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In Vitro Cytotoxicity Assessment of Selenium Nanoparticle-Coated Gutta-Percha Cones for Enhanced Endodontic Biocompatibility

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Abstract

Background: *Successful root canal treatment depends on thorough cleaning, disinfection, and effective obturation of the root canal system. Persistent microorganisms, particularly Enterococcus faecalis, can survive conventional treatment and lead to endodontic failure. Selenium nanoparticles (SeNPs) possess potent antibacterial activity and low cytotoxicity, making them promising agents for enhancing the antimicrobial properties of gutta-percha (GP) cones.*

Aim: *To evaluate the in vitro cytotoxicity of SeNP-coated gutta-percha cones using human gingival fibroblasts (HGFs) and assess their biocompatibility compared to conventional uncoated GP.*

Methodology: *SeNPs were synthesized via chemical reduction and coated onto size 40 GP cones after surface activation. Primary HGFs were exposed to eluates from SeNP-coated GP and uncoated GP for 24, 48, and 72 hours. Cytotoxicity was assessed using MTT and Live/Dead assays. Cell viability and morphology were analyzed using a microplate reader and fluorescence microscopy, respectively.*

Results: *MTT assay showed high cell viability across all groups, with SeNP-coated GP maintaining approximately 101% viability compared to 95.8% for uncoated GP and 100% for controls. Live/Dead assay confirmed predominantly green fluorescence in the SeNP-coated group, indicating excellent cell viability and normal fibroblast morphology. No significant cytotoxicity was observed for either SeNP-coated or uncoated GP.*

Conclusion: *SeNP-coated gutta-percha demonstrated excellent biocompatibility and maintained or slightly enhanced cell viability compared to uncoated GP. Coupled with their known antibacterial properties, SeNP-coated GP may serve as a safe and effective modification to improve the antimicrobial performance of root canal fillings. Further in vivo studies are warranted to confirm clinical applicability.*

Keywords: *Biocompatibility, Cytotoxicity, Gutta Percha, Obturation, Selenium nanoparticles,*

INTRODUCTION

Successful endodontic treatment relies on proper canal shaping, thorough cleaning, effective disinfection, and a tight seal of the root canal system[1]. The primary purpose of root canal obturation is to block coronal and apical leakage while trapping any residual bacteria that survive after treatment[2]. Disinfectants may fail to penetrate areas like isthmuses, dentinal tubules, and canal ramifications where bacteria can persist[3,4]. A study by Lin et al. on 150 cases of endodontic treatment failure identified a correlation between bacterial infection in the canals and periradicular rarefaction[3]. Secondary endodontic infections are mainly associated with facultative anaerobes like Enterococcus faecalis, whereas primary endodontic infections are typically polymicrobial and dominated by Gram-negative anaerobic rods[5]. Endodontic infections involve multiple microbial species[6]. Hence, after thorough chemo-mechanical preparation, an appropriate three-dimensional obturation is essential to create a fluid-tight seal and prevent microbial reinvasion.

Although gutta-percha (GP) cones are manufactured under aseptic conditions, several studies have reported bacterial contamination in newly opened boxes, which can increase due to improper handling, storage, or exposure to aerosols. Immersing GP cones in disinfectants is a common practice to eliminate contaminants while providing antimicrobial properties. Sodium hypochlorite is frequently used for this purpose because of its broad-spectrum activity and low cost; however, it can alter the elasticity, tensile strength, and elongation of GP cones, potentially affecting the quality of root canal obturation[7,8]. Modifications to gutta-percha are intended to enhance the effectiveness, safety, and durability of root canal treatments, ultimately improving patient outcomes. Consequently, various physicochemical

approaches have been explored to increase the antibacterial properties of GP cones while maintaining their filling performance. These methods include incorporating antimicrobial agents such as chlorhexidine, calcium hydroxide, or bioactive phosphate glasses, as well as applying nanoscale techniques like forming nanodiamond-GP composites[9,10]. This modification is crucial for lowering the bacterial load within the root canal, reducing the risk of reinfection, and enhancing overall clinical outcomes. Incorporating antibacterial materials into GP decreases the chance of post-treatment infections, and the sustained release of these agents helps prevent secondary infections from any bacteria that survive after the initial treatment[11].

