Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Evaluation of the Antibiofilm Activity of Selenium nanoparticles Coated Gutta-Percha: An InVitro Study

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Cite this paper as: Manobharathi G, Sandhya Raghu, (2025) Evaluation of the Antibiofilm Activity of Selenium nanoparticles Coated Gutta-Percha: An InVitro Study. *Journal of Neonatal Surgery*, 14 (32s), 8356-8362.

ABSTRACT

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

Materials And Methods: SeNPs were synthesized by a chemical reduction method and used to coat sterilized GP cones through 24-hour immersion for uniform deposition. This in vitro study evaluated and compared the antibiofilm efficacy of selenium nanoparticle-coated gutta-percha (Group 1) and uncoated gutta-percha (Group 2) against *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923). Standardized gutta-percha segments were tested using a 96-well microtiter plate model with biofilm formation induced in BHI broth supplemented with 1% glucose. After 24-hour incubation, dislodged biofilms were serially diluted and plated on Mueller-Hinton agar for CFU quantification. Group 1 showed a significant reduction in CFU counts compared to Group 2, indicating enhanced antibiofilm activity.

Results: The antibiofilm activity of selenium nanoparticle-coated gutta-percha (C-GP) was evaluated against *Staphylococcus aureus*, *Enterococcus faecalis* using serial dilutions (10^{-1} to 10^{-3}) and CFU analysis. Representative agar plates showed that C-GP significantly reduced biofilm formation, with countable colonies (252×10^2 CFU/mL) at 10^{-3} dilution, while uncoated gutta-percha (U-GP) showed too numerous to count (TNTC) growth at all dilutions. These results demonstrate that SeNPs coating imparts enhanced antibiofilm properties to gutta-percha compared to the conventional form.

Conclusion: Selenium nanoparticle coating significantly enhanced the antibiofilm activity of gutta-percha compared to uncoated controls. This surface modification demonstrates promising potential as a novel obturation material to reduce bacterial colonization and improve the success of root canal therapy

Keywords: *Colony forming unit, Gutta Percha, Nanoparticles, Obturation, Selenium*

1. INTRODUCTION

The goal of endodontic treatment is to resolve an infection and avoid reinfection in the tooth's infected pulp. The root canal space is cleaned, shaped, and filled with a core root filling material following the removal of the infected pulp tissue[1, 2]. The outcome of endodontic treatment depends on comprehensive biomechanical preparation and irrigation of the root canal system[3,4]. The objective of root filling is to preserve the aseptic chain that was established during the earlier stages of root canal therapy. Due to its many advantageous properties, including biological compatibility, flexibility, dimensional stability, radio opacity, ease of removal, and cost effectiveness, gutta percha is the most often utilized filling material for core root canal therapy[5]. Endodontic gutta percha filler comprises approximately 66% zinc oxide (filler), 20% gutta percha (matrix), 11% heavy metal sulfates (radiopacifier), and 3% waxes or resins (plasticizer)[6]. However, the main disadvantage is that endodontic failures are caused by bacterial microleakage at the gutta percha/sealer tooth interface[7]. The most frequent microbial species that cause endodontic failures and flare-ups include gram-positive bacteria like Staphylococcus aureus and Enterococcus faecalis, gram-negative bacteria like Escherichia coli, and yeasts like Candida albicans[8].

