Review Article

Comparative evaluation of the antimicrobial efficacy of nanoparticle-mediated photodynamic therapy versus photodynamic therapy and conventional disinfection in endodontics: A systematic review and meta-analysis

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Abstract

Introduction: The aim of this systematic review was to assess the antimicrobial activity of nanoparticle-mediated photodynamic therapy (N-PDT) on *Enterococcus faecalis* biofilms in the presence of dentin substrate when compared to photodynamic therapy (PDT) and conventional disinfection protocols.

Materials and Methods: This systematic review was registered in Open Science Framework (10.17605/OSF.IO/GBR3F). Six databases, namely PubMed, Embase, Web of Science, Scopus, Medline, and Google Scholar, were searched for English language articles until June 2022. Laboratory studies assessing the antimicrobial activity of N-PDT against *E. faecalis* biofilm in human or bovine teeth were included. The risk of bias (RoB) was evaluated using the Joanna Briggs Institute tool for quasi-experimental studies. Meta-analysis was performed using the random-effects maximum likelihood model.

Results: The search revealed 2804 articles, out of which 9 studies were included in the final review. Seven articles had low RoB and two had moderate RoB. Chitosan and diode laser at 810 nm were the most commonly used nanoparticle and light source, respectively. The meta-analysis of bacterial reduction log and percentage reduction revealed that N-PDT had better antimicrobial efficacy than the control group. When the bacterial reduction log of N-PDT was compared with PDT, PDT performed better N-PDT, and for percentage reduction, there was no difference.

Conclusion: The currently available evidence is low and inconclusive with regard to the superior efficacy of N-PDT. The type of nanoparticle, incubation time, light source, and exposure time were found to be covariates that influence the antimicrobial efficacy of N-PDT.

Keywords: Antimicrobial; *Enterococcus faecalis*; nanoparticle; photodynamic therapy; photosensitizer

INTRODUCTION

Photodynamic therapy (PDT) is a procedure that inactivates cells, microorganisms, or molecules by light-activated chemical reactions via a photosensitizer

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in the presence of oxygen.[1] This ability of light to eradicate bacteria was first discovered by Oscar Raab.[2] PDT has also been referred to as photo-activated disinfection (PAD), light-activated disinfection, photochemotherapy, and photoactivated antimicrobial chemotherapy.[3]

There are two components involved in PDT, namely a photosensitizer and a light source. The photosensitizer is a dye that can absorb energy from a light source and transfer it to another molecule.[4] The most commonly used photosensitizers are phenothiazines which include toluidine blue O (TBO) and methylene blue (MB). To activate these photosensitizers, diode laser, light-emitting diode (LED), and halogen lamps are commonly employed.[5]

The aim of endodontic therapy is to eliminate or prevent the growth of microorganisms within the root canal. *Enterococcus faecalis* is a facultative, Gram-positive bacterium that is commonly present in the root canal system and often implicated in failed endodontic therapy. It can form biofilms, develop antibiotic resistance, and survive even after disinfection.[6] PDT is used for enhancing the disinfection of the root canal system because it can overcome bacterial resistance and penetrate well into biofilms and has been used as an adjunct for the effective elimination of *E. faecalis* from the root canal system.[7]

To overcome the clustering of photosensitizers in an aqueous environment, nanoparticles have been used as carriers to improve the transfer of photosensitizers to the target tissue.[8] Nanoparticles have a unique physiochemical property due to their nanoscale sizes and high surface area-to-volume ratio that enables better penetration into biofilms, thereby resulting in high antimicrobial activity.[9,10] They are believed to improve the drug delivery in the target area ensuring maximum therapeutic effect. The advantages of nanoparticle-mediated PDT (N-PDT) over conventional PDT have targeted cell selectivity, increased photosensitizer uptake, reduced photosensitizer leakage from target cells, increased stability, and controlled release of reactive oxygen species.[8] Thus, N-PDT can be potent in eliminating bacterial biofilms by overcoming the limitations of conventional disinfection protocols.[11]

However, whether the use of N-PDT will allow for better antimicrobial efficacy than the control group (conventional disinfection) and PDT alone is controversial since these studies have employed different methodologies that may influence the outcome of antibacterial reduction. Thus, the current systematic review and meta-analysis were carried out with the aim to assess the antimicrobial efficacy of N-PDT on *E. faecalis* biofilms.

