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RESEARCH ARTICLE

Management of *Enterococcus faecalis* associated endodontic infection using gold nanogel: An *in-vitro* study

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Abstract

This study evaluates the antimicrobial efficacy of gold nanogel as an endodontic medicament against Enterococcus faecalis in persistent root canal infections. It investigates whether gold nanogel can serve as an effective and biocompatible alternative to conventional medicaments in eliminating *E. faecalis*. Gold nanoparticles (< 100 nm) were tested against *E. faecalis* using the agar diffusion method. Extracted teeth inoculated with *E. faecalis* were treated with gold nanogel (1000 µg/mL), chlorhexidine gluconate (2%), calcium hydroxide, or saline for seven days. Antimicrobial efficacy was assessed on days 1, 3, and 7 using agar diffusion and colony-forming unit (CFU) assays. Gold nanogel significantly reduced E. faecalis biofilm viability, showing greater efficacy than calcium hydroxide and comparable results to 2% chlorhexidine. Chlorhexidine eradicated E. faecalis by day 7, while gold nanogel exhibited sustained antibacterial action with minimal CFUs remaining. Gold nanogel demonstrated superior efficacy over calcium hydroxide and comparable effectiveness to chlorhexidine gluconate (2%) against *E. faecalis*. Further studies are recommended to evaluate its clinical applications and long-term biocompatibility.

Keywords Gold nanogel · Enterococcus faecalis · Intracanal medicament · Antimicrobial efficacy · Endodontic infections

1 Introduction

The adverse effects of microbial invasion and root canal colonization, resulting in irreversible pulpitis and apical periodontitis, have been extensively studied and documented (Wong et al. 2021). *Enterococcus faecalis (E. faecalis),* a Gram-positive bacterium, is frequently responsible for persistent endodontic infections and treatment failures, with a prevalence rate of 79.5% with primary infections and 89.6% in re-infections (Alghamdi and Shakir 2020; Yang et al. 2024). The virulence of *E. faecalis* is largely attributed to its ability to invade dentinal tubules and resist nutritional deficits (Love 2001). The strong attachment of *E. faecalis* cells to root canals, dentinal tubules, including isthmuses, lateral canals, and apical region leads to a thicker biofilm formation (Kishen et al. 2006). Biofilms are well-organised communities of microbial species embedded within an extracellular polymeric substance (EPS) (Zhao et al. 2023). EPS forms a protective scaffold for the cells to grow and protects them from changes in pH, nutritional deficit, mechanical trauma, and antibiotics developing higher antibiotic resistance (Zhao et al. 2023). The exact mechanism of *E. faecalis* biofilm resistance is not well established however, factors such as the low penetration of antibiotics through EPS, slow-growing cells within the biofilm, and bacterial stress response mechanisms appear to play important roles (Yang et al. 2024). Additionally, EPS facilitates cell-to-cell communication known as quorum sensing, resulting in a synergistic behaviour among microbial cells and subsequent increased antibiotic resistance (Yang et al. 2024). Also, dead cells and tissue residues in the root canal help in increased *E. faecalis* resistance to antimicrobial agents (Kishen et al. 2006). Various antimicrobial strategies are used to reduce and eliminate *E. faecalis* biofilms associated with endodontic treatment (Yang et al. 2024). Among these, mechanical instrumentation of the root canal system is the most common method for biofilm removal; however, complexities in the