Selenium nanoparticles (SeNPs), an essential trace element at the nanoscale, exhibit potent antibacterial and antioxidant properties while maintaining low toxicity toward human cells[3,4,12]. Their antibacterial activity, driven by the generation of reactive oxygen species, disruption of bacterial membranes, and depletion of intracellular ATP, makes them effective against resilient pathogens such as Enterococcus faecalis, which are often implicated in persistent root canal infections[13]. Given their strong antimicrobial potential and excellent biocompatibility, SeNPs have emerged as promising agents for biomedical applications, including dental materials. Gutta-percha (GP), the standard root canal filling material, lacks intrinsic antimicrobial activity, which can contribute to treatment failure[14]. Surface modification of GP with SeNPs offers a potential strategy to enhance its antibacterial properties without compromising cell compatibility. Therefore, this study aims to assess the in vitro cytotoxicity of SeNP-modified GP cones to evaluate their safety for potential endodontic applications.

MATERIALS AND METHODS

Preparation of SeNPs coated Gutta Percha

Size 40 gutta-percha cones (Dentsply Maillefer, Switzerland) from freshly opened boxes were placed in a laminar airflow chamber for 30 min prior to coating. Selenium nanoparticles (SeNPs) were synthesized via chemical reduction, as described by Verma and Maheshwari (2018). In this method, selenous acid (H_2SeO_3) is reduced to elemental SeNPs. To enhance nanoparticle attachment, the surface of the gutta-percha cones was pre-activated through mild physical/chemical treatment, increasing surface roughness and reactivity. For nanoparticle preparation, 0.2 g of selenous acid (H_2SeO_3; Sigma Aldrich, USA) was dissolved in 100 mL of distilled water with continuous stirring for 30 min at room temperature.

Separately, 0.5 g of sodium borohydride ($NaBH_4$; Sigma Aldrich, USA) was dissolved in 100 mL of distilled water to serve as the reducing agent. The two solutions were then combined under vigorous stirring, producing a colloidal suspension of SeNPs, which was further stirred for 15 min and stored in an amber glass container to prevent light-induced degradation. Activated GP cones were immersed in the SeNP suspension for 24 h to allow deposition. During this process, the colloidal nanoparticles adhered to the GP surface, and stabilizing agents present in the medium minimized aggregation, promoting a uniform coating. After incubation, the cones were rinsed with distilled water to remove loosely bound particles and air-dried, ensuring that only firmly attached nanoparticles remained, thereby forming a stable SeNP coating.

Cytotoxicity Analysis

MTT Assay

The MTT assay was conducted to evaluate the cytocompatibility of selenium-coated gutta-percha (Se-GP) and conventional gutta-percha (C-GP) using primary human gingival fibroblasts (HGFs). HGFs were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and incubated at 37°C in a humidified atmosphere with 5% CO_2.

Cells at passages 3 to 6 were used to ensure consistency. Gutta-percha samples from Group 1 and Group 2 were cut into uniform discs (5 mm in diameter and 1 mm thick), sterilized via UV irradiation for 30 minutes on each side, and rinsed with sterile phosphate-buffered saline (PBS) to remove residual particles. To simulate clinical leaching, material extracts (eluates) were prepared by incubating the gutta-percha discs in serum-free DMEM at a ratio of 0.2 g/mL for 24 hours at 37°C. The eluates were filtered through 0.22 µm syringe filters and used in subsequent assays. HGFs were seeded in 96-well plates at a density of 1×10^4 cells/well in 100 µL of complete DMEM and incubated for 24 hours (Fig 1).

After attachment, the medium was replaced with 100 µL of the respective eluates: Group 1 (Se-GP eluate), Group 2 (C-GP eluate), and a control group (fresh complete medium only). Cells were exposed to the eluates for 24, 48, and 72 hours. Following each incubation period, 10 µL of MTT solution (5 mg/mL) was added to each well, and plates were incubated for 4 hours at 37°C in the dark to allow for formazan

crystal formation. After incubation, the medium was carefully removed, and 100 µL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the crystals. Plates were gently shaken for 10 minutes, and absorbance was measured at 570 nm with a reference wavelength of 630 nm using a microplate reader. Cell viability (%) was calculated using the formula: (Mean absorbance of test group / Mean absorbance of control group) × 100. All tests were performed in triplicate and repeated three times, with results expressed as mean ± standard deviation (SD).