Additionally, despite the fact that GP cones are made in an aseptic environment, multiple investigations have found microbes in recently opened boxes; this contamination rises with incorrect handling, storage, and aerosols[9]. It is usual practice to immerse GP cones in various disinfectants, which provides antibacterial action and decontaminates the material. Since sodium hypochlorite (NaOCl) is inexpensive and has broad-spectrum activity, it is typically utilized in this kind of decontamination[10]. Nevertheless, it might change the GP cones' elasticity, tensile strength, and elongation rate, which could have an impact on the results of root filling[11],[12]. As a result, several physicochemical strategies have been documented with the goal of boosting GP cones' antimicrobial effectiveness while maintaining their filling specifications[10,13]. Biofilms are a complex network of bacteria that develop over time on dental surfaces, which can stimulate an inflammatory host response, resulting in the degradation of supporting periodontal tissues and subsequent tooth loss[14]. The field of nanotechnology offers treatment alternatives for the restoration of damaged, infected, absent, and fractured teeth. The capacity of metallic nanoparticles to function through several routes at once is their greatest advantage as antibacterial agents. Among the several potential processes is the induction of reactive oxygen species (ROS), which can impede the synthesis of amino acids and DNA, ultimately leading to the breakdown of the bacterial cell membrane[15]. Due to its remarkable antibacterial action and favorable characteristics like biocompatibility, bioavailability, and low toxicity, selenium nanoparticles, or SeNPs, have attracted a lot of attention. While research has demonstrated that SeNPs have remarkable antibacterial action, little is known about their antibiofilm activity against S. mutans and if NP size may affect biofilm penetration and, in turn, biofilm breakdown[16]. The present work proposes a novel approach to increase the antibacterial and antibiofilm efficacy of GP cones by coating GP cones with selenium nanoparticles.

2. MATERIALS AND METHODS

Preparation of SeNP-Coated Gutta-Percha

GP cones (size 40, Dentsply Maillefer, Switzerland) were aseptically handled in a laminar flow chamber and surface-modified with selenium nanoparticles (SeNPs) synthesized via chemical reduction. Briefly, 0.2 g selenous acid was dissolved in distilled water and reacted with freshly prepared 0.5 g sodium borohydride under vigorous stirring, with polyvinylpyrrolidone (0.1% w/v) added as a stabilizer to prevent aggregation, yielding a red SeNP colloidal sol. GP cones were chemically activated in 1 M NaOH for 10 min, then immersed in the SeNP sol for 24 h at room temperature to achieve uniform coating, rinsed with distilled water, and dried at 37 °C.

Antibiofilm Activity

This *in vitro* study was designed to assess and compare the antibiofilm activity of Group 1 (Selenium nanoparticles coated Gutta Percha) and Group 2 (Uncoated Gutta Percha) against biofilms formed by *E.faecalis* and *S.aureus*, utilizing the serial dilution method. The test groups included Group 1(C-GP) and Group 2 (U-GP) , serving as the control, with each group tested against both bacterial species in triplicates. Standard bacterial strains, *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923), were revived in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours to obtain fresh overnight cultures (Figure 1) . Gutta-percha cones were sectioned into standardized 10 mm segments, with Group 1 specimens coated with SeNPs according to an established protocol, and all samples were sterilized using UV exposure for 30 minutes prior to testing.

For the biofilm inhibition assay, overnight bacterial cultures were diluted to a 0.5 McFarland standard (~1.5×10^8 CFU/mL) using sterile phosphate-buffered saline (PBS), followed by a 1:100 dilution in BHI broth supplemented with 1% glucose to enhance biofilm formation. In 96-well flat-bottom microtiter plates, 180 µL of the bacterial suspension was added to each well, and a single gutta-percha segment (from either group) was immersed in the suspension. Plates were incubated aerobically at 37°C for 24 hours to allow biofilm development on the gutta-percha surfaces. Following incubation, each specimen was transferred into a sterile tube containing 1 mL of PBS, vortexed for 1 minute, and sonicated for 5 minutes to dislodge the biofilm without compromising bacterial viability. Serial 10-fold dilutions (10^{-1} to 10^{-6}) of the resulting suspension were prepared (Figure 2)(Figure 3), and 100 µL from each dilution was plated onto Mueller-Hinton agar using the spread plate method. These plates were incubated at 37°C for 24 hours, after which colony-forming units (CFUs) were manually counted or quantified using a digital colony counter.

Negative controls (wells containing only bacterial suspension without gutta-percha) were included to evaluate normal biofilm growth, while sterility controls (wells containing only sterile BHI broth and gutta-percha) ensured the absence of contamination. The antibiofilm activity of each test group was determined by comparing the reduction in CFU counts recovered from gutta-percha surfaces, with results expressed as $\log_{10}$ CFU/mL for statistical comparison between the groups.

Figure 1: Antibiofilm activity of gutta-percha samples against *S.aureus (SA)* and *E.faecalis (EF)* using a serial dilution method in 6-well plates.

