Keywords: Photodynamic therapy, Toluidine blue O, Enterococcus faecalis, Diode laser, Nanoparticles, Chitosan nanoparticles,Silver nanoparticles, Root canal disinfection

Authors
Smitha Reddy[1]
Professor, Department of conservative dentistry and Endodontics, Sri Sai College of Dental Surgery, Vikarabad, Telangana, smithalok@rediffmail.com
ORCID ID: 0009-0006-1966-8096

Pundari deveneni[2]
Post graduate student, Department of Conservative dentistry and Endodontics, Sri Sai College of Dental Surgery, Vikarabad, Telangana, pundaridevenenI@gmail.com
ORCID ID: 0009-0001-5432-003X

Priti maurya[3*]
Postgraduate Department of conservative dentistry and Endodontics, Sri Sai College of Dental Surgery, Vikarabad, Telangana, drpritimaurya98@gmail.com
ORCID ID: 0009-0005-8044-4086

Sohail Mahmood[4]
Post Graduate student, Department of conservative dentistry and endodontics, Sri Sai College of Dental Surgery, Vikarabad, Telangana, sohailmahmood63@gmail.com
ORCID ID: 0009-0001-9008-2438

Aisha Habeeb[5]
Associate Professor Department of Conservative Dentistry and Endodontics Sri Sai College of Dental Surgery, Vikarabad, Telangana aishahabeeb9@gmail.com
ORCID ID: 0009-0004-8176-1844

G Deepshika[6]
Postgraduate Student Department of conservative dentistry and Endodontics, Sri Sai College of Dental Surgery, Vikarabad, Telangana, govuladeepshi@gmail.com
ORCID ID: 0009-0008-4491-7933

Received: 07.03.2026
Revised: 19.04.2026
Accepted: 24.03.2026
DOI:10.1922/ejprd.v34i2s.1379

Evaluation Of Antimicrobial Efficacy of Photosensitizer Loaded with Various Nanoparticles Mediated Photo Dynamic Therapy - An In Vitro Study

ABSTRACT

Aim: To evaluate the antimicrobial efficacy of toluidine blue O (TBO)-mediated photodynamic therapy (PDT) combined with various nanoparticle carriers in comparison with sodium hypochlorite (NaOCl) for disinfecting root canals infected with *Enterococcus faecalis*.

Materials and Methods: One hundred freshly extracted single-rooted human teeth were decoronated and standardized to a root length of 13 mm. Following biomechanical preparation using rotary instrumentation, the samples were sterilized and inoculated with *E. faecalis*. Specimens were incubated at 37°C for 21 days to allow biofilm formation. The samples were randomly allocated into five groups (n=20): Group 1 - 5.25% NaOCl , Group 2 - TBO + diode laser (DL); Group 3 - Chitosan-TBO nanoparticles (Chit-TBO NPs) + DL; Group 4 - Silver-TBO nanoparticles (Ag-TBO NPs) + DL; and Group 5 - TBO-Chitosan-Gold-Silver nanoparticles (TBO-Chit-Au-Ag NPs) + DL. Antimicrobial efficacy was assessed using colony-forming unit (CFU) counts. Statistical analysis was performed using the Shapiro–Wilk test for normality and the Tukey post hoc test for group comparisons, with the significance level set at $p < 0.05$.

Results: Group 1 demonstrated the lowest mean CFU count, indicating superior antimicrobial efficacy, while Group 2 exhibited the highest CFU values. All nanoparticle-mediated PDT groups (Groups 3–5) showed significantly reduced CFU counts compared to TBO alone ($p<0.0001$). Among them, the Ag-TBO NP group demonstrated the lowest bacterial counts; however, no statistically significant differences were observed among the nanoparticle groups ($p>0.05$).

Conclusion: NaOCl remains the most effective irrigant for root canal disinfection. Nanoparticle-assisted PDT significantly enhances antimicrobial efficacy compared to TBO alone and shows potential as an adjunctive approach, although it does not surpass NaOCl under the conditions of this study.