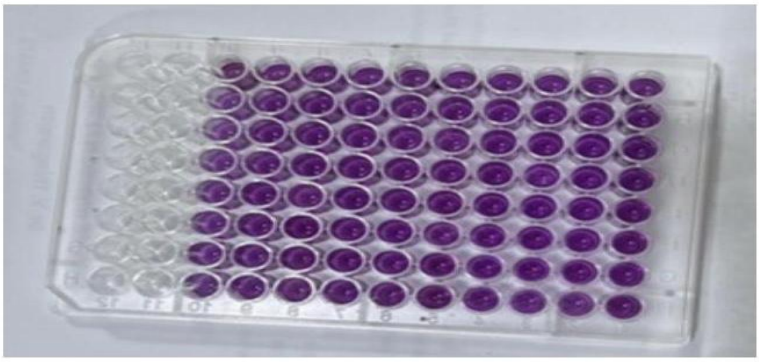

Figure 1: MTT assay performed using a 96-well plate.

Live and Dead cell Assay

The Live/Dead cell viability assay was employed to assess the biocompatibility of Group 1 and Group 2 using primary human gingival fibroblasts (HGFs). HGFs were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained at 37°C in a humidified 5% CO_2 incubator. Upon reaching 80% confluence, cells were used for the assay. Gutta-percha samples from both groups were cut into standardized discs (5 mm diameter, 1 mm thickness), sterilized by ultraviolet (UV) irradiation for 30 minutes on each side, and rinsed with sterile phosphate-buffered saline (PBS) to remove contaminants. HGFs were seeded into 24-well plates at a density of 1×10^5 cells per well and allowed to adhere for 24 hours. After cell attachment, gutta-percha discs were placed in the wells designated for Group I , Group 2, and Group 3 (control group with no material). Incubation periods of 24, 48, and 72 hours were used to assess time-dependent cytotoxicity. At the end of each incubation, the medium was aspirated, and the wells were rinsed with PBS.

Cell viability was evaluated using a Live/Dead Viability/Cytotoxicity Kit containing Calcein-AM and Ethidium homodimer-1 (EthD-1). A freshly prepared staining solution (2 µM Calcein-AM and 4 µM EthD-1 in PBS) was added (200 µL/well), and cells were incubated for 30 minutes at room temperature in the dark. Fluorescence microscopy was used to capture images, where live cells fluoresce green (Calcein-AM), and dead cells fluoresce red (EthD-1). Images from three randomly selected fields per well were analyzed using ImageJ software, and the percentage of viable cells was calculated using the formula: (Number of live cells / Total number of cells) × 100.

RESULTS

Cytotoxicity Analysis

MTT Assay

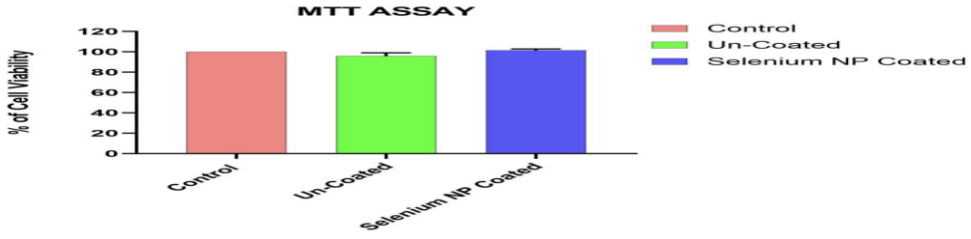

Figure 2: MTT assay showing percentage of cell viability for control, uncoated gutta-percha, and SeNPs-coated gutta-percha.

The MTT assay was performed to quantitatively evaluate the cell viability after exposure to three groups: Control (untreated cells), Uncoated Gutta-Percha, and SeNPs-Coated Gutta-Percha.