MATERIALS AND METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol registration of the systematic review was done in Open Science Framework with the platform number 10.17605/OSF.IO/GBR3F.

Research question

The search was defined based on the PICOS strategy (Population – P, Intervention – I, Comparison – C, Outcome – O, and Study design – S).

- P – Endodontic biofilms comprising *E. faecalis*
- I – N-PDT
- C – Control and PDT
- O – Microbial load reduction: Colony-forming units (CFU/ml) and percentage of microorganisms in the biofilm
- S – Laboratory studies.

Literature search

A literature search of five databases, PubMed, Embase (using Ovid interface), Web of Science, Scopus, and Medline, was performed. For gray literature, Google Scholar was searched. All databases were searched for articles published in the English language till June 2022. The keywords used during the search were photodynamic therapy, nanoparticles, *E. faecalis*, biofilms, and endodontics. The search strategy for each database is listed in Table 1.

Table 1: Search strategy

Database	Search strategy
PubMed	((((((“Photochemotherapy”[Mesh]) OR “Photosensitizing Agents”[Mesh]) OR “Phototherapy”[Mesh]) OR (“light activated disinfection”)) OR (“photo activated disinfection”)) AND ((((“Endodontics”[Mesh]) OR “Biofilms”[Mesh]) OR “Anti-Infective Agents”[Mesh]) OR “Dental Caries”[Mesh])) AND (“Nanoparticles”[Mesh])
Web of Science	(TS=(photodynamic therapy OR phototherapy OR photosensitizing agents OR light activated disinfection OR photo activated disinfection)) AND (TS=(Endodontic* OR biofilm* OR antimicrobial)) AND (TS=(nanoparticle*))
Scopus	(“photodynamic therapy” OR “phototherapy” OR “light activated disinfection” OR “photo activated disinfection”) AND (endodontic* OR biofilm* OR antimicrobial* OR “root canal”) AND (nanoparticle*)
Embase	((photodynamic therapy or photosensitizing agents or phototherapy or light activated disinfection or photo activated disinfection) and (endodontic* or biofilm* or antimicrobial*) and (nanoparticle*)
Medline	(“Photochemotherapy” OR “Photosensitizing Agents” OR “Phototherapy” OR “light activated disinfection” OR “photo activated disinfection”) AND (“Endodontics” OR “Biofilms” OR “Anti-Infective Agents” OR “Dental Caries”) AND (“Nanoparticles”)
Google Scholar	Photodynamic therapy or photosensitizing agents or phototherapy or light activated disinfection or photoactivated disinfection or endodontics or biofilms or anti-infective agents or dental caries and nanoparticles

Laboratory studies reporting *E. faecalis* load before and after N-PDT in human or bovine teeth, regardless of the type of photosensitizer, light source, duration of exposure, sampling method, and use of tissue inhibitors were included in this review. Experiments conducted against planktonic bacteria, case reports, literature reviews, editorials, articles in languages other than English, opinion/personal comments, conference abstracts, thesis, dissertations, and books were excluded from this review.

Data extraction

Two reviewers (SY and RJ) performed the searches. Following this, screening of titles and abstracts was done, and duplicates were removed using Zotero (version 5.0.8). Full-text reading of the remaining articles was then carried out, and those fulfilling the predefined inclusion criteria were selected. In case of any disagreements, the senior investigators (SRP and VN) were consulted and the final decision was made through discussion until a consensus was reached.

The following variables were considered for the data extraction from the included articles:

(i) Study characteristics – Author/year of publication and type of article
(ii) Sample – Tooth type and sample material
(iii) Intervention characteristics – Comparator group characteristics, nanoparticles, light source, control groups, microorganisms, use of tissue inhibitors, and sampling method
(iv) Outcome – Assessment method and microbial load.

Data extraction was done in an Excel spreadsheet by two reviewers (SY and RJ). Data extraction was verified for accuracy by the senior investigators (SRP and VN) [Table 2].

