenium nanoparticles (SeNPs) have numerous applications in nanomedicine. Moreover, they exhibit lower cytotoxicity compared to many commonly used silver nanoparticles.[10][11] While SeNPs have been utilized in various biomedical applications, their antimicrobial potential in the field of endodontics remains largely unexplored. Chemically, SeNPs are typically synthesized through the reduction of selenite or selenous acid using reducing agents such as glutathione (GSH), hydrazine, sodium borohydride ($NaBH_4$), stannous chloride ($SnCl_2$), L-cysteine, ascorbic acid, sodium thiosulfate ($Na_2S_2O_3$), and sodium dodecyl sulfate (SDS).[12] Surface coating GP with SeNPs represents a promising approach to enhance its antimicrobial effectiveness. This strategy aims to inhibit microbial colonization without altering the core physical and mechanical properties of GP, thereby maintaining its suitability as a root canal filling material. The aim of this study is to enhance the antimicrobial effectiveness of conventional GP by modifying its surface with SeNPs.

2. MATERIALS AND METHODS

Surface Modification of Gutta Percha

GP cones (size 40, Dentsply Maillefer, Switzerland) from freshly opened GP boxes were firstly sterilized in the laminar air flow chamber for 30 mins. The synthesis of SeNPs was conducted using the chemical reduction method.[13] The chemical reduction method involved reducing selenous acid (H_2SeO_3) to elemental SeNPs. Before coating, the surface of GP was activated to improve adhesion. This can involve physical or chemical treatments to make the surface more reactive or rougher, enhancing the interaction with the nanoparticles. 0.2 g of selenous acid (H_2SeO_3) (Sigma Aldrich, United states) was dissolved in 100mL of distilled water and stirred until complete dissolution for 30 minutes at room temperature. Next, a reducing agent solution was prepared by dissolving 0.5 g of sodium borohydride (Sigma Aldrich, United states) in 100mL of water. After the complete dissolution of the substances, both solutions were mixed with vigorous stirring. The resulting sol of SeNPs was stirred for 15 minutes. The resulting mixture was poured into an opaque brown glass container. The activated GP was immersed in the SeNPs solution. The nanoparticles, which are now in a colloidal state due to the reduction reaction, are attracted to and adhere to the surface of the GP. As the GP remains in the solution for 24 hrs, SeNPs deposit onto its surface. Stabilizing agents in the solution help prevent aggregation of the nanoparticles, ensuring a uniform coating. After coating, the GP was rinsed to remove any unbound nanoparticles and then dried (Figure 1). This ensured that only nanoparticles firmly attached to the surface remain, providing a stable coating.

Figure 1 : Selenium nanoparticles coated Gutta Percha

Antimicrobial activity

This *in vitro* experimental study was conducted to evaluate and compare the antimicrobial efficacy of SeNPs coated GP (Group 1) and conventional GP cones (Group 2) against two common endodontic pathogens: *E.faecalis* and *S.aureus*. The antimicrobial activity was assessed using the Direct Contact Test and effectiveness was determined by measuring the zone of inhibition (ZOI) on Mueller-Hinton agar (MHA) plates. The test microorganisms included standard strains of *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923), which were obtained from a recognized microbiological culture collection and revived in Brain Heart Infusion (BHI) broth under appropriate conditions. All cones were sectioned into standardized 10 mm lengths using sterile scissors. SeNPs were synthesized and applied to Group 1 cones using a previously established coating protocol, and the coated cones were air-dried under sterile conditions. MHA was prepared as the culture medium according to the manufacturer's instructions, sterilized by autoclaving, poured into sterile 90 mm Petri dishes (approximately 25 mL per plate), and allowed to solidify. Plates were incubated at 37°C for 24 hours to ensure sterility before use. Fresh bacterial suspensions were prepared and adjusted to a 0.5 McFarland standard (~1.5 × 10^8 CFU/mL), and lawn cultures were created on MHA plates using sterile cotton swabs. Once the surfaces dried slightly, GP specimens from both groups were aseptically placed at the center of each plate. Four experimental setups were established to test both groups against each microorganism: Group 1 with *E. faecalis*, Group 1 with *S. aureus*, Group 2 with *E. faecalis*, and Group 2 with *S. aureus* (Figure 2). All plates were incubated aerobically at 37°C for 24 hours. Following incubation, antimicrobial activity was evaluated by measuring the diameter of the ZOI (including the GP cone) in millimeters using a digital Vernier caliper. Each sample was tested in triplicate to ensure consistency, and the mean ZOI was calculated for statistical analysis.