Control Group (Untreated Cells):
Observation: The cells not exposed to any gutta-percha samples exhibited 100% cell viability (Fig 2). Interpretation: This indicates that the baseline health of the cell culture was optimal, and the assay conditions were suitable for assessing cytotoxic effects. The intense purple coloration observed in the wells reflects active mitochondrial metabolism, confirming the presence of viable, healthy cells.
Uncoated Gutta-Percha:
Observation: The cells exposed to uncoated gutta-percha exhibited a slight reduction in viability, with viability measured around 96–97% compared to the control (Fig 2). Interpretation: This minor reduction suggests that uncoated gutta-percha exerts minimal cytotoxicity on the cells. Gutta-percha is known to be generally biocompatible; however, certain chemical additives (plasticizers, stabilizers, or coloring agents) used in the manufacturing process may leach out and cause slight cellular stress or metabolic inhibition. The lighter purple coloration observed in some wells indicates a very mild reduction in mitochondrial activity. Importantly, a viability above 90% is typically considered non-cytotoxic according to ISO 10993-5 standards for biocompatibility testing. Therefore, uncoated gutta-percha remains within acceptable safety margins.
Selenium Nanoparticle-Coated Gutta-Percha:
Observation: Cells exposed to SeNPs-coated gutta-percha showed a slightly higher viability, approximately 100%, compared to the control group (Fig 2). Interpretation: Interestingly, the SeNPs coating not only preserved cell viability but appeared to slightly enhance it. SeNPs at low concentrations are known to possess antioxidant properties and can promote cellular protection against oxidative stress, which might explain the observed enhancement in viability. The deep purple coloration in these wells suggests strong mitochondrial activity, indicating excellent cell health. The results suggest that coating gutta-percha with selenium nanoparticles does not introduce cytotoxicity and may, in fact, offer additional cytoprotective benefits.
The optical density (O.D) measurements indicated high cell viability across all groups. The control group demonstrated 100% viability, while the uncoated gutta-percha exhibited a mean viability of 95.82% and the SeNPs coated gutta-percha showed a slightly higher mean viability of 101%. The difference in viability between the control and selenium NP coated group was statistically insignificant ($p = 0.0759$), indicating that the SeNPs coating did not induce cytotoxic effects and maintained excellent biocompatibility. Similarly, the uncoated gutta-percha also showed no significant cytotoxicity ($p = 0.0776$).
Live dead cell Assay

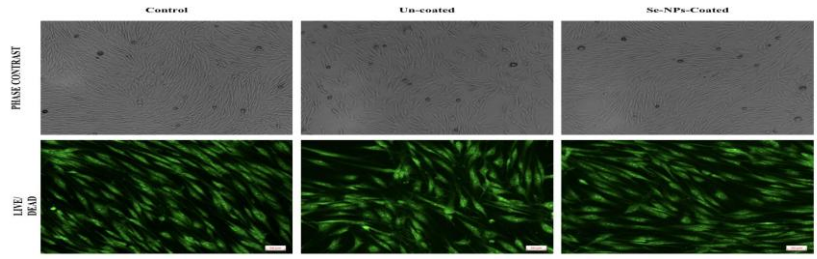

Figure 3: Live/Dead cell assay and phase contrast images showing high cell viability in fibroblasts exposed to SeNPs-coated gutta-percha, comparable to control and uncoated groups.
The Live/Dead cell assay was conducted to evaluate the cytocompatibility of conventional gutta-percha and SeNPs coated gutta-percha using human gingival fibroblast (HGF) cells. Phase-contrast microscopy revealed that cells in the control group maintained a healthy, spindle-shaped morphology with extensive cytoplasmic extensions and formed a dense, confluent monolayer, indicative of active proliferation. Similarly, cells exposed to both uncoated and SeNPs coated gutta-percha retained normal fibroblastic morphology, with good adherence and spreading, although a slight reduction in cell density was observed in the uncoated group compared to the control. Live/Dead fluorescence imaging demonstrated predominant green fluorescence in all groups, indicating a high proportion of viable cells. The uncoated gutta-percha group showed slightly decreased fluorescence intensity relative to the control, suggesting a minor reduction in cell viability, whereas the SeNP-coated gutta-percha group exhibited intense green fluorescence comparable to the control, and slightly higher than the uncoated group, with minimal evidence of dead cells (Fig 3).
Overall, both uncoated and SeNP-coated gutta-percha demonstrated excellent cytocompatibility with human gingival fibroblasts, with the selenium nanoparticle-coated material supporting slightly higher cell viability and better maintenance of normal cell morphology. These findings suggest that selenium

nanoparticle coating does not compromise the biocompatibility of gutta-percha and may offer a slight biological advantage by promoting enhanced fibroblast survival, highlighting its potential benefit for endodontic applications.