Quality assessment of selected studies

A quality assessment of selected studies was performed using the Joanna Briggs Institute (JBI) critical appraisal tool for quasi-experimental studies. Two reviewers (SY and RJ) independently scored the articles. In case of any disagreements, the senior investigators (SRP and VN) were consulted and the final decision was made through discussions. Out of the nine questions in JBI, one question was excluded as the question pertaining to follow-up was irrelevant to the *in vitro* nature of the studies, and hence, the scoring was done out of eight questions. A senior endodontist (VN) validated the modified JBI critical appraisal tool. The percentage of positive answers (yes) was used to calculate the final score. The risk of bias (RoB) was categorized as "high" (score equal to or lower than 49%), "moderate" (50%–69%), or low (higher than 70%).[12]

Statistical analysis

It was decided a priori that if the statistical pooling of data from the included studies was justifiable, a meta-analysis would be carried out. Meta-analysis was performed using STATA SE version 17 (STATA Corporation, College Station, Texas, USA). In the present meta-analysis, the random effects maximum likelihood model was adopted to estimate all the pooled estimates because it produces an unbiased, nonnegative estimate of between-study heterogeneity. As Higgins I^2 is a better measure for evaluating the percentage of variability across the studies due to true heterogeneity, it was preferred over Cochran's Q statistic. $P < 0.05$ or $I^2 > 50\%$ indicated the presence of heterogeneity.[13] A tau's square test was also used to assess heterogeneity in the random-effects model.[14] All P values were two-sided with $\alpha = 5\%$, except for the test of between-study heterogeneity ($\alpha = 10\%$). Standardized mean difference (SMD) "Hedge's g" was used as the measure of effect size for the pooled estimates from the included studies. Hedge's and Olkin's bias correction factors were applied during the calculation of SMD. Metaprop and metapreg packages were used to determine the pooled estimates expressed as percentages. In a subgroup analysis, studies were stratified according to the incubation period, type of nanoparticle used, presence of tissue inhibitors, photosensitizer used, light source, and duration of exposure to the light source. In case of missing data, the authors were contacted through E-mail for information regarding the light source, duration of exposure to the light source, bacterial reduction log (mean standard deviation [SD]), and percentage reduction of bacteria after the N-PDT.

A meta-regression analysis was also planned *a priori* to determine the effect of the covariates (incubation period, type of nanoparticle used, presence of tissue inhibitors, photosensitizer used, light source, and duration of exposure to light source) on the pooled outcome measure. The residual heterogeneity across the studies after accounting for all the covariates affecting the outcome measure was determined.

RESULTS

A total of 2804 articles were identified through electronic databases and gray literature searches. After the removal of duplicates and screening abstracts, 2764 articles were eliminated and 40 full-text articles were included. Subsequently, after the full-text reading of 40 articles, only 9 articles were included in the final analysis since 31 articles did not meet the inclusion criteria. A flow diagram of the search strategy is presented in Figure 1 according to PRISMA guidelines.

Study characteristics

Tooth type

Extracted human permanent teeth were used in six of the included articles, out of which five articles used

Table 2: Data extraction

Author	Tooth type	Sample	Organism	NP	Light source
Pagon`is *et al*., 2010	Extracted single-rooted human teeth decoronated to 12 mm (*N*=32, *n*=4)	Microtubes from root canals	*E. faecalis*	PLGA	Diode laser 665 nm/1 W/5 min
Shrestha *et al*., 2012	Human third molars and bovine teeth	NM	*E. faecalis*	CSNP	5 J/cm^2/1.66 min and 10 J/cm^2/3.33 min
DaSilva *et al*., 2013	Bovine incisors decoronated to 4 mm (*N*=35, *n*=6–8)	NM	*E. faecalis*	CS	540 nm/40 J/cm^2/4 min
Shrestha *et al*., 2014a	BSA, pulp from bovine teeth, dentin powder, and matrix from extracted human third molars	NM	*E. faecalis*	CS+RB/MB	5 J/cm^2/1.66 min and 10 J/cm^2/3.33 min
Shrestha *et al*., 2014b	Extracted human molars	NM	Biofilm containing *E. faecalis, P. intermedia, A. naeslundii, S. oralis*	CSRB	540 nm/60 J/cm^2
Afkami *et al*., 2016	Extracted single-canal human teeth (*N*=65, *n*=9)	Paper points and files from root canals	*E. faecalis*	AgNP	Diode laser 810 nm/200 mW/30 s
Camacho- Alonso *et al*., 2017	Extracted single-rooted human teeth decoronated to 12 mm (*N*=102, *n*=17)	Paper points from root canals	*E. faecalis*	CSNP	660 nm at 100 mW/60 s
Golmohamadpour *et al*., 2018	Extracted human mandibular 1st premolar (*n*=20)	Tungsten carbide burs from root canals	*E. faecalis*	ICG loaded Nano - MOF (Fe 101, Fe 88, Al 101)	Diode laser 810/250 mW
Aydin *et al*., 2021	Extracted human mandibular molars - decoronated to 13 mm (*N*=120, *n*=20)	Paper points from root canals	*E. faecalis*	AgNP	LED 620–640 nm/2000–4000 W/cm^2/30 or 60 s