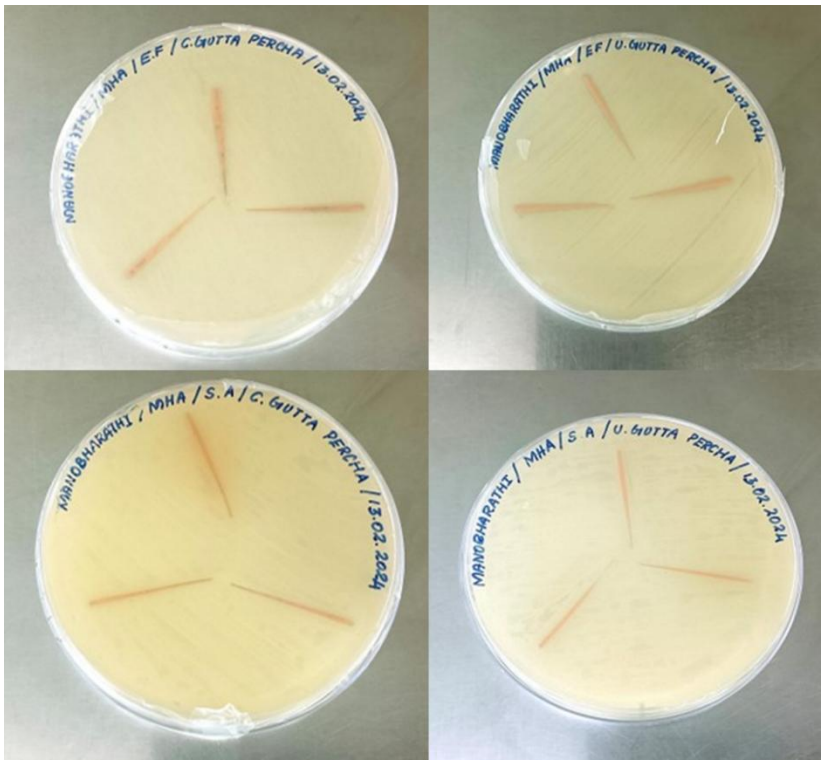


Figure 2: Antimicrobial activity of gutta-percha cones tested against *S. aureus* and *E.faecalis* using Mueller-Hinton Agar (MHA)

3. RESULTS

The antimicrobial efficacy of SeNPs coated GP was evaluated against *S. aureus* and *E. faecalis* through zone of inhibition (ZOI) analysis, compared with uncoated GP specimens. As shown in the agar diffusion assay, the SeNPs coated samples demonstrated larger ZOI for both microorganisms, indicating enhanced antibacterial properties. Specifically, against *S. aureus*, the ZOI measured 29 mm (30 µL) and 28 mm (20 µL), while against *E. faecalis*, the ZOI was 25 mm (30 µL) and 24 mm (20 µL). These values are markedly higher than typically observed for standard GP, which is known to possess limited intrinsic antimicrobial activity.[14] SeNPs well-documented redox activity and ability to generate reactive oxygen species contribute significantly to this enhanced bactericidal effect.[15, 16]

