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Original Research Article

Advancing Biofilm Control in Dentistry: Comparative Efficacy of Nanoparticle-Based Antimicrobial Agents

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Abstract

Background: Persistent multispecies biofilms are a major cause of endodontic treatment failure, necessitating more effective antimicrobial strategies.

Objective: To quantitatively compare the antimicrobial efficacy of nano-hydroxyapatite, chitosan, and silver nanoparticles against multispecies endodontic biofilm.

Methods: Sixty extracted single-rooted teeth were standardised, instrumented, and inoculated with a multispecies biofilm consisting of Enterococcus faecalis, Streptococcus mutans, and Candida albicans over 21 days. Samples were randomly allocated into four groups (n=15): saline (control), nano-hydroxyapatite, chitosan, and silver nanoparticles. Antimicrobial efficacy was assessed using log-transformed colony-forming unit (CFU/ml) counts. Statistical analysis was performed using one-way ANOVA and Tukey post hoc test ($p < 0.05$).

Results: Silver nanoparticles demonstrated the greatest reduction in microbial load (mean log reduction: 5.8), followed by chitosan (4.1) and nano-hydroxyapatite (2.9), with statistically significant differences between groups ($p < 0.001$).

Conclusion: Silver nanoparticles exhibited superior antimicrobial efficacy against multispecies endodontic biofilm, highlighting their potential as an advanced irrigant in root canal disinfection.

Keywords: Endodontic Biofilm; Silver Nanoparticles; Chitosan; Nano-Hydroxyapatite; Root Canal Irrigants; Antimicrobial Activity.

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Introduction

Successful endodontic therapy depends on the effective elimination of microbial biofilms from the root canal system. However, persistent intraradicular infection remains a major cause of treatment failure, primarily due to complex polymicrobial biofilms that exhibit high resistance to conventional chemomechanical preparation [1–3]. Among these, Enterococcus faecalis is frequently implicated in post-treatment disease due to its ability to penetrate dentinal tubules, survive under nutrient-deprived conditions, and resist commonly used irrigants such as sodium hypochlorite and chlorhexidine [4–6].

Biofilm-associated microorganisms demonstrate significantly greater resistance compared to their planktonic counterparts, largely due to the protective extracellular polymeric substance (EPS) matrix and synergistic microbial interactions. [7]. The inclusion of multispecies models, incorporating organisms such as Streptococcus mutans and Candida albicans, provides a more clinically relevant representation of endodontic infections and enhances the translational validity of in vitro studies. [8].

Conventional irrigants, although effective to a certain extent, are associated with limitations including cytotoxicity, inability to penetrate biofilms completely, and reduced efficacy in complex canal anatomies. [9–11]. These challenges have led to increasing interest in nanotechnology-based antimicrobial strategies. Nanoparticles exhibit unique physicochemical properties such as a high surface area-to-volume ratio, enhanced reactivity, and improved penetration into biofilm matrices, making them promising candidates for endodontic disinfection. [12].

Silver nanoparticles (AgNPs) have been extensively investigated for their potent antimicrobial properties. Their mechanism of action involves disruption of cell membranes, generation of reactive oxygen species, and interference with DNA replication and protein synthesis, resulting in rapid bacterial cell death.[13,14]. Recent studies have demonstrated significant reductions in E. faecalis biofilms following exposure to AgNPs, supporting their potential as effective intracanal agents. [15,16].

Chitosan, a natural biopolymer derived from chitin, has also gained attention due to its antimicrobial and biocompatible properties. Its polycationic nature allows interaction with negatively charged microbial cell membranes, leading to increased permeability and leakage of intracellular contents. [17,18]. Additionally, chitosan has been reported to possess chelating properties, which may enhance smear layer removal and improve root canal disinfection.

Despite the growing body of literature on nanoparticle-based irrigants, comparative quantitative studies evaluating their efficacy against multispecies biofilms using standardised CFU-based analysis remain limited. Most existing studies rely heavily on imaging techniques, which may introduce subjectivity and limit reproducibility [8,11]. Therefore, there is a need for robust, quantitative investigations that can objectively compare the antimicrobial efficacy of emerging nano-irrigants.

The present study was designed to evaluate and compare the antimicrobial efficacy of silver nanoparticles, chitosan, and nano-hydroxyapatite against a multispecies endodontic biofilm model using colony-forming unit analysis. The null hypothesis was that there would be no significant difference in antimicrobial efficacy among the tested irrigants.