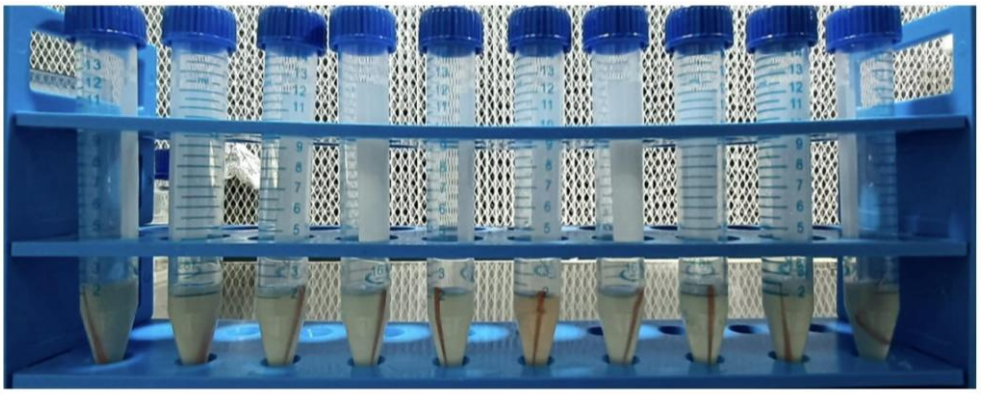

Figure 2 : Serial dilution of SeNPs coated and conventional gutta-percha samples for antibiofilm testing.

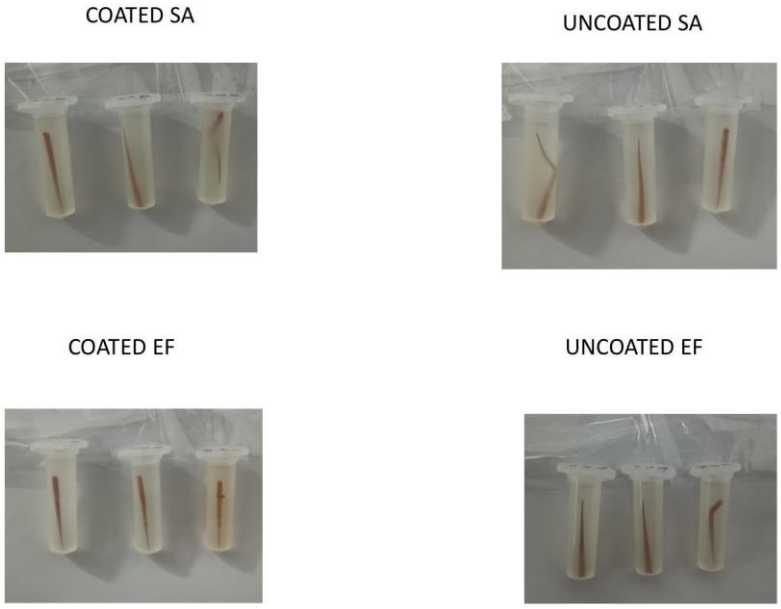

Figure 3 : Gutta-percha samples post serial dilution showing experimental grouping—coated and uncoated samples exposed to *S. aureus (SA) and E. faecalis (EF).*

3. RESULTS

The antibiofilm potential of selenium nanoparticle-coated gutta-percha (C-GP) was compared with conventional uncoated gutta-percha (U-GP) using *S. aureus* as a biofilm-forming model organism. The findings demonstrated a clear difference in biofilm inhibition between the two groups across serial dilutions (10^{-1}, 10^{-2}, 10^{-3}). The antibiofilm activity of selenium nanoparticle-coated gutta-percha (C-GP) was compared with conventional uncoated gutta-percha (U-GP) using *Staphylococcus aureus* as a biofilm-forming model organism. A distinct difference in biofilm inhibition was observed between the two groups across serial dilutions (10^{-1}, 10^{-2}, 10^{-3}). Representative agar plates illustrate biofilm formation by *S. aureus* at 10^{-1} dilution, showing sparse colonies with C-GP (left) compared to dense growth with U-GP (right) after 24 hours (Figure 4). Similar trends were noted at 10^{-2} (Figure 5) and 10^{-3} (Figure 6) dilutions.