INTRODUCTION

The main etiologic agents that play a significant role in the pathogenesis of pulp and periapical diseases are microorganisms and their metabolic waste, which extensively influence the outcome of endodontic treatment [1]. Effective root canal treatment (RCT) is essential in the achievement of the elimination of microbes in the root canal system. Nevertheless, even with the development of chemo-mechanical preparation, irrigation treatment, intracanal medicaments, and hermetic obturation, the canal disinfection is still a clinical problem [2].

Primary endodontic infections normally comprise a polymicrobial flora that is predominantly composed of gram-negative non-aerobic bacteria. Persistent or secondary infections, on the other hand, are mainly caused by gram-positive organisms and especially by *Enterococcus faecalis* (*E. faecalis*) [3]. *E.*

faecalis
is a bacterium that is often linked to cases of treatment resistance, remarkable survival characteristics such as the ability to penetrate dentinal tubulewithstanding severe conditions such as high pH and salinity, resistance to intracanal disinfectants, biofilm formation, and survival in nutrient-depleted conditions [4].
Conventional irrigants such as sodium hypochlorite and chlorhexidine demonstrate little activity with debriding of anatomic complex areas such as lateral canals, isthmuses, fins, and accessory canals [5]. This has increased the need to develop more effective, bio-compatible, and resistance-free disinfection methods.
Photodynamic therapy (PDT) is a recently introduced minimally invasive adjunctive procedure in endodontic disinfection. PDT involves the use of a photosensitizer (PS), which is Toluidine Blue O (TBO), a photosensitizer that is activated by a wavelength of light and that produces reactive oxygen species (ROS) that are capable of destroying microbial cells. In spite of the possibility, conventional PSs are restricted by such aspects as low water solubility, insufficient tissue penetration, and insufficient specificity [6].
Nanoparticles (NPs) are particles between 1-100 nm that provide superior drug delivery by increasing tissue penetration and the release of treatment agents that last longer [7]. It is possible that nano- encapsulated materials (especially endodontic-specific) can be used to improve the antimicrobial properties of PDT. Chitosan (Chit), Silver nanoparticles (Ag NPs), and Chitosan-coated gold-silver core-shell nanoparticles (Chit-Au-Ag NPs) were used as the three nanoparticle carriers in delivering TBO in this study. Chitosan is a natural polycationic polymer that interacts with the microbial membranes using electrostatic energy to disrupt them [9]. Silver nanoparticles have a powerful oxidative and antimicrobial effect [10], and gold nanoparticles lead to high biocompatibility and good surface-area-volume rates.
The Chit-Au-Ag NPs have synergistic benefits, such as a high degree of surface area and physicochemical stability [12]. Such NPs conjugated with TBO could be even more effective in antimicrobial properties, particularly against the endodontic pathogens with resistance [8].
This paper will be focused on assessing the effectiveness of nanoparticle-assisted PDT as an adjunctive measure to conventional cleaning and shaping, filling the gaps in the penetration of PS and light delivery into the complex structure of the root canal system.

METHODOLOGY

Synthesis of Nanoparticles

1. Chitosan-Toluidine Blue Nanoparticles (Chit-TBO NPs):Chitosan nanoparticles were prepared by pouring a Toluidine Blue (TBO) solution (4g in 5 mL water) with the following reagents: 15 mL of 1% tripolyphosphate (TPP) solution, 500 mg ethylenediamine carbodiimide, and 950 mg N-hydroxy succinimide (both 5 mM). This solution was dropwise added to the chitosan solution in a 1:1 ratio and probe sonicated for 30 minutes. The solution was then dialyzed against distilled water on an 8000 Da cellulose membrane for 24 hours and freeze-dried at -45°C for 48 hours.
Characterization:
The particle size was 313-385 nm in diameter, and the zeta potential was +20.8 to +29.8 mV. The efficiency of entrapment of drugs ranged at 70-78% [13].

2. Silver-Toluidine Blue Nanoparticles (Ag-TBO NPs):
The production of silver nanoparticles was carried out by dissolving 0.21 g silver nitrate in water, after which polyvinylpyrrolidone (PVP) and potassium bromide were added drop by drop. To achieve an AgNP solution of 1000 ppm AgNP solution, the solution was stirred for 5 hours at room temperature and then diluted to 10 ppm. It was combined with a 20 ppm TBO solution and incubated under constant shaking for 24 hours. Centrifugation of the mixture was performed at 6000 rpm for 40 min, and rinsing of the pellet, followed by re-dissolution in water, was done.
Characterization:
The average particle size was 52 nm. The analysis of the UV-vis spectra did not reveal a change in the absorption peak of TBO (600 nm), which demonstrates the stability of conjugation [14].