Author	Control	Other groups	Sample assessment	*P*	Result
Pagon`is *et al*., 2010	Without MB + light + NP	MB + PLGA, MB + PLGA + Light	CFUs	<0.05	Bacterial reduction: MB + PLGA + Light (significant) > MB + PLGA
Shrestha *et al*., 2012	Water	CSNP + MB/RB	NM	<0.05	Antibacterial activity inhibition: Dentin matrix > BSA >pulp > dentin powder > LPS
DaSilva *et al*., 2013	ZOE	ZOE + CS, ZOE + CS + surface treatment, CSRB + PDT, ZOE + CSRB + surface treatment + PDT	SEM, CLSM	>0.05	1 week - No significant difference; 4 weeks – ZOE + CSRB + CS + PDT: > Biofilm inhibition
Shrestha *et al*., 2014a	CSRB NP without inhibitors vehicle control: CSRB	MB, MB + BSA, MB + BSA for 24 h, MB + pulp, MB + pulp for 24 h; CSRB, CSRB + BSA, CSRB + BSA for 24 h, CSRB + pulp, CSRB + pulp for 24 h; RB, RB + BSA, RB + BSA for 24 h, RB + pulp, RB + pulp for 24 h	CFUs	<0.05	Pulp and BSA - Significant. inhibited antibacterial activity of photosensitizers
Shrestha *et al*., 2014b	NM	CSRB/RB + PDT	SEM, CLSM	<0.05	CSRB + PDT - Significant bacterial reduction
Afkami *et al*., 2016	NaOCl	Diode laser, AgNP, ICG + Diode, ICG + AgNP + Diode	CFUs	<0.05	AgNP + ICG + Diode laser: > highest reduction *n* colony count
Camacho- Alonso *et al*., 2017	Positive control: No treatment Negative control: No bacteria, no treatment	NaOCl, PDT, CS, PDT + CS	CFUs, SEM	<0.05	CS + PDT: > Antibacterial effect
Golmohamadpour *et al*., 2018	Bacterial suspension	Fe 101, Fe 88, Al 101, Fe 101 + ICG + PDT, Fe 88 + ICG + PDT, Al 101 + ICG + PDT	CFUs	<0.05	ICG-loaded MOFs mediated aPDT: Significant reduction of *E. faecalis*
Aydin *et al*., 2021	Positive control: NaOCl	TBO + light (30/60 s), AgNP, TBO + AgNP, AgNP + light (30/60 s), TBO + AgNP + light (30/60 s)	CFUs	<0.004	NaOCl (control): Statistically significant reduction of *E. faecalis* than other groups

NP=Nanoparticle, RB=Rose bengal, CS=Chitosan, PDT=Photodynamic therapy, AgNP=Silver NP, aPDT=Antimicrobial PDT, BSA=Bovine serum albumin, CSNP=CS NP, CSRB=Conjugated with RB, CLSM=Confocal laser scanning microscopy, CFU=Colony-forming unit, ICG=Indocyanine green, LED=Light-emitting diode, MB=Methylene blue, MOFs=Metal organic frameworks, NM=Not mentioned, PLGA=Polylactic glycolic acid, SEM=Scanning electron microscope, TBO=Toluidine blue O, ZOE=Zinc oxide eugenol, *E. faecalis=Enterococcus faecalis, P. intermedia=Prevotella intermedia, A. naeslundii=Actinomyces naeslundii, Streptococcus oralis=Streptococcus oralis*, NaOCl=Sodium hypochlorite