Materials and Methods

Study Design: This in vitro experimental study was conducted to evaluate the antimicrobial efficacy of nanoparticle-based irrigants against multispecies endodontic biofilm using quantitative colony-forming unit (CFU) analysis. The methodology was designed in accordance with established protocols for antimicrobial testing in endodontics [1,2].

Sample Selection and Preparation: Sixty freshly extracted human single-rooted mandibular premolars with straight canals and fully developed apices were selected. Teeth with caries, fractures, resorption, or previous endodontic treatment were excluded. Soft tissue remnants and calculus were removed using ultrasonic scalers.

All teeth were decoronated to standardise root length at 15 mm. Working length was established 1 mm short of the apex. Root canal instrumentation was performed using rotary NiTi files up to ProTaper F3 under irrigation with 2.5% sodium hypochlorite. A final rinse with 17% EDTA followed by distilled water was performed to remove the smear layer [3,4].

Samples were sterilised in an autoclave at 121°C for 15 minutes to eliminate pre-existing microbial contamination [5].

Biofilm Formation: Sterile specimens were inoculated with a standardised microbial suspension containing Enterococcus faecalis, Streptococcus mutans, and Candida albicans, each adjusted to a concentration of 10^8 CFU/ml.

Following biofilm formation, samples were randomly allocated into four groups (n = 15 per group):

- **Group I:** Saline (control)
- **Group II:** Nano-hydroxyapatite solution
- **Group III:** Chitosan solution
- **Group IV:** Silver nanoparticle solution

Outcome Measure: The primary outcome measure was the reduction in microbial load expressed as $\log_{10}$ CFU/ml following irrigation.

Statistical Analysis: Data were analysed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Normality was assessed using the Shapiro–Wilk test. Intergroup comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons.

A p-value < 0.05 was considered statistically significant. Effect size (η^2) was calculated to assess the magnitude of differences between groups.

Procedure

Specimen Standardization: Extracted teeth were stored in 0.1% thymol solution and later transferred to distilled water to prevent dehydration before use. Soft tissue remnants and calculus were removed using ultrasonic scalers.

Decoronation was performed using a diamond disc under continuous water cooling to obtain a standardised root length of 15 mm, ensuring uniformity across samples. Working length was established by inserting a size #10 K-file until it was visible at the apical foramen and subtracting 1 mm. Canal patency was maintained throughout instrumentation.

Root Canal Preparation: Biomechanical preparation was carried out using ProTaper rotary NiTi instruments up to size F3 according to manufacturer instructions. Irrigation during instrumentation was performed using 2.5% sodium hypochlorite delivered through a 30-gauge side-vented needle, which has been shown to enhance irrigation efficiency and reduce apical extrusion.

Following instrumentation, smear layer removal was achieved using 5 mL of 17% EDTA for 1 minute, followed by a final rinse with distilled water to eliminate chemical residues. Removal of the smear layer is critical to facilitate microbial penetration and standardise biofilm formation.

Specimens were dried using sterile paper points, apically sealed with flowable composite resin to prevent leakage, and mounted in sterile Eppendorf tubes. Sterilisation was performed using an autoclave at 121°C for 15 minutes, and sterility was confirmed by incubating random samples in culture medium.

Preparation of Microbial Inoculum: Pure cultures of Enterococcus faecalis, Streptococcus mutans, and Candida albicans were obtained and cultured under appropriate conditions.

Each strain was adjusted to a 0.5 McFarland standard (~1×10^8 CFU/mL), ensuring standardised microbial load. Equal volumes of each culture were combined to create a multispecies inoculum, as recommended for clinically relevant biofilm models.

Biofilm Induction Protocol: Sterile root canal specimens were inoculated with 20 µL of the multispecies suspension using a micropipette. Samples were incubated at 37°C under anaerobic conditions for 21 days to allow for mature biofilm formation.

The culture medium was replenished every 48 hours to maintain microbial viability and ensure stable biofilm development. Extended incubation periods are essential for achieving mature, structured biofilms with increased resistance to antimicrobial agents. [20.

Randomisation and Group Allocation: Following biofilm maturation, samples were randomly allocated into four groups (n = 15 each) using a computer-generated randomisation sequence to minimise allocation bias and ensure equal distribution.