3.TBO-Chitosan-Gold-Silver Nanoparticles (TBO-Chit-Au-Ag NPs):
Gold nanoparticles were produced by combining $HAuCl_4$ and chitosan at 50 °C to produce red-colored spherical AuNPs. The addition of silver nitrate and ascorbic acid was done gradually to create a silver shell on the gold core. Dark dropwise addition of TBO was done.
Characterization:
The nanoparticles' formation was established by UV-Vis analysis. TEM demonstrated an indistinct gold core and silver shell. SEM-EDS established the composition of elements (Au: 13.26%, Ag: 9.98%, C: 24.12%, O: 52.64%). Chit-AuNPs, chit-Au-AgNPs, and TBO-loaded NPs had a particle size of 129.4 nm, 131 nm, and 134 nm, respectively. The presence of stability and compatibility indicated that the zeta potential was positive (+37.6 to +41.2 mV) [12].

Tooth Sample Preparation:
One hundred freshly extracted teeth with straight canals, obtained following periodontal or orthodontic extractions, were collected. An ultrasonic scaler was used to clean the teeth, which were stored in saline and decoronated to a 12 mm long standardized root length. Apical patency was assessed using a #15 K-file(MANI, INC).
The root canals were irrigated using a standardized protocol with 5.25% sodium hypochlorite during instrumentation, followed by 17% EDTA for smear layer removal and a final rinse with saline. Biomechanical preparation was completed using ProTaper Gold rotary files (Dentsply Sirona, North Carolina, USA) up to size F4. The apical foramina were then sealed, and each sample was placed in 1 mL of phosphate buffer solution within sterile

microcentrifuge tubes (Satya Moulds, India). Sterilization was achieved by autoclaving at 121°C for 15 minutes.

Bacterial Inoculation and Biofilm Formation:
All the microbiological operations were carried out aseptically in a laminar flow cabinet. *Enterococcus faecalis* (ATCC 29212) was grown using frozen stock, further sub cultured on trypticase soy broth, and incubated by placing it on blood agar at 37°C after 24 hours. A Gram stain test and a bile esculin test were done to confirm the identification.
A 10 µL volume of *E. faecalis* suspension (5×10^4 CFU/mL) was added to each sterilized root canal. Biofilm formation on the samples was carried out by incubation at 37 °C for 21 days, with the culture broth replaced daily. Canals were dried using sterile paper points and incubated, after which they were aspirated.

Samples were divided into five groups (n=20):
Group 1: 5.25% NaOCl for 120 seconds
Group 2: TBO followed by diode laser (620-640 nm) for 120 seconds
Group 3: Chitosan-TBO nanoparticles (Chit-TBO NPs) + DL
Group 4: Silver-TBO nanoparticles (Ag-TBO NPs) + DL
Group 5: TBO-Chitosan-Gold-Silver nanoparticles (TBO-Chit-Au-Ag NPs) + DL

Microbiological Evaluation:
After the treatment procedures, every root canal was rinsed with 1 mL of sterile saline to remove the remaining agents. The canals were subsequently inoculated with saline, and the aliquots of the dilute suspension streaked on blood agar plates to analyze colony-forming unit (CFU).