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root canal system, such as lateral canals, isthmus, and fins, often require adjunct antimicrobial therapies (Gomes et al. 2023). Dynamic irrigation of antimicrobial agents using ultrasonic, apical negative pressure, and laser enhances the removal of microbial pathogens and biofilms from the root canal system (Wong et al. 2021). Sodium hypochlorite (NaOCl), chlorhexidine gluconate (CHX, 2%), and calcium hydroxide ($Ca(OH)_2$) are widely used antimicrobial agents against *E. faecalis*-related endodontic infection (Boutsioukis and Arias-Moliz 2022; Kim and Kim 2015). NaOCl (5.25%) and CHX (2%) are often used in combination due to their potent antibacterial and antifungal efficacy against *E. faecalis* infection (Stuart et al. 2006). However, NaOCl compromises the resin composite polymerization due to its oxidizing effect on dentin, decreasing the dentin-resin bonding (Abuhaimed and Abou Neel 2017). When combined with CHX, NaOCl also causes the formation of orange-brown precipitates that discolor the tooth and create a smear layer on the dentinal tubules, which interferes with the root canal sealer (Homayouni et al. 2014). Furthermore, a major drawback of NaOCl is its toxicity when it extrudes the root canal.

Accidental contact of NaOCl with oral mucosa causes inflammation, pain, and tissue necrosis (Echeverri and Alderete 2015). In contrast, CHX (2 %) has demonstrated long-term efficacy for up to 12 weeks when used as a root canal irrigant, without adverse effects on composite-adhesive bonds (Kanisavaran 2008). CHX is a strong cation, and its long-term antimicrobial activity is attributed to its ability to be absorbed onto negatively charged surfaces, such as teeth, mucosa, and restorative materials, and be slowly released, known as substantivity (Kanisavaran 2008). Mechanistic studies are not conclusive, but it appears that the protonated amine groups of CHX interact with the negatively charged phosphates, carboxylic, and hydroxyl groups of the dentinal collagen matrix, resulting in prolonged efficacy of CHX (Carrilho et al. 2010). However, there is growing concern regarding CHX toxicity (Liu et al. 2018). CHX also inhibits cell proliferation and affects the morphology of human gingival. A significant decrease in cell migration and viability is observed in fibroblasts, myoblasts, and osteoblasts treated with CHX (2%) *in vitro* fibroblast cells in a time-dependent manner (Wyganowska-Swiatkowska et al. 2016). Other than NaOCl and CHX, $Ca(OH)_2$ is another commonly used agent in treating endodontic infections. $Ca(OH)_2$ dissociates into calcium and hydroxyl ions when in contact with aqueous fluid. The alkaline nature of the hydroxyl ions is mainly attributed to the antibacterial property of $Ca(OH)_2$. The hydroxyl free radicals can also damage bacterial cell membranes and denature proteins and enzymes, inducing DNA damage (Yang et al. 2024). The pH in the root canal reaches its maximum of approximately 10.3 when $Ca(OH)_2$ is used as an intracanal medicament due to the buffering effect of the radicular dentin. However, E. faecalis exhibits increased resistance to $Ca(OH)_2$. The pH in the root canal reaches its maximum of approximately 10.3 when $Ca(OH)_2$ is used as an intracanal medicament due to the buffering effect of the radicular dentin. Despite this, E. faecalis can sustain a high pH of up to 11.5 due to the use of proton pumps in its plasma membranes, which help maintain cytoplasmic equilibrium and resistance to $Ca(OH)_2$ (Punathil et al. 2020). Furthermore, an alkaline pH enhances Ca2+ absorption and increases biomass thickness, colony size, and volume when *E. faecalis* is treated with $Ca(OH)_2$ (Momenijavid et al. 2022). This indicates that $Ca(OH)_2$ can cause a denser *E. faecalis* biofilm rather than decreasing it. Also, long-term use of $Ca(OH)_2$ reduces the fracture resistance of the teeth (Sireesha et al. 2017). Hence, there is a need for a new effective intracanal medicament against *E. faecalis-related* persistent endodontic infections. Noble nanoparticles (NPs) such as gold (AuNPs) and silver (AgNPs) show promising antimicrobial effects in dentistry due to their ultra-small size (0–100 nm), large surface-area-to-volume ratio, and variable surface chemistry. These properties lead to cell death primarily through plasma membrane disruption and oxidative stress. (Samiei et al. 2016). AgNPs are potent antimicrobials against *E. faecalis;* however, toxicity issues restrict their widespread clinical use (Samiei et al. 2016; Asharani et al. 2011). In contrast, enhanced biofilm inhibitory efficacy and low toxicity of AuNPs make them a potential therapeutic agent, but the scarcity of literature against *E. faecalis* infection does not allow any conclusion (Pradeepa et al. 2017; Riaz et al. 2020). The present study assessed the sustained effect of AuNPs in a non-toxic, biodegradable gel (hydroxypropyl methylcellulose, HPMC) as an intracanal medicament.