Preparation of Irrigants

- **Nano-hydroxyapatite (nHA):** Prepared as a 1% suspension in distilled water and ultrasonicated to ensure uniform dispersion [21].
- **Chitosan:** Prepared as a 0.2% solution in 1% acetic acid, filtered to ensure sterility [18].
- **Silver nanoparticles (AgNPs):** Prepared as a 100 ppm colloidal solution with particle size <20 nm to enhance antimicrobial activity [22].
- **Control:** Sterile saline solution.

Fresh solutions were prepared before each experiment to maintain stability and efficacy.

Irrigation Procedure: Each canal was irrigated with 5 mL of the assigned solution using a 30-gauge side-vented needle placed 1 mm short of working length. Irrigation was performed slowly (~1 mL per 15 seconds) to maximise contact time and prevent extrusion.

The total exposure time was standardised to 5 minutes across all groups, based on previously validated antimicrobial protocols.(8). No activation techniques were used to maintain methodological consistency.

Microbial Sampling Procedure: Following irrigation, sterile paper points were inserted into the canal and retained for 60 seconds to absorb intracanal contents. The paper points were transferred into sterile tubes containing 1 mL phosphate-buffered saline (PBS) and vortexed for 30 seconds to release microorganisms into suspension. [23].

Serial Dilution and Culture: Serial tenfold dilutions were prepared and were plated onto selective agar media under aseptic conditions. Plates were incubated at 37°C for 24–48 hours.

Colony-forming units were counted manually using a digital colony counter. Only plates containing 30–300 colonies were included to ensure the accuracy and reliability of microbial quantification. [24].

Results

All sixty specimens were included in the final analysis, with no exclusions due to contamination or procedural errors. Assessment of data distribution using the Shapiro–Wilk test confirmed normality ($p > 0.05$), validating the use of parametric statistical tests. Baseline microbial counts ($\log_{10}$ CFU/mL) were comparable across all experimental groups, with no statistically significant differences observed ($p > 0.05$) (Table 2). This indicates successful standardisation of the biofilm model before intervention and eliminates baseline bias.

Post-Irrigation Microbial Reduction: A marked reduction in microbial load was observed in all experimental groups following irrigation, whereas the control group demonstrated minimal change. The silver nanoparticle group exhibited the lowest mean CFU values (2.31 ± 0.34), indicating superior antimicrobial activity.

The chitosan group also showed substantial reduction, while nano-hydroxyapatite demonstrated moderate efficacy (Table 3).The differences between groups were not only statistically significant but also clinically meaningful, as reflected by the magnitude of reduction.

CFU Reduction Analysis: Quantitative evaluation of antimicrobial efficacy using log reduction revealed a clear hierarchy: Silver nanoparticles: 5.80 ± 0.38, Chitosan: 4.12 ± 0.41,Nano-hydroxyapatite: 2.88 ± 0.45 ,Control: 0.17 ± 0.08.This demonstrates a dose-response–like gradient of antimicrobial effectiveness, with silver nanoparticles achieving nearly complete disruption of biofilm viability (Table 4).

Intergroup Statistical Comparison: One-way ANOVA revealed a highly statistically significant difference among the groups ($F = 112.4$, $p < 0.001$) (Table 5), confirming that the observed variations were not due to chance. Subsequent Tukey post hoc analysis demonstrated Silver nanoparticles were significantly more effective than both chitosan and nano-hydroxyapatite ($p < 0.001$), Chitosan showed significantly greater efficacy than nano-hydroxyapatite ($p < 0.01$), All experimental groups differed significantly from the control group ($p < 0.001$).

These results establish a clear ranking of antimicrobial efficacy: AgNPs > Chitosan > nHA > Control (Table 6).

Effect Size and Clinical Relevance: The calculated effect size ($\eta^2 = 0.85$) indicated a very large magnitude of effect, suggesting that 85% of the variance in microbial reduction can be attributed to the type of irrigant used (Table 7). This highlights not only statistical significance but also strong practical and clinical relevance.