In the C-GP group, plates at 10^{-1} and 10^{-2} dilutions exhibited growth that was too numerous to count (TNTC), which was expected given the higher initial bacterial concentration. However, at 10^{-3} dilution, colonies were countable, yielding 252 × 10^2 CFU/mL. This demonstrates that although C-GP did not completely eliminate the bacterial population, it substantially reduced the viable bacterial load compared to U-GP. The reduction reflects the antibiofilm properties of selenium nanoparticles, which act by generating reactive oxygen species (ROS), disrupting bacterial cell walls, and interfering with quorum sensing—mechanisms that collectively impair *S. aureus* biofilm development.

In contrast, all dilutions in the U-GP group (10^{-1}, 10^{-2}, and 10^{-3}) displayed TNTC colonies, confirming that conventional gutta-percha lacks inherent antibacterial or antibiofilm properties. The persistence of dense biofilm formation on U-GP underscores its susceptibility to microbial colonization, a major contributor to endodontic treatment failure.

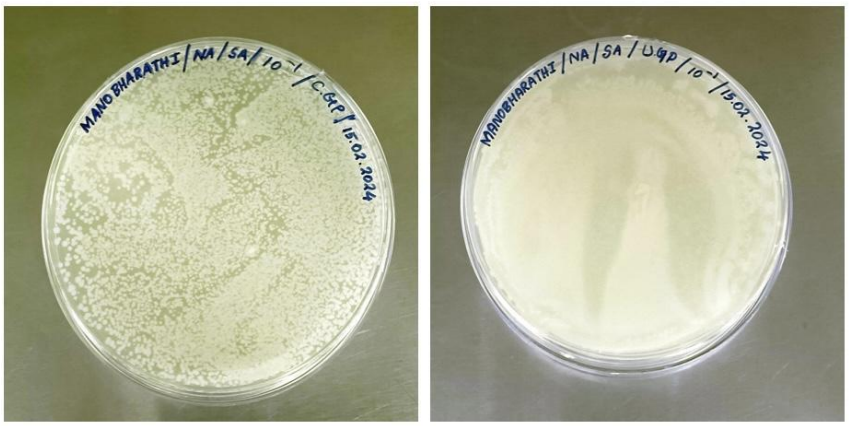

Figure 4 : Antibiofilm activity of selenium nanoparticle-coated gutta-percha (C-GP) compared to conventional gutta-percha (U-GP) against *S.aureus.*

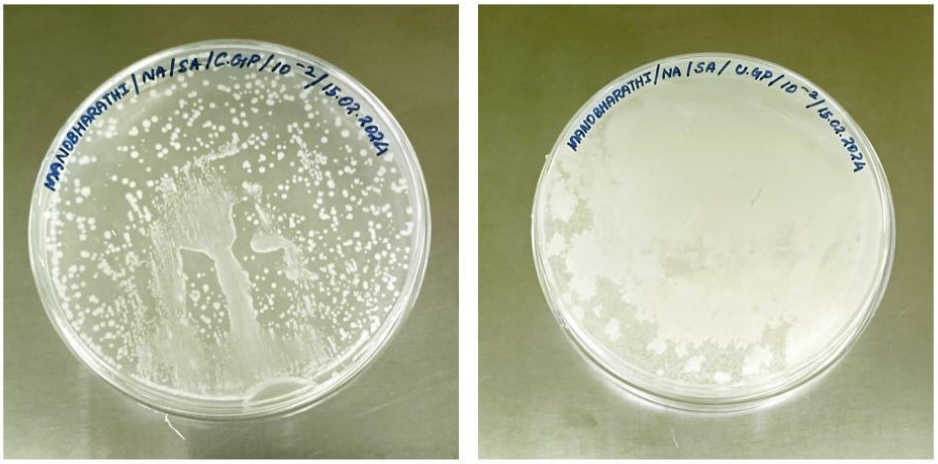

Figure 5: Antibiofilm activity of selenium nanoparticle-coated gutta-percha (C-GP) compared to conventional gutta-percha (U-GP) against *S.aureus.*