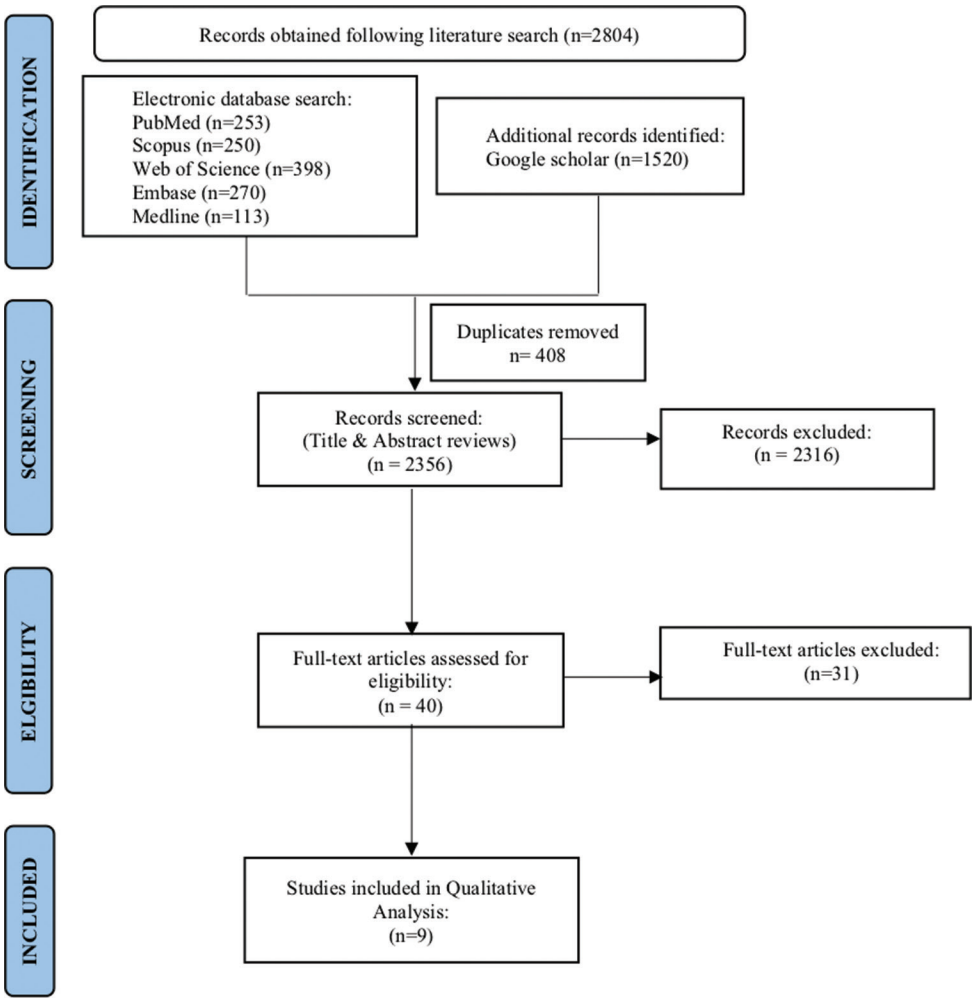

Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the study search and identification

single-rooted teeth.[8,15-18] A combination of human and bovine teeth was used in two articles.[19,20] One article each used extracted human permanent multirooted teeth[21] and bovine teeth.[22]

Photosensitizer used in the studies

The photosensitizers used included TBO, MB, indocyanine green (ICG), rose bengal (RB), and polylactic co-glycolic acid (PLGA). RB was used as a photosensitizer in four articles,[19-22] whereas MB was used as a photosensitizer in three articles.[17,21,22] Two articles[16,18] used ICG as the photosensitizer. TBO[8] and PLGA[15] were used in one article each.

Laser parameter of the included articles

The wavelength of the light source used ranged from 540 to 810 nm. The duration of exposure to a light source ranged from 30 s to 5 min. Diode laser was used as the light source in three articles.[15,16,18] LED was used as the light source in one article.[8] Five of the included articles did not mention the light source used.[17,19-22]

Sample and sampling method

From root canals, the sample was collected using absorbent paper points in three articles and files, tungsten carbide bur, and microbrush was used in one article each.[8,16,17] Two articles used dentin disc[21,22] and dentin powder (obtained by mechanical grinding).[19,20]

Sample assessment

CFUs were used to measure microbial load reduction in six articles.[8,15-18,21] Percentage reduction was calculated using the formula:

$$\frac{CFUs(before\ \ treatment) - CFUs(after\ \ treatment)}{CFUs(before\ \ treatment)} \times 100$$