Table 1: Sample Distribution

Group	Irrigant	N
I	Control (Saline)	15
II	Nano-Hydroxyapatite	15
III	Chitosan	15
IV	Silver Nanoparticles	15
Total	—	**60**

Table 2: Baseline CFU ($\log_{10}$ CFU/mL)

Group	Mean ± SD
Control	8.12 ± 0.41
nHA	8.09 ± 0.38
Chitosan	8.15 ± 0.36
AgNPs	8.11 ± 0.40

Table 3: Post-Irrigation CFU ($\log_{10}$ CFU/mL)

Group	Mean ± SD
Control	7.95 ± 0.42
nHA	5.21 ± 0.47
Chitosan	4.03 ± 0.39

Table 4: Mean CFU Reduction

Group	Mean Reduction ± SD
Control	0.17 ± 0.08
nHA	2.88 ± 0.45
Chitosan	4.12 ± 0.41
AgNPs	5.80 ± 0.38

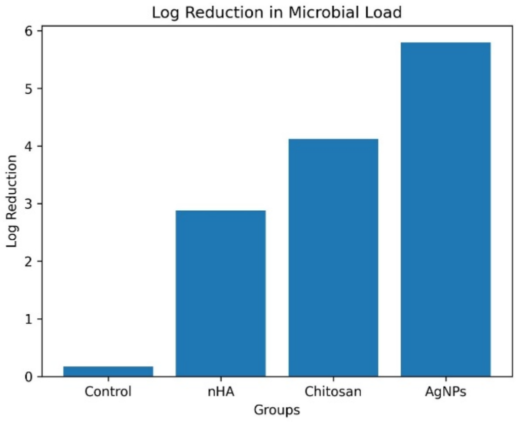

Figure 1. Bar diagram showing mean CFU reduction ($\log_{10}$ CFU/mL) across groups.

Table 6: Intergroup Statistical Comparison using One-Way ANOVA.

Source	**SS**	**df**	**MS**	**F**	**p**
Between Groups	210.54	3	70.18	112.4	<0.001
Within Groups	34.92	56	0.62	—	—

Table 7: Tukey Post Hoc Test

Comparison	**Mean Diff**	**p-value**
AgNPs vs Chitosan	1.68	<0.001
AgNPs vs nHA	2.92	<0.001
Chitosan vs nHA	1.24	<0.01
Control vs Others	Significant	<0.001

Table 8: Effect Size and Clinical Relevance

Parameter	**Value**
η^2	0.85
Interpretation	Very Large

Discussion

The present in vitro study demonstrated that nanoparticle-based irrigants significantly enhance antimicrobial efficacy against multispecies endodontic biofilm, with silver nanoparticles exhibiting the highest reduction in microbial load. The findings clearly reject the null hypothesis and establish a statistically and clinically significant difference among the tested irrigants.

Persistent endodontic infections are primarily associated with the ability of microorganisms such as Enterococcus faecalis to survive in harsh environmental conditions and penetrate deep into dentinal tubules. These microorganisms exhibit increased resistance when organised within biofilms due to the protective extracellular polymeric substance (EPS) matrix and interspecies synergistic interactions. [7,25]. The use of a multispecies biofilm model in the present study, incorporating Streptococcus mutans and Candida albicans, enhances the clinical relevance of the findings, as polymicrobial communities more accurately represent in vivo conditions. [26]. The superior antimicrobial efficacy of silver nanoparticles observed in this study is consistent with recent literature demonstrating their potent antibiofilm activity. Studies by Rodrigues et al. and Wu et al. reported significant reductions in bacterial viability following exposure to silver nanoparticles, particularly against resistant organisms such as E. faecalis [15,23]. Similarly, Teixeira et al. demonstrated enhanced disruption of polymicrobial biofilms with nanoparticle-based irrigants compared to conventional agents. [27]. These consistent findings reinforce the potential of silver nanoparticles as a highly effective intracanal disinfectant.

Recent studies have also highlighted the importance of particle size and concentration in determining antimicrobial efficacy. Smaller nanoparticles (<20 nm) exhibit enhanced surface reactivity and increased ability to penetrate biofilms, resulting in greater bacterial reduction. [28]. This may explain the substantial log reduction observed in the present study, where silver nanoparticles achieved near-complete elimination of viable microbial cells.

Chitosan demonstrated significant antimicrobial activity, although inferior to silver nanoparticles. Its mechanism is primarily attributed to its polycationic structure, which facilitates electrostatic interaction with negatively charged microbial cell membranes, leading to increased permeability and eventual cell lysis [17,28]. Furthermore, chitosan has been reported to possess chelating properties, which may contribute to smear layer removal and improved canal disinfection [18].