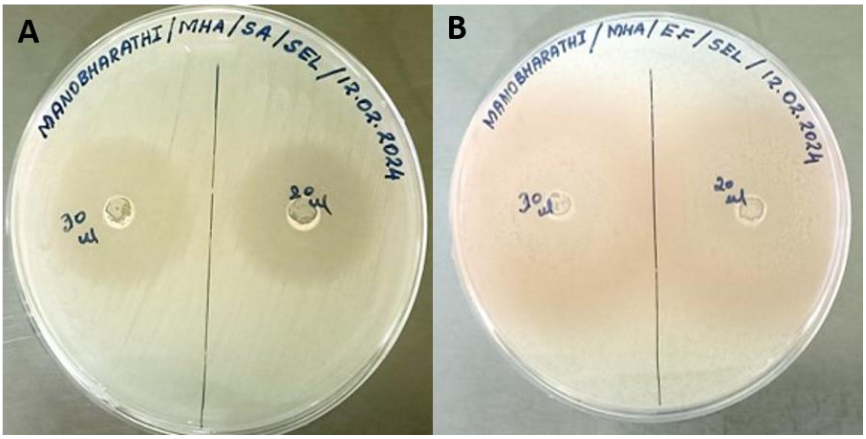
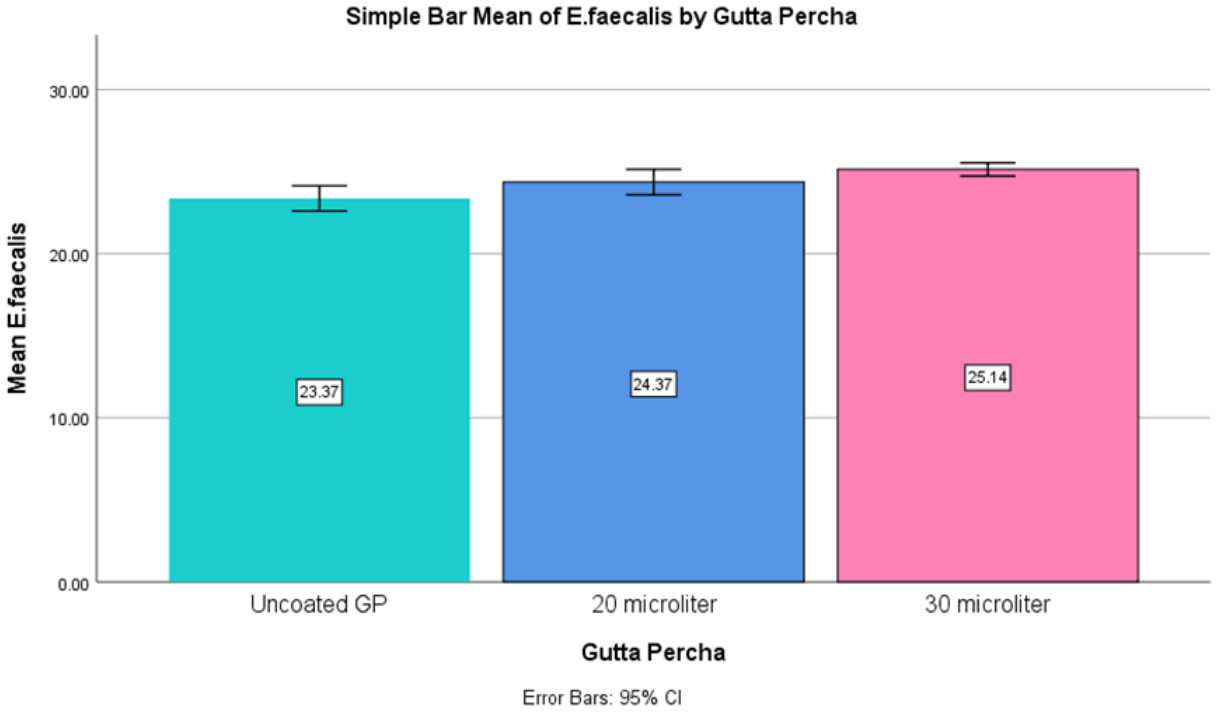
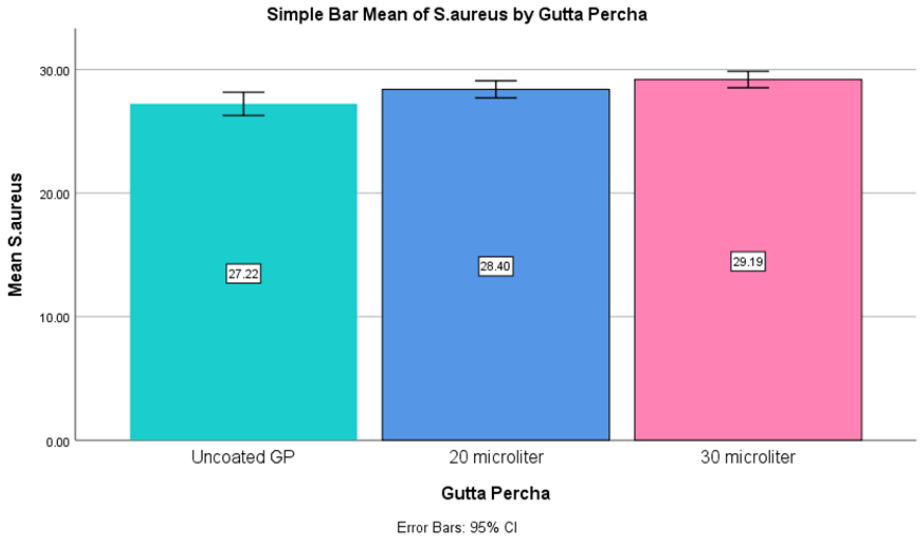