A combination of scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) was used in two articles.[21,22] SEM was used to assess the percentage of biofilm-covered interface, uniformity, thickness, and abundance of extracellular polymeric substance in biofilm. CLSM was used to assess the biofilm area at the sealer–dentin interface, biofilm structure, and uptake

of CSNP by biofilm. One article alone used SEM for the assessment of the percentage of area with contamination and debris in relation to the total area.[17]

Microbiological assessment

All of the included articles evaluated the antimicrobial efficacy against *E. faecalis*. In addition, one article[21] evaluated the efficacy of PDT against multispecies biofilm containing *E. faecalis*, *Prevotella intermedia, Actinomyces naeslundii,* and *Streptococcus oralis.*

Quality assessment of included studies

Risk of bias

The methodological quality assessment was done using the modified JBI critical appraisal checklist for quasi-experimental studies. The agreement between the two reviewers (SY and RJ) calculated using Cohen's kappa inter-examiner reliability was 0.8266 [Supplementary Figure 1]. Overall, based on the percentage of positive answers (yes), seven articles[15-19,21] had a low RoB, whereas two articles[21,22] had a moderate RoB [Figure 2].

Figure 2: Risk of bias scoring of individual articles and overall risk of bias of individual articles using Joanna Briggs Institute tool

Quantitative assessment of included studies

The corresponding authors were contacted via E-mail for the data regarding the bacterial reduction log (SD and mean),[18,20] percentage reduction (SD and mean),[15,17,19] light source, and its duration of exposure.[17,20-22] Despite contacting the authors twice within a span of 1 month, the necessary data could not be procured. The meta-analysis was performed for the parameters: bacterial reduction log (four studies)[8,15-17] and percentage reduction (three studies).[8,16,18] A network meta-analysis for comparison of antimicrobial activity between N-PDT versus PDT versus control was not possible as the control groups were different in the concentrations of NaOCl used, besides the methodology used for checking the antimicrobial activity varied considerably.

Bacterial reduction log

In four studies (eight comparisons), the bacterial reduction log was compared between N-PDT versus the control group. The CFU/mL was reported to be higher in the case of the control group when compared to the N-PDT group; however, the extent of heterogeneity was very high[15-17] (SMD = −3.46, 95% confidence interval = [−5.46–−1.46] [I^2 = 94.68%]) [Figure 3].

In three studies, the bacterial reduction log was measured in the PDT and N-PDT groups.[8,16,19] The CFU/mL was reported to be higher in the case of the N-PDT when compared to PDT; however, the extent of heterogeneity was very high (SMD = 0.70, 95% confidence interval = [0.05–1.34] [I^2 = 69.55%]) [Figure 3].

Bacterial percentage reduction

Two studies[8,16] found no significant difference in the percentage reduction of biofilm in either of the comparisons, N-PDT versus control group (95% confidence interval = [−4.95–24.18] [I^2 = 99.82%]) [Figure 4] or N-PDT versus PDT (95% confidence interval = [−2.22–8.72] [I^2 = 99.21%]) [Figure 4].

Subgroup analysis

A subgroup analysis based on incubation period, type of nanoparticle, presence of tissue inhibitors, photosensitizer, light source, and duration of exposure to the light source used was performed to ascertain the reasons for the high heterogeneity values observed across the studies.

On subgroup analysis of comparison of N-PDT versus control groups based on the light source for percentage reduction (95% confidence interval = [−4.95–24.18] [I^2 = 99.82%]) and bacterial reduction log [95% confidence interval = [−5.46–−1.46] [I^2 = 94.68%]), diode laser at 810 nm performed better when compared to other light sources [Supplementary Figures 2 and 3].