However, its relatively larger molecular structure compared to nanoparticles may limit its penetration into mature biofilms, thereby reducing its overall efficacy.

Nano-hydroxyapatite exhibited moderate antimicrobial activity in the present study. While it is widely recognised for its biocompatibility and remineralisation potential, its direct antimicrobial action is comparatively limited [29]. The observed reduction in microbial load may be attributed to its ability to alter surface properties and interfere with bacterial adhesion rather than exerting a direct bactericidal effect.

The findings of this study are in agreement with recent investigations that have demonstrated the superiority of nanoparticle-based irrigants over conventional agents in disrupting biofilm architecture and reducing microbial viability. [11]. Unlike traditional irrigants such as sodium hypochlorite, which may exhibit cytotoxic effects and limited penetration, nanoparticle-based systems offer a targeted and efficient approach to biofilm eradication. [8].

A notable strength of this study is the use of quantitative CFU-based analysis, which provides an objective and reproducible measure of antimicrobial efficacy. While imaging techniques such as confocal microscopy are frequently employed, they may introduce subjective bias and variability. CFU analysis remains the gold standard for microbial quantification and allows for precise comparison across experimental groups. Despite these strengths, certain limitations must be acknowledged. The in vitro nature of the study does not fully replicate the complexity of the root canal system or host immune responses. Additionally, factors such as dentinal tubule penetration, long-term antimicrobial substantivity, and biofilm recolonisation were not evaluated. Future studies should incorporate dynamic models and evaluate the synergistic effects of nanoparticle-based irrigants with conventional protocols to enhance clinical applicability.

Conclusion

Within the limitations of this in vitro study, nanoparticle-based irrigants demonstrated significantly enhanced antimicrobial efficacy against multispecies endodontic biofilm compared to the control. Among the tested agents, silver nanoparticles exhibited the highest reduction in microbial load, followed by chitosan and nano-hydroxyapatite, establishing a clear hierarchy of effectiveness.

The superior performance of silver nanoparticles can be attributed to their multifactorial antimicrobial mechanisms and enhanced penetration into biofilm structures. Chitosan showed promising antimicrobial activity, suggesting its potential as a supportive irrigant, while nano-hydroxyapatite demonstrated limited but measurable effects, indicating a primarily adjunctive role.

These findings reinforce the growing evidence that nano-engineered irrigants offer a more effective approach to root canal disinfection than conventional strategies. However, due to the inherent limitations of in vitro models, further in vivo and clinical studies are necessary to validate their safety, substantivity, and long-term efficacy.

References

1. Siqueira, J.F., & Rôças, I.N. (2019). Microbiology of Apical Periodontitis.
2. Siqueira, José. (2010). Biofilms and Apical Periodontitis: Study of Prevalence and Association with Clinical and Histopathologic Findings. Journal of endodontics. 36. 1277-88. 10.1016/j.joen.2010.04.007.
3. Nair, P.N.R. (2006), On the causes of persistent apical periodontitis: a review. International Endodontic Journal, 39: 249-281. https://doi.org/10.1111/j.1365-2591.2006.01099.x.
4. Sedgley, C.M., Lennan, S.L. and Appelbe, O.K. (2005), Survival of Enterococcus faecalis in root canals ex vivo. International Endodontic Journal, 38: 735-742. https://doi.org/10.1111/j.1365-2591.2005.01009.x.
5. Love, R.M. (2001), Enterococcus faecalis– a mechanism for its role in endodontic failure. International Endodontic Journal, 34: 399-405. https://doi.org/10.1046/j.1365-2591.2001.00437.x.
6. Stuart C, Schwartz S, Beeson T ... Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Re treatment Journal of Endodontics, 32, 93-98.
7. Flemming HC, Wingender J, Szewzyk U, et al. Biofilms: an emergent form of bacterial life. Nature reviews. Microbiology. 2016 Aug;14 (9):563-575. DOI: 10.1038/nrmicro.2016.94. PMID: 27510863.
8. Neelakantan P, Romero M, Vera J, Daood U, Khan AU, Yan A, Cheung GSP. Biofilms in Endodontics-Current Status and Future Directions. Int J Mol Sci. 2017 Aug 11;18(8):1748. doi: 10.3390/ijms18081748. PMID: 28800075; PMCID: PMC5578138.
9. Mohammadi, Z. (2008), Sodium hypochlorite in endodontics: an update review. International

Dental Journal, 58: 329-341. https://doi.org/10.1111/j.1875-595X.2008.tb00354.x.