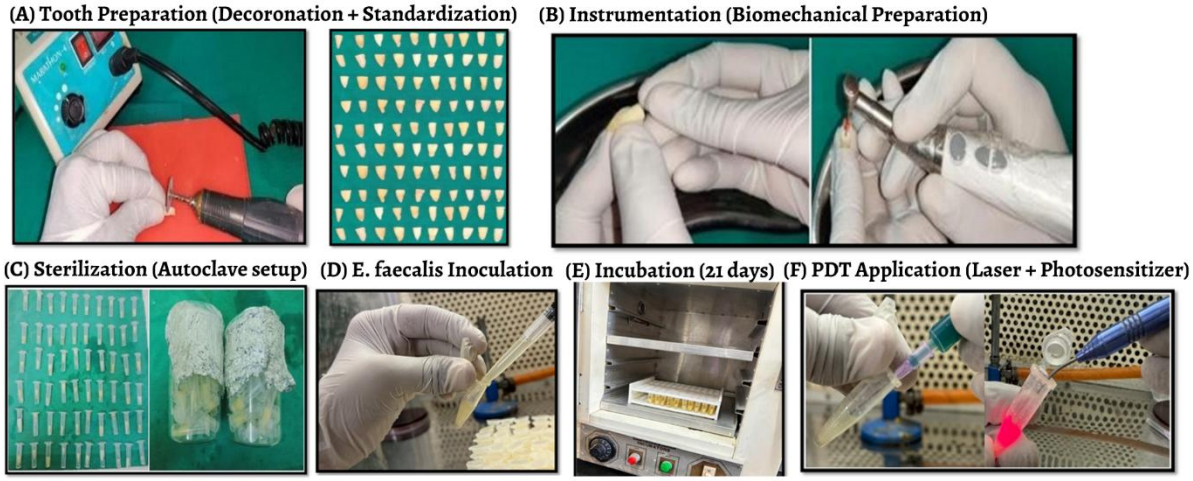

Figure 1. Sample preparation, biofilm formation, and photodynamic therapy: Experimental workflow. (A) Tooth preparation with decoronation and standardization of root length; (B) Biomechanical preparation with rotary instrumentation (ProTaper system); (C) Sterilization of samples in autoclave using microcentrifuge tubes; (D) Inoculation of root canals with suspension of *E. faecalis* under aseptic conditions; (E) Incubation of samples in 37°C and 21 days to allow the formation of biofilms; (F) Application of photodynamic therapy using toluidine blue O-based photosensitizer and diode laser irradiation.

Figure 1 demonstrates the general experimental workflow that was used in the current study. First, the extracted teeth were decorated and standardized to a constant root length so that all samples would have the same root length. This was then followed by biomechanical preparation with rotary instrumentation. The ready samples were subsequently sterilized under autoclaving conditions in order to remove already existing microbial contamination. Root canals were then inoculated with *E faecalis*, after which they were left to incubate over a period of 21 days to allow biofilm development. After incubation, the samples were then treated according to their respective treatment procedures, such as photodynamic therapy using toluidine blue O in the presence of diode laser irradiation, to assess the antimicrobial effectiveness.

RESULTS

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was assessed using the Shapiro–Wilk test. Colony-forming unit (CFU) counts across all groups were summarized using descriptive statistics, including mean, median, standard deviation, standard error, and 95% confidence intervals. Differences in mean values among groups were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant

Group	N	Mean CFUs	Median	Standard Deviation	Standard Error	95% Confidence Interval for mean	
						Lower Bound	Upper Bound
Group 1 (Control)	20	6.05	6.0	2.395	0.535	4.924	7.176
Group 2	20	79.0	79.5	13.673	3.057	72.503	85.497
Group 3	20	40.9	43.0	9.346	2.089	36.464	45.336
Group 4	20	33.6	31.5	9.011	2.015	29.325	37.875
Group 5	20	37.0	37.0	6.374	1.425	33.989	40.011

Table 1. Descriptive Statistics of Mean CFU Counts Across Experimental Groups

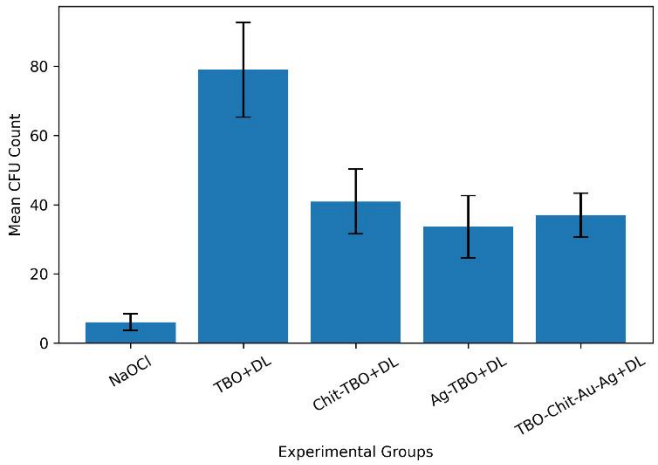

Figure 2. Comparison of mean colony-forming unit (CFU) counts across experimental groups

Figure 2 demonstrates the effectiveness of the antimicrobial action in the experimental groups. Group 1 (5.25% NaOCl) exhibited the lowest mean CFUs count, whereas Group 2 (TBO + DL) exhibited the highest. The values of the CFU in nanoparticle-mediated photodynamic therapy groups are intermediate, with the lowest counts in Group 4 (Ag-TBO NPs + DL). The nanoparticle groups did not show statistically significant differences.