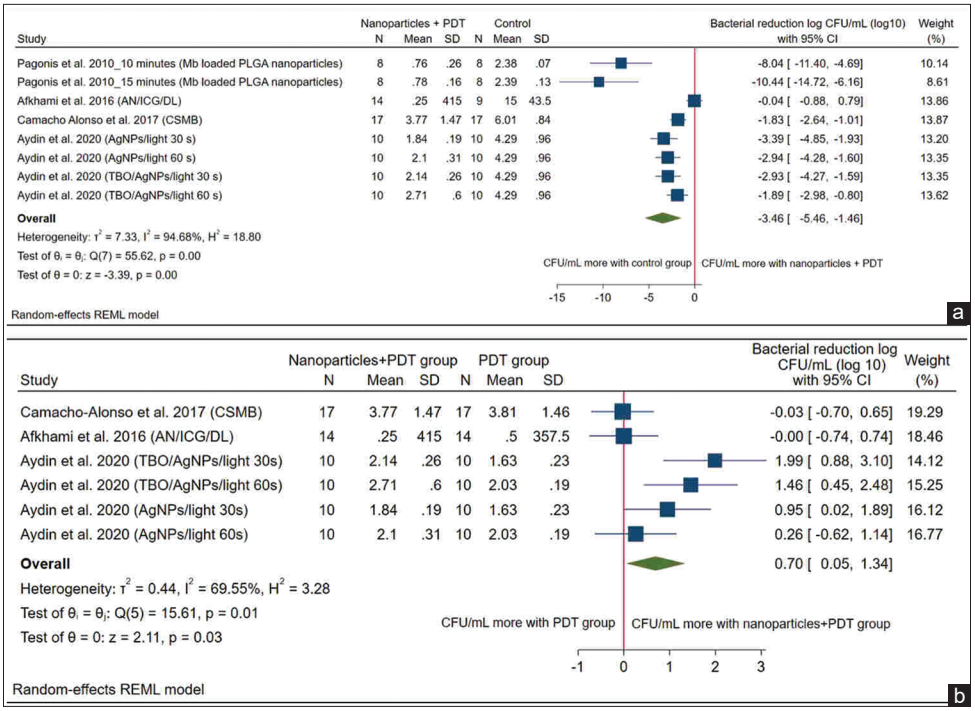

Figure 3: (a) Meta-analysis forest plot for random-effects maximum likelihood model (REML) of bacterial reduction log comparing nanoparticle-mediated photodynamic therapy (N-PDT) and control groups, (b) Meta-analysis forest plot for REML model of bacterial reduction log comparing photodynamic therapy and N-PDT groups. PDT = Photodynamic therapy, SD = Standard deviation, CFU = Colony-forming unit, CI = Confidence interval, PLGA = Polylactic glycolic acid, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood

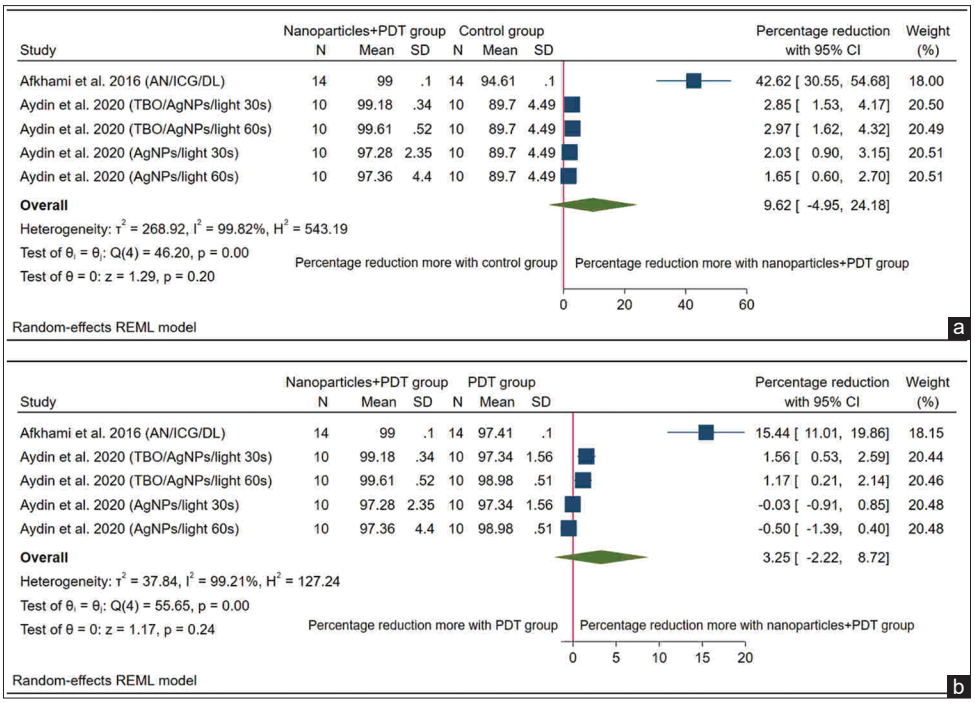

Figure 4: (a) Meta-analysis forest plot for random-effects maximum likelihood model (REML) of bacterial percentage reduction of nanoparticle-mediated photodynamic therapy (N-PDT) and control groups, (b) Meta-analysis forest plot for REML model of percentage reduction of bacteria comparing photodynamic therapy and N-PDT groups. PDT = Photodynamic therapy, SD = Standard deviation, CI = Confidence interval, ICG = Indocyanine green, REML = Random-effects maximum likelihood