2 Materials and methods

2.1 Antimicrobial susceptibility testing of AuNPs

The study used AuNPs (< 100 nm) obtained from Sigma-Aldrich, USA. The antibiotic sensitivity of AuNP was tested at various concentrations using the agar diffusion method to determine the optimum concentration against *E. faecalis*. Freshly grown *E. faecalis* (ATCC 29212) colonies were suspended in brain heart infusion (BHI) broth, and the turbidity was adjusted to the McFarland standard of 0.5. The suspension was evenly spread over a tryptic soy agar plate (TSA) and left for 5 min to dry. Pre-sterilized Whatman discs (6 mm) (ThermoFischer Scientific, USA) were soaked in 20 µL of different concentrations of each medicament: Group 1: AuNPs (50–1000 µg/mL); Group 2: CHX solution at 2%, 1%, 0.2% (Consepsis, Ultradent Inc, USA); Group 3: $Ca(OH)_2$ with distilled water at 1:1, ¾:1, ½:1, ¼:1 ratio (Sigma-Aldrich, MERCK, USA); Group 4: saline as a

control. The discs were then placed on the *E. faecalis* inoculated TSA plate and incubated at 37 °C for 24 h, and the zones of inhibition were measured. Triplicate data for each concentration were obtained, and the results were averaged.

2.2 Formulation of intracanal medicaments

Intracanal dressings were formulated by incorporating the medicaments at a concentration demonstrating maximum zone of inhibition into a non-toxic hydroxypropyl methylcellulose (HPMC) gel. Briefly, all test compounds at the optimum concentration were mixed with 1 mL of HPMC continuously on a magnetic stirrer till a homogenous gel was formed. Additionally, the shape and size of the AuNPs in the gel were assessed using a transmission electron microscope (Hitachi HT7700 high-resolution TEM; Hitachi, Japan). A sample of AuNPs gel was loaded onto a carbon-coated copper grid without pre-treatment, air-dried for 5 min, and inserted into the TEM. The images were captured at an accelerating voltage of 80 kV and magnification of 15,000x (Fig. 1).

2.3 Preparation of teeth specimens

Forty freshly extracted single-rooted mandibular premolars were collected from patients with informed consent under a protocol approved by the Institutional Review Board of International Medical University. Following a previously described study by Haapasalo and Orstavik (Haapasalo and Orstavik 1987), teeth were stored in 0.1% thymol at 4℃ to prevent bacterial growth and used within one month. Teeth specimens (8 mm) were obtained by removing the crowns (2–3 mm from the cement-enamel junction) and apical portion of the root (3–5 mm) using a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA). The specimens were placed in an ultrasonic bath of 17% EDTA followed by 5.25% sodium hypochlorite for 4 min to remove the smear layer, then rinsed with sterile water (1 min) and autoclaved for 30 min at 121℃. The external surface was coated with nail varnish and immersed in 70% alcohol for sterilization, and then allowed to dry at room temperature for one hour in a biosafety cabinet, followed by room temperature drying for one hour in a biosafety cabinet.