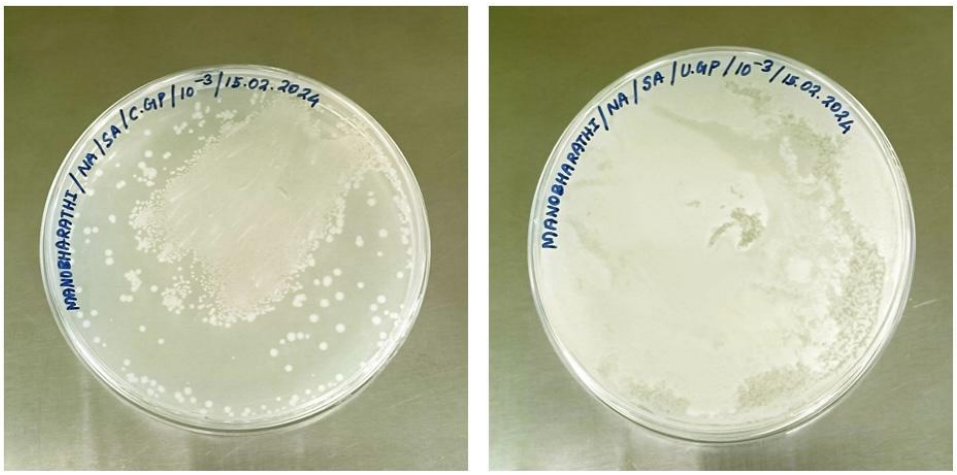

Figure 6: Antibiofilm activity of selenium nanoparticle-coated gutta-percha (C-GP) compared to conventional gutta-percha (U-GP) against *S.aureus.*

In the case of C-GP, plates at 10^{-1} and 10^{-2} dilutions displayed growth that was too numerous to count (TNTC), which was expected due to the higher initial bacterial concentration. However, at 10^{-3} dilution, colonies became countable, with a CFU of 252×10^2 CFU/mL. This indicates that while C-GP did not completely eradicate the bacterial population, it significantly reduced the viable bacterial load compared to U-GP. Such reduction reflects the antibiofilm effect of selenium nanoparticles, which are known to generate reactive oxygen species (ROS), disrupt bacterial cell walls, and interfere with quorum sensing, thereby compromising the ability of *S. aureus* to establish robust biofilms.

Conversely, in the U-GP group, all tested dilutions (10^{-1}, 10^{-2}, and 10^{-3}) showed TNTC colonies, signifying that the conventional material lacks inherent antibacterial or antibiofilm activity. The persistence of heavy biofilm formation in U-GP highlights the vulnerability of untreated gutta-percha to microbial colonization, which is a critical factor in endodontic treatment failure.

4. DISCUSSION

Achieving a three-dimensional seal of the root canal system is crucial for the success of root canal treatment, as it helps prevent leakage from both the coronal and apical ends[17]. Failure of endodontic treatment can occur due to microorganisms that survive the chemical and mechanical debridement of the root canal or persist within the filling materials[18]. To overcome this challenge, the present work investigated a novel strategy to improve the antimicrobial performance of commercial GP cones. Conventional chairside disinfection of gutta-percha cones by immersion in sodium hypochlorite (NaOCl) has been shown to induce significant surface alterations, including irregular topography caused by component loss, variations in particle or grain size, and the deposition of numerous surface residues[19]. These observations are consistent with earlier reports demonstrating that NaOCl disinfection can compromise the physical and mechanical properties of GP cones, thereby weakening the quality of the obturation seal and predisposing the surface to biofilm colonization[20] .