Group 1 had the lowest mean CFU count among all the groups tested, a fact that shows that the group has the best antimicrobial efficacy. The statistical analysis proved that this population was strongly different from all the rest ($p < 0.0001$), which proved its high disinfection power again. However, Group 2 had the largest mean CFU count, which indicated the least antimicrobial activity, and was significantly different from the rest of the groups ($p < 0.0001$). The three Groups 3, 4, and 5 nanoparticles-based photodynamic therapy (PDT) exhibited intermediate antimicrobial activities. All these groups were much more effective than Group 2 ($p < 0.0001$), but they were not as effective as Group 1. Notably, no statistically significant differences were observed between the three nanoparticles themselves ($p > 0.05$), which indicates that there were no significant differences between these formulations in terms of bacterial reduction.

DISCUSSION

Enterococcus faecalis commonly causes persistent root canal infections that are resistant to the traditional disinfection processes because the bacteria form biofilm, infiltrate dentinal tubules, and are also resistant to alkali tolerance [3]. The fact that it remains resilient makes it a good model to be used to test other methods, such as antimicrobial photodynamic therapy (aPDT).

The antimicrobial effectiveness of toluidine blue O (TBO)-mediated PDT, in mono and in conjugation with different nanoparticles (NPs), against the traditional irrigant sodium hypochlorite (NaOCl) was compared in this study. The results showed that the use of 5.25% NaOCl was much better than all PDT-based groups, which substantiated its use as the gold standard in endodontic irrigation because it has tissue-dissolving and high oxidizing abilities. The oxidative damage caused by the use of hypochlorite ions has been associated with rapid protein degradation/membrane disruption/bacterial lysis mechanisms, which are clearly described in the literature [15].

Nevertheless, even though it is effective, NaOCl also has significant disadvantages, such as cytotoxicity, inability to penetrate deep dentinal tubules, and extrusion risk to periapical tissues [16]. In order to deal with these shortcomings, another potential modality has come to the forefront: PDT as an adjunctive (or alternative) modality. PDT is a site-specific disinfection plan that utilizes the light activation of a photosensitizer (PS) in oxygen to produce reactive oxygen species (ROS), which destroy bacterial membranes, proteins, and DNA [17].

Group 2, which incorporated TBO and only a diode laser (DL), had the highest bacterial load compared to other groups, and this implied the lowest efficacy. Although TBO is an effective cationic PS that is reported to exhibit better membrane interaction and ROS generation than methylene blue [18], it's penetration into biofilms and dentinal tubules is, nevertheless, poor when applied without nanocarriers [19]. This could be the reason behind the poor result of TBO + DL as a single practice, which underlines the need to implement stronger delivery mechanisms of PS.

To address these limitations, NP have been considered useful PS carriers as they are more soluble, can be targeted, and deliver to the intracellular levels [20]. The groups in this paper, i.e., nanoparticle-based PS, Chit-TBO NPs (Group 3), Ag-TBO NPs (Group 4), and TBO-Chit-Au-Ag NPs (Group 5), showed considerably better antimicrobial activity than TBO, but still, less statistically significant than 5.25% NaOCl.

The Ag-TBO NPs (Group 4) showed the lowest mean number of colony-forming units (CFUs), but statistical analysis showed no significant difference was present between the three NP groups. The antimicrobial effects of silver nanoparticles are well documented because the nanoparticles can disrupt the bacterial membranes, induce intracellular ROS, and disrupt the bacterial replication process [21]. Their positive charge on the surface boosts their affinity to negatively charged bacterial cell walls, leading to increased cellular uptake [22]. Moreover, they are nanoscale (52 nm), which is beneficial in penetrating deeper into dentinal tubules and has the potential to enhance bactericidal efficacy [21].