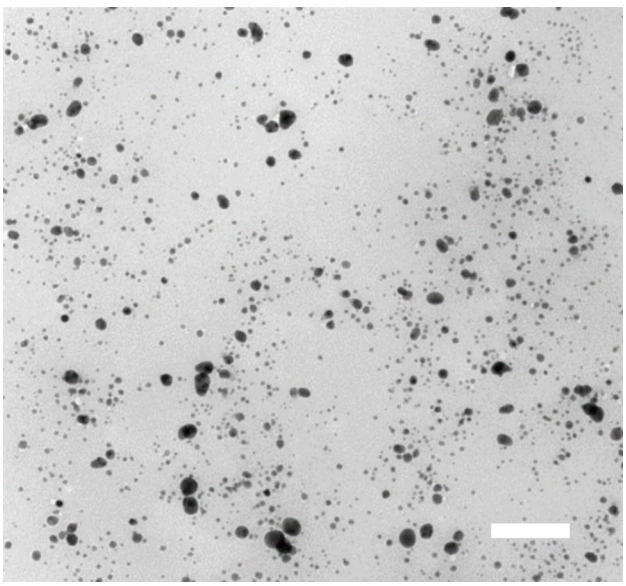

Fig. 1 Transmission electron microscopy showing well-distributed AuNP in the gel (bar 100 nm)

2.4 Bacterial inoculation of teeth specimens

E. faecalis was freshly grown on BHI agar and incubated anaerobically at 37 °C for 24 h. Teeth specimens were incubated anaerobically for 4 weeks at 37 °C in a Falcon tube containing 3 mL *E. faecalis* suspension (1.0×10^8 ml^{-1}) in BHI medium. Fresh BHI broth was replaced every 48 h to remove dead cells and to ensure bacterial viability. After the incubation period, the specimens were rinsed with phosphate-buffered saline (PBS) to remove the culture medium and non-adherent bacteria.

2.5 Intracanal dressings and antibacterial assay

The specimens were randomly divided into four groups (n=10) and subjected to HPMC gels incorporated with the following intracanal medicaments: Group 1:AuNPs; Group 2: CHX, Group 3: $Ca(OH)_2$; Group 4: saline. Each dressing was applied using a 23-gauge needle till the medicaments filled the root canal and were extravasated. The control group was irrigated with saline. After the application of medicaments, the coronal and apical orifices of the specimens were sealed with Cavit G (3 M, ESPE), a temporary restorative material. The specimens were then incubated at 37 °C for 1 day, 3 days, and 7 days. After each incubation period, the dentinal fragment samples from a depth of 100–200 µm were collected from the inner diameter of root canals using Gates Glidden burs #6 using a rotary handpiece (200 rpm, 1 N, electric motor Endo Plus, Driller: Sao Paulo, Brazil) (Delgado et al. 2010). The dentinal fragment samples were harvested in Eppendorf tubes containing 0.5 ml saline and vortexed for 10 s to obtain a homogenized suspension. Samples were then analysed for antibacterial activity using the colony-forming unit (CFU) assay. For each group of specimens, three micro dilutions were prepared, and 20 µL from each dilution was inoculated onto the TSA plates. After 24 h of incubation at 37 °C, the number of CFUs was manually counted. All assays were performed in triplicate, and results are presented as the means and standard deviations. Data was analysed using the Statistical Package for

Social Sciences (SPSS v 21.0, IBM). Significant differences ($P < 0.05$) between multiple groups were analysed using the Kruskal–Wallis Chi-square test. Multiple pairwise comparison was performed using Bonferroni's Post Hoc Tests ($P < 0.05$).

3 Results

3.1 Size and distribution of AuNPs in HPMC gel

The TEM micrograph of AuNPs in HPMC gel confirmed that the particles were uniformly distributed and ranged from 11 to 26 nm with an average size of 18 nm (Fig. 1).