The antibiofilm evaluation demonstrated a marked difference between selenium nanoparticle-coated gutta-percha (SeNP-GP) and conventional gutta-percha (C-GP). Across all serial dilutions (10^{-1} to 10^{-3}), SeNP-GP showed significantly reduced biofilm formation, with only sparse bacterial colonies visible on agar plates, indicating strong inhibition of *Staphylococcus aureus* biofilm development. In contrast, C-GP samples displayed dense, confluent bacterial growth, reflecting limited resistance to biofilm formation. The superior antibiofilm effect of SeNP-GP can be attributed to the antimicrobial action of selenium nanoparticles, which compromise microbial cell walls, disrupt protein functions, and interfere with quorum sensing, thereby preventing biofilm initiation and maturation. These findings suggest that SeNP-coated gutta-percha holds promise as a more effective alternative in endodontic therapy, offering enhanced protection against persistent root canal infections often linked to biofilm-forming bacteria. The antimicrobial activity of nanoparticles operates through multiple mechanisms, including disruption of cell membrane integrity, release of toxic ions that interfere with metabolic processes, generation of reactive oxygen species (ROS) that damage cell membranes, and inhibition of bacterial proliferation by causing DNA strand breakage [21].

Selenium is an essential trace element that contributes to cellular defense by being incorporated into selenoproteins, which play a key role in neutralizing reactive oxygen species (ROS) and minimizing oxidative stress[22]. In nanoparticle form, selenium demonstrates improved bioavailability and stability, while maintaining low toxicity toward mammalian cells . To

address the limitations of conventional gutta-percha—such as poor adhesion and limited antimicrobial activity research has focused on modifying GP with antimicrobial and bioactive agents[23]. Additives such as zinc oxide (ZnO) enhance antibacterial effects and radiopacity, cetylpyridinium chloride (CPC) provides long-lasting antimicrobial action, glass ionomer cement (GIC) improves adhesion to dentin and reduces microleakage, silver nanoparticles (AgNPs) deliver broad-spectrum antimicrobial activity, and nanocurcumin contributes anti-inflammatory and antibacterial properties with low cytotoxicity[24,25][26][26,27] .

Selenium nanoparticles (SeNPs) have been shown to possess 4–6 times lower toxicity than selenium oxyanions such as SeO_3^{2-} and SeO_4^{2-}[28] . Significant toxicity from SeNPs occurs only at relatively high doses, with the median lethal dose (LD_{50}) reported as 92.1 mg Se/kg considerably higher than the concentration employed in this study (1 mg/mL) [29]. Beyond their safety profile, SeNPs exhibit notable anticancer activity and strong free radical scavenging capacity[30]. Biologically synthesized SeNPs, which display even lower cytotoxicity, have been investigated against a range of cell lines, including human non-small cell lung cancer, HeLa (cervical cancer), SKOV-3 (ovarian cancer), human keratinocytes, and MCF-7 (human breast cancer) cells[28] . Moreover, Silva et al. (2020), in their systematic review, reported that the biocompatibility of root canal sealers differs according to their composition and setting characteristics, underscoring the need to assess novel materials such as SeNP-GP . Taken together, the evidence indicates that SeNP-GP holds strong potential for endodontic use, offering both antimicrobial, antibiofilm effectiveness and favorable biocompatibility.

Among these modifications, selenium nanoparticles (SeNPs) have attracted particular interest due to selenium's strong antimicrobial, antioxidant, and anti-inflammatory capabilities, especially in inhibiting biofilm formation by resistant species such as *Enterococcus faecalis*. These enhancements collectively strengthen disinfection, sealing ability, biocompatibility, and the long-term success of root canal therapy. The synergistic effects of these additives especially selenium play a crucial role in improving the physicochemical properties and clinical performance of gutta-percha, highlighting its potential as a promising avenue for future research and clinical application in endodontics.

5. CONCLUSION

Within the limitations of this in vitro study, selenium nanoparticle-coated gutta-percha (SeNP-GP) demonstrated significantly enhanced antibiofilm activity compared to conventional gutta-percha (C-GP). The incorporation of selenium nanoparticles effectively reduced bacterial colonization and inhibited biofilm formation by *Staphylococcus aureus* and *Enterococcus faecalis*, two key pathogens implicated in endodontic treatment failures. This improvement can be attributed to the unique antimicrobial, antioxidant, and anti-inflammatory properties of selenium at the nanoscale, which disrupt bacterial cell walls, interfere with quorum sensing, and limit biofilm maturation.

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