DISCUSSION

Achieving a three-dimensional seal of the root canal system is crucial for the success of root canal treatment, as it prevents leakage from both the coronal and apical directions[15]. Failure of endodontic treatment can occur due to microorganisms that survive the chemical and mechanical cleaning of the root canal, as well as those that persist within the filling materials[16]. To overcome this challenge, the present study investigated a novel strategy to improve the antimicrobial effectiveness of commercial GP cones. Immersion of gutta-percha cones in sodium hypochlorite (NaOCl), a commonly used chairside disinfectant, leads to significant surface alterations, such as irregular topography due to component loss, greater variability in particle or grain size, and the formation of numerous surface deposits[17]. These results are consistent with earlier studies showing that this method of disinfection can modify the physical and mechanical properties of GP points, which may compromise the integrity of the obturation seal and heighten susceptibility to biofilm development[18]. Given their strong antibacterial activity, nanoparticles are extensively investigated for their effectiveness against Enterococcus faecalis, a resilient microorganism capable of surviving under harsh conditions and in nutrient-deprived environments.

Surface modification of GP cones produced substantial alterations in surface topography. In comparison with untreated GP cones, these modifications markedly enhanced surface characteristics such as surface area-to-volume ratio, free energy, and reactivity[19]. Several nanoparticles, including chitosan, bioactive glass, silver, zinc oxide, and quaternary ammonium polyethyleneimine, have been investigated in endodontics for their antibacterial potential. Chitosan nanoparticles have also been tried but they require prolonged treatment time for antibacterial effects[20][20,21]. Silver and ZnO nanoparticles have been tested against E. faecalis biofilms, with 1% AgNPs and 26% ZnONPs demonstrating comparable antibiofilm effectiveness to conventional irrigating solutions[22].

Selenium, an essential trace element, has demonstrated strong antibacterial and anticancer properties when utilized at the nanoscale[23]. Compared to other synthesis methods, biosynthesized SeNPs exhibit lower cytotoxicity toward normal cell lines, making them a more suitable candidate for use in human studies[24][25]. However, the antibacterial and antibiofilm effectiveness of SeNPs against E. faecalis—for potential use as an endodontic disinfectant—has not yet been explored, which is why they were selected for this study. Their antibacterial activity is attributed to the generation of reactive oxygen species (ROS), depletion of intracellular ATP, and disruption of membrane potential, ultimately resulting in bacterial cell death[26]. Because of their low toxicity and anticancer potential, SeNPs have demonstrated therapeutic benefits in various conditions, including arthritis, nephropathy, diabetes, and cancer[27]. In a study, the MIC_{80} of SeNPs against E. faecalis was determined to be 25 μg/ml, which is considerably lower than the value reported by Alam et al. through cytotoxicity studies. Similarly, the MIC_{99} of biosynthesized SeNPs against P. aeruginosa, S. aureus, E. coli, and S. pyogenes was reported as 125, 100, 100, and 250 μg/ml, respectively—values notably higher than those observed in the present study[28].

Nanotoxicology has been a major concern since the introduction of nanoparticles in biomedical applications. Selenium nanoparticles exhibit 4–6 times lower toxicity compared to selenium oxyanions such as SeO_3^{2-} and SeO_4^{2-} (Zhang et al., 2005; Zhang, Wang & Xu, 2008). Significant toxicity from SeNPs occurs only at higher concentrations, with a median lethal dose (LD_{50}) of 92.1 mg Se/kg for Nano-Se—substantially higher than the concentration used in this study (1 mg/ml)[24][29]. Furthermore, SeNPs have demonstrated remarkable anticancer activity and free radical scavenging potential. Biologically synthesized SeNPs show even lower cytotoxicity and have been evaluated against several cell lines, including human non-small cell lung cancer, HeLa (cervical cancer) and SKOV-3 (ovarian cancer) cells, human keratinocytes, and human breast cancer cells (MCF-7). Their cytotoxicity has been reported to be lower than that of the widely used silver nanoparticles[30][31].

CONCLUSION

Selenium nanoparticle-coated gutta-percha cones showed excellent compatibility with human gingival fibroblasts and did not cause cytotoxic effects. Instead, they maintained or slightly improved cell viability compared to uncoated cones. With their known antibacterial properties and low toxicity, SeNP-coated gutta-percha may offer a useful way to enhance the antimicrobial performance of root canal fillings while remaining safe for clinical use. Further studies are needed to confirm their effectiveness in vivo.

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