3.2 Antimicrobial activity of AuNPs

The antimicrobial activity of all tested compounds is shown in terms of mean and standard deviation (Table 1). All test compounds demonstrated a concentration-dependent increase in antibacterial effects. Among all, AuNPs (1000 µg/mL) exhibited maximum antimicrobial efficacy with the largest zone of inhibition (7.27 mm) (p=0.003) (Table 2). A pairwise comparison of the zone of inhibition was also performed using Bonferroni's Post Hoc Tests, which revealed that there was a statistically significant difference (p<0.01) seen between most pairs of groups.

Number of colony-forming units (CFU) Comparison of CFUs in the dentin block model exposed to AuNPs (1000 µg/ml), CHX (2%), and $Ca(OH)_2$ (1:1) on day 1, 3, and 7 showed a significant difference in antibacterial effect on *E. faecalis* (Table 3). All medicaments showed a time-dependent reduction in the number of CFUs from days 1–7. While CHX completely eradicated *E. faecalis* ($p < 0.001$) on day 7, a minimal number of CFUs were found in the group treated with AuNPs compared to $Ca(OH)_2$. All test compounds exhibited significantly reduced colony count when compared to the control group ($p < 0.01$); however, not much difference was seen between the test groups, even though lower values were noted for AuNPs and CHX compared to $Ca(OH)_2$.

Table 1 Zones of inhibition demonstrated by AuNPs, CHX, and $Ca(OH)_2$

Test compounds	Mean	Std. Deviation
AuNP 50 µg/mL	4.4843	0.00142
AuNP 100 µg/mL	4.5608	0.00069
AuNP 150 µg/mL	5.9768	0.56710
AuNP 200 µg/mL	6.0630	0.57735
AuNP 250 µg/mL	5.7749	0.00036
AuNP 500 µg/mL	6.8643	0.00453
AuNP 750 µg/mL	6.8800	0.00020
AuNP 1000 µg/mL	7.2795	0.57735
CHX 0.2%	0.9773	0.02288
CHX 1%	2.9340	0.00051
CHX 2%	3.8875	0.00009
$Ca(OH)_2$ ¼:1	2.6451	0.00150
$Ca(OH)_2$ ½:1	3.5930	0.00023
$Ca(OH)_2$ ¾:1	4.4891	0.00012
$Ca(OH)_2$ 1:1	5.3560	0.00505

Table 2 Antimicrobial efficacy of AuNPs using disc diffusion method

AuNPs concentrations	*p value < 0.05	Mean SD	Kruskal Wallis Chi-square	p-value
1000 µg/mL	4.48	0.00	21.773	0.003*
750 µg/mL	4.56	0.00		
500 µg/mL	5.97	0.56		
250 µg/mL	6.06	0.57		
200 µg/mL	5.77	0.00		
150 µg/mL	6.86	0.00		
100 µg/mL	6.88	0.00		
50 µg/mL	7.27	0.57		

Table 3 Mean colony-forming unit (CFU/mL) in the dentin block model

Day	Group	Mean (CFU/mL)	SD	Kruskal Wallis Chi-square	*p*-value
1	AuNPs (1000 µg/mL)	6.57	0.00	10.385	0.016*
	CHX (2%)	3.55	0.01		
	$Ca(OH)_2$ (1:1)	5.73	0.03		
	Saline	8.92	0.02		
3	AuNPs (1000 µg/mL)	5.07	0.57	9.462	0.024*
	CHX (2%)	2.43	0.01		
	$Ca(OH)_2$ (1:1)	4.81	0.03		
	Saline	7.91	0.04		
7	AuNPs (1000 µg/mL)	1.61	0.02	10.569	0.014*
	CHX (2%)	0.00	0.00		
	$Ca(OH)_2$ (1:1)	2.72	0.01		
	Saline	4.92	0.02		