Ag-TBO had a slightly lower performance compared to the Chit-TBO NPs (Group 3), yet it was also significantly less effective than the latter. Chitosan is a mucoadhesive, biocompatible polymer, which increases PS delivery, decreases degradation mediated by collagenase, and increases collagen crosslinkage in dentin [23, 24]. It also interacts with the negatively charged dentin matrix through the interaction of its positive surface charge, which enables retention of the drug [25]. It was argued that the antimicrobial effect of this group was caused not only by an intrinsic antibacterial effect of chitosan but also by an improved delivery of TBO.

Group 5 (TBO-Chit-Au-Ag NP) presented moderate antibacterial activity, which was a little higher than Chit-TBO and a little lower than Ag-TBO. Even though the gold nanoparticles possess the ability to have properties that are the most desirable, such as low toxicity and enhancement of photothermal, it is possible that their size (134 nm) was significantly higher than that of Ag-TBO NPs, so they were not able to penetrate deeper tubules [26]. This combination has, however, been promising in other biofilm models, like in diabetic foot ulcers, whereby severe reduction of bacteria and healing of the wound were recorded [12].

A mind-grabbing point was that the groups of nanoparticles were not statistically different, even though their sizes and compositions differed. This implies that nanoparticle conjugation, together with size, does increase the efficacy of PDT, but the exact formulation and surface characteristics, including zeta potential, functionalization, and kinetics of release, may have a greater impact than size itself [27].

These results indicate that PDT using nanoparticles can be used as an adjunctive modality to the regular endodontic treatment. NaOCl appeared to be more effective, but its use with nanoparticle-assisted PDT could be able to make use of the merits of the two approaches. NaOCl has the potential to offer immediate debridement and disinfection, whereas PDT could aid in eliminating residual bacteria in those areas that are not accessible to the irrigants, like lateral canals, isthmus, and deep dentinal tubules. This combination may improve overall treatment efficacy and reduce reinfection risk.

Limitations of the Study

This in vitro experiment on extracted teeth might not be a complete replication of in vivo conditions. It evaluated the antimicrobial activity only against *E. faecalis* in terms of CFU counts, without considering the depth of biofilms and the prolonged outcomes. Variability might have been caused by anatomical variations and manual procedures.

CONCLUSION

Under the limitations of this in-vitro study, 5.25% NaOCl was found to be the most effective antimicrobial agent against *E. faecalis*. TBO was the least effective, whereas nanoparticle-based PDT (Chit-TBO, Ag-TBO, TBO-Chit-Au-Ag) was much more effective in bacterial reduction. Ag-TBO NPs exhibited the highest efficacy, likely due to their smaller size and enhanced antimicrobial activity. Although differences among NP groups were not significant, all showed comparable efficacy, suggesting potential as adjuncts. The synergistic disinfection could be provided through the combination of NaOCl with NP-based PDT. Additional clinical research of polymicrobial biofilms and optimal NP preparations is justified.

REFERENCES

1. Wieczorkiewicz K, Jarząbek A, Bakinowska E, Kiełbowski K, Pawlik A. Microbial dynamics in endodontic pathology: from bacterial infection to therapeutic interventions—a narrative review. *Pathogens*. 2024;14(1):12.
2. Wong J, Manoil D, Näsman P, Belibasakis GN, Neelakantan P. Microbiological aspects of root canal infections and disinfection strategies: an update review on current knowledge and challenges. *Front Oral Health*. 2021; 2:672887.
3. Siqueira JF Jr, Rôças IN. Microbiology and treatment of endodontic infections. *Int Endod J*. 2022;55(1):12–34.
4. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed

endodontic treatment and outcome of conservative retreatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85(1):86–93.
5. Siqueira JF Jr. Aetiology of root canal treatment failure: why well-treated teeth can fail. *Int Endod J*. 2001;34(1):1–10.
6. Soukos NS, Chen PS, Morris JT, Ruggiero K, Abernethy AD, Som S, et al. Photodynamic therapy for endodontic disinfection. *J Endod*. 2006;32(2):979–984.
7. Samiei M, Farjami A, Dizaj SM, Lotfipour F. Nanoparticles for antimicrobial purposes in endodontics: a systematic review of in vitro studies. *Mater Sci Eng C Mater Biol Appl*. 2016; 58:1269–1278.
8. Pagonis TC, Chen J, Fontana CR, Devalapally H, Ruggiero K, Song X, et al. Nanoparticle-based endodontic antimicrobial photodynamic therapy. *J Endod*. 2010;36(8):322-8.
9. Huang N, Lin J, Li S, Deng Y, Kong S, Hong P, Yang P, Liao M, Hu Z. Preparation and evaluation of squid ink polysaccharide-chitosan as a wound-healing sponge. *Materials Science and Engineering*: C. 2018 1; 82:354-62.
10. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*. 2009;27(1):76-83.
11. Dykman LA, Khlebtsov NG. Gold nanoparticles in biology and medicine: recent advances and prospects. *Acta Naturae*. 2011;3(2):34-55.
12. Akhtar F, Khan AU, Qazi B, Kulanthaivel S, Mishra P, Akhtar K, Ali A. A nano phototheranostic approach of toluidine blue conjugated gold-silver core-shells mediated photodynamic therapy to treat diabetic foot ulcer. *Sci Rep*. 2023; 13:1490.
13. Hegde V, Srilatha S, Vangala A, Khandwawalla N, Mujawar A. Antimicrobial efficacy of triple antibiotic-loaded chitosan nanoparticles activated with photochemical disinfection: A microbiological and confocal microscopic analysis. *J Conserv Dent* 2022; 25:252-7.
14. Aydın H, Er K, Kuştarcı A, et al. Antibacterial activity of silver nanoparticles activated by photodynamic therapy in infected root canals. *Dent Med Probl.* 2020;57(4):393-400.
15. Gulabivala K, Ng YL, Gilbertson M, Eames I. The fluid mechanics of root canal irrigation. Physiological measurement. 2010 1;31(12): R49-84.
16. Hülsmann M, Hahn W. Complications during root canal irrigation-literature review and case reports. *International Endodontic Journal*. 2000 1;33(3).
17. Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. *Biochem J.* 2016 15;473(4):347-64.
18. Usacheva MN, Teichert MC, Biel MA. Comparison of the methylene blue and toluidine blue photobactericidal efficacy against gram-positive and gram- negative microorganisms. *Lasers Surg Med*. 2001; 29:165-73.
19. Balhaddad AA, Garcia IM, Ibrahim MS, Rolim JPML, Gomes EAB, Martinho FC, Collares FM, Xu H, Melo MAS. Prospects on nano-based platforms for antimicrobial photodynamic therapy against oral biofilms. *Photobiomodul Photomed Laser Surg*. 2020; 38:481-96.
20. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity, and regulations. *Beilstein J Nanotechnol*. 2018; 9:1050-74.
21. Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MMQ. Nanosilver particles in medical applications: synthesis, performance, and toxicity. *Int J Nanomedicine*. 2014; 9:2399-407.
22. Abbaszadegan A, Nabavizadeh M, Gholami A, et al. Positively charged imidazolium-based ionic liquid-protected silver nanoparticles: a promising disinfectant in root canal treatment. *Int Endod J.* 2015;48(8):790-800.
23. Mohammed MA, Syeda JT, Wasan EK. An overview of chitosan nanoparticles and their application in non-parenteral drug delivery. *Pharmaceutics*. 2017; 9:53.
24. Shrestha A, Friedman S, Kishen A. Photodynamically cross-linked chitosan-incorporated dentin collagen. *J Dent Res*. 2011; 90:1346-51.
25. Taravel MN, Domard A. Relation between the physicochemical characteristics of collagen and its interactions with chitosan: *I. Biomaterials*. 1993; 14:930-8.
26. Basudan AM. Nanoparticle-based periodontal drug delivery-a review on current trends and future perspectives. *The Saudi dental journal*. 2022 1;34(8):669-80.
27. Hada AM, Potara M, Suarasan S, Vulpoi A, Nagy-Simon T, Licarete E, Astilean S. Fabrication of gold-silver core-shell nanoparticles for performing as ultrabright SERS-nanotags inside human ovarian cancer cells. *Nanotechnology.* 2019 3;30(31):315701.