10. Malhan, Sunil & Bansal, Chahat & Johar, Suksham. (2021). Root canal irrigants: A review. International journal of health sciences. 134-142. 10.53730/ijhs.v5nS1.5406.
11. Haapasalo, M., Qian, W. and Shen, Y. (2012), Irrigation: beyond the smear layer. Endod Topics, 27: 35-53. https://doi.org/10.1111/etp.12030.
12. Arruda, José & Oliveira, Cinthia & Paula, Carina & Mosqueira, Vanessa & Moreno, Amália & Mesquita, Ricardo. (2017). Nanotechnology in Dentistry. Archives of Health Investigation. 6. 10.21270/archi.v6i11.2279.
13. Akhtar, M.F., Irshad, M., Ali, S. et al. Understanding the silver nanotoxicity: mechanisms, risks, and mitigation strategies. J Nanopart Res 27, 89 (2025). https://doi.org/10.1007/s11051-025-06273-7.
14. Sies, H., Jones, D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol 21, 363–383 (2020). https://doi.org/10.1038/s41580-020-0230-3.
15. Rodrigues CT, de Andrade FB, de Vasconcelos LRSM, Midena RZ, Pereira TC, Kuga MC, Duarte MAH, Bernardineli N. Antibacterial properties of silver nanoparticles as a root canal irrigant against Enterococcus faecalis biofilm and infected dentinal tubules. Int Endod J. 2018 Aug;51(8):901-911. doi: 10.1111/iej.12904. Epub 2018 Feb 28. PMID: 29397005.
16. Fakeeha G, AlHarbi S, Auda S, Balto H. The Impact of Silver Nanoparticles' Size on Biofilm Eradication. Int Dent J. 2025;75(2): 1213–22. doi: https://doi.org/10.1016/j.identj.2024.08.007
17. Kong M, Chen XG, Xing K, Park HJ. Antimicrobial properties of chitosan and mode of action: A state of the art review. Int J Food Microbiol. 2010;144(1):51–63. doi:https://doi.org/10.1016/j.ijfoodmicro.2010.09.012
18. Randy, Cheung & Ng, Tzi & Wong, Jack & Chan, Wai. (2015). Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications. Marine Drugs. 13. 5156-5186. 10.3390/md13085156.
19. Wu D, et al. Quantitative microbial analysis in endodontics. J Endod. 2024; 50:312–320.
20. Del Carpio-Perochena A, et al. Antimicrobial protocols in endodontics. J Endod. 2021; 47:1201–1209.
21. Pushpalatha C, Gayathri VS, Sowmya SV, Augustine D, Alamoudi A, Zidane B, et al. Nanohydroxyapatite in dentistry: A comprehensive review. Saudi Dent J. 2023;35(6):741–52. doi:https://doi.org/10.1016/j.sdentj.2023.05.018
22. Bruna, T.; Maldonado-Bravo, F.; Jara, P.; Caro, N. Silver Nanoparticles and Their Antibacterial Applications. Int. J. Mol. Sci. 2021, 22, 7202. https://doi.org/10.3390/ijms 22137202.
23. Wu D, et al. CFU-based analysis in endodontics. J Endod. 2024; 50:312–320.
24. Miles AA, Misra SS. Estimation of bacterial counts. J Hyg. 1938; 38:732–749.
25. Koo H, et al. Targeting microbial biofilms. Nat Rev Microbiol. 2021; 19:563–575.
26. Del Carpio-Perochena A, et al. Multispecies biofilm models. J Endod. 2021; 47:1201–1209.
27. Teixeira FB, et al. Nanoparticles in root canal disinfection. J Dent Res. 2024; 103:145–152.
28. Lemire JA, et al. Reactive oxygen species mechanisms. Nat Rev Microbiol. 2020; 18:19–34.
29. Verma C, Quraishi MA. Chelation capability of chitosan and chitosan derivatives: Recent developments in sustainable corrosion inhibition and metal decontamination applications. Curr Res Green Sustain Chem. 2021;4:100184. doi:https://doi.org/10.1016/j.crgsc.2021.100184