*$p < 0.05$

4 Discussion

In this laboratory-based study, AuNPs (~ 20 nm) at 1000 µg/mL demonstrated a significant antibacterial effect against *E. faecalis,* suggesting a potential therapeutic effect. The antibacterial effect of AuNP in HPMC gel was evident even after seven days of application medicament. *E. faecalis* is the most common microorganism associated with root canal failure (Alghamdi and Shakir 2020; Yang et al. 2024). *E. faecalis* persists in the root canal even after optimum cleaning, shaping, and use of intracanal medicaments. This persistence is due to its ability to invade dentinal tubules and adhere to inter-tubular collagen (Love 2001). Furthermore, *E. faecalis* can survive under nutritional deficit conditions, especially in biofilms, and is highly resistant to phagocytosis, antibodies, and antimicrobials, necessitating alternative treatment strategies (Yang et al. 2024). AuNPs were used as a new therapeutic agent against *E. faecalis* in the present study. AuNPs exert antibacterial effects against through several mechanisms, including cell membrane damage, DNA damage, and disruption of metabolic pathways, ultimately leading to bacterial cell death (Samiei et al. 2016). Their small particle size increases direct cellular interaction due to a greater surface area to volume ratio that increases the surface-active atoms, leading to increased reactivity and cellular response (Samiei et al. 2016). AuNPs have demonstrated excellent biocompatibility both in vitro and in vivo (Asharani et al. 2011). Additionally, the ease of conjugation with biomolecules provides AuNPs with strong anti-inflammatory and antimicrobial effects, suggesting therapeutic potential as an intracanal medicament in persistent endodontic infections (Verma and Stellacci 2010). However, there is limited evidence of AuNPs against *E. faecalis* in endodontic infection. Furthermore, the AuNPs capped with an antimicrobial peptide nisin are potent bactericidal against various *E. faecalis* strains while being nontoxic to human red blood cells with negligible haemolytic activity even at a higher concentration of 150–200 mg/ml (Pradeepa et al. 2017). Furthermore, biosynthesized, flavonoid coated AuNPs also significantly reduce *E. faecalis* in vitro and in vivo mice models (Riaz et al. 2020). The antimicrobial effect is also enhanced when AunPs are combined with Nd; YAG lasers against *E. faecalis*-infected human dentine (Kushwaha et al. 2018). In line with these results, AuNPs have demonstrated an increased antimicrobial effect against *E. faecalis-infected* dentine when compared with the control. As expected, the positive control CHX (2%) consistently exhibited excellent reduction of *E. faecalis* when compared to the control throughout the seven-day study period, completely eliminating the bacteria at day seven. Evidence shows CHX (2%) demonstrates equal efficacy to NaOCl (5.25%) as a root canal medicament against various pathogens, including *Staphylococcus aureus, Candida albicans*, *E. faecalis, Porphyromonas endodontalis*, *Porphyromonas gingivalis*, and *Prevotella intermedia,* both in solution and gel form (Homayouni et al. 2014). However, the antimicrobial effect was influenced by the type, concentration, and delivery of the antimicrobial agent. The current study used AuNPs (1000 µg/mL) in HPMC gel to increase their long-term release and efficacy. HPMC is a nontoxic, biodegradable, hydrophilic cellulose ether derivative used for controlled oral-mucosal sustained drug delivery (Javanbakht and Shaabani 2019). The main characteristic of HPMC is the high swellability, which significantly affects the drug release kinetics. Once in contact with the biological fluid, the fluid diffuses into the gel, resulting in polymer chain relaxation with volume expansion and diffusion of the drug (Javanbakht and Shaabani 2019). A controlled drug release is achieved using HPMC by altering the type and amount of drug,