Figure 3 : Antibacterial activity of SeNP-coated gutta-percha against *S. aureus*(A) and *E. faecalis*(B) , showing dose-dependent zones of inhibition.



Graph 1 : Mean zone of inhibition (mm) of *E. faecalis* around uncoated and SeNPs coated GP, demonstrating a slight dose-dependent increase in antimicrobial activity.

The Graph 1 illustrates the mean ZOI (in millimeters) exhibited by *E.faecalis* in response to three types of GP: uncoated GP, 20 µL SeNPs coated GP, and 30 µL SeNPs coated GP. The antimicrobial efficacy was assessed by measuring the diameter of the inhibition zones, with error bars representing 95% confidence intervals. Among the tested groups, the uncoated GP demonstrated the lowest antimicrobial activity, with a mean inhibition zone of 23.37 mm. The 20 µL SeNPs coated GP exhibited a modest increase in antimicrobial effect, showing a mean inhibition zone of 24.37 mm. The 30 µL SeNPs coated GP group presented the highest antimicrobial activity, with a mean ZOI of 25.14 mm. This progressive increase in zone diameter with increasing SeNPs volume suggests a dose-dependent enhancement of antimicrobial properties. Although the differences in means among the groups appear relatively small, the consistent upward trend in inhibition zone sizes with higher SeNPs concentrations implies that coating enhances the antibacterial efficacy of GP against *E. faecalis*.



Graph 2 : Mean zone of inhibition (mm) of *S.aureus* around uncoated and SeNPs coated GP cones, showing a dose-dependent increase in antimicrobial activity.

The Graph 2 illustrates the mean zone of inhibition of *S. aureus* exhibited by uncoated and SeNPs coated GP. The uncoated GP showed a mean ZOI of 27.22 mm, whereas GP coated with 20 µL and 30 µL of SeNPs demonstrated larger zones of 28.40 mm and 29.19 mm, respectively. These findings indicate a dose-dependent improvement in antimicrobial activity with increasing concentrations of SeNPs. The enhanced ZOI suggests that surface modification of GP with SeNPs may effectively increase its antibacterial efficacy against *S. aureus*, supporting its potential use in endodontic applications to reduce the risk of microbial persistence.

4. DISCUSSION

Ensuring a three-dimensional seal of the root canal system is essential for the success of root canal treatment, as it prevents both coronal and apical leakage.[17] Endodontic treatment failure may result from microorganisms that withstand the chemical and mechanical cleaning of the root canal, as well as those that remain within the filling materials.[18] To address this challenge, this work explored a novel approach to enhance the antimicrobial efficacy of commercial GP cones. Immersing GP cones in sodium hypochlorite (NaOCl), a commonly used chairside disinfection method, causes notable surface changes, including irregular topography from component loss, increased variation in particle or grain size, and the presence of numerous surface deposits (Rico D. Short et al ., 2003.[19] These findings align with previous studies indicating that such disinfection can alter the physical and mechanical properties of GP points, potentially compromising the quality of the obturation seal and increasing vulnerability to biofilm formation. [20]

The antibacterial activity of the modified GP cones was tested against E. faecalis and S. aureus due to the high percentages of recovery from infected canals in endodontic failures and following the cones' storage and handling, respectively. [21, 22] GP cones coated with the SeNPs deposited directly on its surface presented higher antibacterial activity compared with the control cones. Several nanoparticles, including chitosan, bioactive glass, silver, zinc oxide, and quaternary ammonium polyethyleneimine, have been explored in endodontics for their antibacterial potential. [23, 18]. Silver and zinc oxide nanoparticles (AgNPs and ZnONPs) have been tested against *E. faecalis* biofilms, with studies showing that 1% AgNPs and 26% ZnONPs exhibit comparable antibiofilm efficacy to conventional irrigating solutions.[24]