polymer, and additives. During the seven-day study period, a steady significant decline in the number of CFUs of *E. faecalis* was observed after treatment with both AuNPs (1000µg/mL) and CHX (2%) in HPMC gel when compared with the control group, suggesting a prolonged drug release. Though AuNPs'efficacy was slightly lower than CHX (2%), the difference was statistically insignificant, suggesting AuNPs-HPMC gel could be a potential intracanal agent against persistent *E. faecalis* infection. The bacteria inoculation and sample collection in this study followed a previously established method by Delgado et al. that allowed the recovery of bacterial samples within biofilms inside dentinal tubules instead of planktonic microorganisms suspended at the lumen of the root canal (Delgado et al. 2010). Furthermore, evidence suggests that CHX (2%) gel removes *E. faecalis* from the superficial layers of dentinal tubules up to 100 μm^3 but *E. faecalis* can survive in intratubular collagen. Therefore, the current study explored the effect of the medicaments against *E. faecalis* at the intratubular depth of 100–200μm^3. It has been suggested that dentin should be treated for up to seven days to eradicate *E. faecalis* from dentinal tubules and the root canal space (Kanisavaran 2008). Furthermore, the substantivity of CHX (2%) gel can last up to 8 weeks. Keeping these factors in mind, the effects of AuNP on the *E. faecalis* CFUs in this present study were analysed up to seven days at different time intervals (1, 3, and 7 days) to get a comparative result with CHX (2%). The results concur with previous studies as no colony units were detected after exposure to CHX (2%) gel for seven days. Additionally, CHX (2%) and AuNPs gel demonstrated significantly increased antimicrobial effect in terms of CFUs when compared to $Ca(OH)_2$ and the negative control (saline). The antimicrobial effect of CHX is attributed to its

substantivity, but the antibacterial mechanism of AuNPs is unclear. It has been suggested that AuNPs lead to membrane destabilization and pore formation, followed by intracellular fluid leakage, resulting in *E faecalis* cell death (Pradeepa et al. 2017). $Ca(OH)_2$ demonstrated the least antimicrobial activity among the tested medicaments throughout the study. It is hypothesized that *E. faecalis* pumps proton ions coupled with the buffering nature of dentin, reducing the alkalinity of $Ca(OH)_2$ within the dentinal tubules, which deactivates $Ca(OH)_2$ (Punathil et al. 2020). This could explain the reduced efficacy of $Ca(OH)_2$ in the present study. The study suggests that AuNP might be as effective as CHX (2%) in the eradication of *E. faecalis*. However, there are a few shortcomings that need to be kept in mind while evaluating the results. Although the potent antibacterial efficacy of AuNP has been shown, the biocompatibility of AuNP has not been tested against mammalian cells. It is imperative to evaluate the efficacy and safety of AuNP for clinical use. Also, the mechanism of action of AuNP gel was not evaluated. Mechanistic studies of AuNP-E. faecalis interactions and long-term AuNP release profiles will provide a better understanding of future AuNP formulations against *E. faecalis-associated* infections. This study has some limitations, where the standard *E. faecalis* strain was used rather than clinical isolates from endodontic infections. Cytotoxicity on oral tissues, such as gingival fibroblasts or keratinocytes, was not evaluated. Additionally, the *in vitro* setting may not fully mimic clinical conditions. Future research should include clinical isolates, *in vivo* studies, and detailed cytotoxicity assessments.

5 Conclusion

AuNP-HPMC gel is an effective antimicrobial and could be a potential therapeutic agent against *E. faecalis-associated* persistent endodontic infection. AuNP (1000 µg/mL) has increased efficacy than $Ca(OH)_2$ as an intracanal medicament in vitro and can have a comparable effect as that of CHX (2%) gel against *E. faecalis*. Further mechanistic studies on AuNP -*E. faecalis* interaction and long-term AuNP release and safety are needed for the clinical use of AuNP as an intracanal medicament in routine endodontic therapy.

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Amalraj Fabian Davamani: Validation, Formal Analysis, Supervision
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Minati Choudhury: Writing – Review & Editing

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare that they have no financial or non-financial competing interests related to this study.

Ethics approval The study was conducted in accordance with the ethical guidelines of the institution and approved by the Institutional Review Board of International Medical University.

Informed consent Informed consent was obtained from all subjects involved in the study for the use of extracted teeth in research.

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