Selenium, an essential trace element, has demonstrated strong antibacterial and anticancer properties when in its nano-sized form.[25] Biosynthesized SeNPs, in comparison to other synthesis methods, exhibit lower cytotoxicity towards normal cell lines, making them a preferred material for human studies.[26]. However, the antibacterial and antibiofilm effectiveness of SeNPs against *E. faecalis* for potential use as a disinfectant in endodontics has not yet been explored. Therefore, this study utilized SeNPs to evaluate their efficacy. The antibacterial activity of these nanoparticles is attributed to their generation of reactive oxygen species (ROS), depletion of intracellular ATP, and disruption of membrane potential, ultimately resulting in bacterial cell death. [27]

One study found the MIC_{80} of SeNPs against *E. faecalis* to be 25 µg/ml, a concentration notably lower than that reported by Alam et al. in their cytotoxicity assessments.[28] The low MIC_{80} value observed in the present study suggests minimal or no potential toxicity to human or animal cells. The MIC_{80} of 25 µg/ml reported here is comparable to that of the commercial antibiotic gentamicin, which has an MIC_{80} of 17 µg/ml. The antimicrobial effect of nanoparticles is due to various

mechanisms, including creating leaks in the cell membrane, releasing toxic ions that affect the metabolism system, producing reactive oxygen species (ROS) that damage the cell membrane, and inhibiting bacterial reproduction by breaking the DNA strands (Dalal M. Ridha et al ., 2024). SeNPs exhibit 4 to 6 times lower toxicity compared to selenium oxyanions like SeO_3^{2-} and SeO_4^{2-}[29]. Severe toxicity from SeNPs is observed only at high doses. The median lethal dose (LD_{50}) for SeNPs is 92.1 mg Se/kg, which is significantly higher than the concentration used in this study (1 mg/ml).[29] Additionally, SeNPs have demonstrated remarkable anticancer and free radical scavenging properties.

Furthermore, the systematic review by Silva et al. (2020) highlighted that the biocompatibility of root canal sealers can vary based on composition and setting conditions, emphasizing the importance of evaluating new materials like SeNPs coated GP.[30] Collectively, these findings suggest that SeNPs coated GP is a promising material for endodontic applications, combining antimicrobial efficacy with favorable biocompatibility. Selenium is an essential trace element that plays a crucial role in cellular defense mechanisms through its incorporation into selenoproteins, which help neutralize reactive oxygen species (ROS) and reduce oxidative stress. When used in nanoparticle form, selenium exhibits enhanced bioavailability and stability, while maintaining low toxicity to mammalian cells.[31, 32, 33].

Modified GP, enhanced with various antimicrobial and bioactive agents, has shown significant promise in improving the outcomes of root canal therapy by overcoming the limitations of conventional GP, such as poor adhesion and limited antimicrobial activity. Incorporation of materials like zinc oxide (ZnO) improves antibacterial efficacy and radiopacity, cetylpyridinium chloride (CPC) provides sustained antimicrobial action, glass ionomer cement (GIC) enhances adhesion to dentin and reduces microleakage, silver nanoparticles (AgNPs) offer broad-spectrum antimicrobial effects, and nanocurcumin contributes anti-inflammatory and antibacterial benefits with low cytotoxicity. [2,34]. Among these, the addition of SeNPs has gained attention due to selenium's potent antimicrobial, antioxidant, and anti-inflammatory properties, effectively inhibiting biofilm formation by resistant organisms like *E. faecalis*. These modifications collectively improve disinfection, sealing ability, biocompatibility, and long-term success of endodontic treatment.

5. CONCLUSION

Within the limitations of the study, surface modified GP with SeNPs exhibited excellent antimicrobial properties, effectively inhibiting key microorganisms associated with root canal failure such as *E. faecalis* and *S. aureus*. Further ex vivo and in vivo studies, including animal models and tooth-based assessments, are required to substantiate the antimicrobial efficacy of SeNPs GP. Clinical trials are essential to evaluate its biocompatibility and therapeutic potential in endodontic practice.

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