On subgroup analysis based on nanoparticles for bacterial reduction log comparing the N-PDT and control groups, MB-loaded PLGA was more efficacious when compared to the other nanoparticles (95% confidence interval = [−5.46–−1.46] [I^2 = 94.68%]) [Supplementary Figure 4].

On subgroup analysis based on nanoparticles for percentage reduction comparing the N-PDT versus control groups (95% confidence interval = [−4.95–24.18] [I^2 = 99.82%]), AN/ICG/DL performed better than the other nanoparticles [Supplementary Figure 5].

On subgroup analysis based on the light source for percentage reduction comparing the N-PDT versus PDT groups (95% confidence interval = [−2.22–−8.72] [I^2 = 99.21%]), diode laser at 810 nm performed better when compared to other light sources [Supplementary Figure 6]. However, when the bacterial reduction log between the N-PDT and PDT groups was compared, there was no difference irrespective of the light source used (95% confidence interval = [0.05–1.34] [I^2 = 69.55%]) [Supplementary Figure 7].

The subgroup analysis based on nanoparticles for bacterial reduction log comparing N-PDT and PDT revealed that except for CSMB and AN/ICG/DL groups, all the other nanoparticles had lesser antibacterial activity when compared to the PDT group (95% confidence interval = [0.05–1.34] [I^2 = 69.55%]) [Supplementary Figure 8].

On subgroup analysis based on nanoparticles for percentage reduction comparing the N-PDT versus PDT groups (95% confidence interval = [−2.22–−8.72] [I^2 = 99.21%]), AN/ICG/DL performed better than the other nanoparticles [Supplementary Figure 9].

Meta-regression

The results of the meta-regression analysis showed that the type of nanoparticles ($P < 0.001$) and light source used ($P < 0.001$) were identified as covariates influencing the outcome measure "Bacterial reduction log" when the N-PDT group was compared with the control group. The residual heterogeneity across the studies after accounting for the influence of covariates was 0% (observed heterogeneity [I^2 = 94.68%]) [Table 3].

For the outcome measure "Bacterial reduction log," when the N-PDT group was compared with the PDT group, the type of nanoparticles ($P = 0.027$) and exposure time ($P = 0.034$) were identified as significant covariates. The residual heterogeneity across the studies after accounting for the influence of covariates was 0% (observed heterogeneity [I^2 = 69.55%]) [Table 3].

In addition, the results of the meta-regression analysis also showed that the type of photosensitizer ($P = 0.009$), incubation time ($P = 0.034$), light source ($P = 0.005$), and the exposure time used ($P = 0.041$) were identified

as covariates influencing the outcome measure "Percentage reduction" for the N-PDT group. The residual heterogeneity across the studies after accounting for the influence of covariates was still 100% (observed heterogeneity [I^2 = 100%]) [Table 4].

For the outcome measure "Percentage reduction," when the N-PDT group was compared with the control and PDT groups, incubation time ($P < 0.0001$) was identified as a significant covariate. The residual heterogeneity across the studies after accounting for the influence of covariates was 0% (observed heterogeneity [I^2 = 99.82% and 99.21%, respectively]) [Table 4].

DISCUSSION

To the best of our knowledge, this is the first systematic review to evaluate the efficacy of N-PDT with PDT and control groups in reducing *E. faecalis* microbial load in the presence of dentin substrate by assessing the bacterial reduction log and percentage reduction.

According to the JBI critical appraisal tool, seven of the included articles in this systematic review had a low RoB[8,15-19,21] and two articles had moderate RoB[20,22] owing to the fact that multiple outcome measurements, multiple investigators, and investigator calibration not being done and the statistical analysis done was inadequate. Others like CRIS and QUIN tools can be used for assessing RoB; however, in our review, we have used a modified JBI critical appraisal tool for quasi-experimental studies as it is most widely used.

Among the articles included in this review, the most commonly used nanoparticle was chitosan.[17,19-22] The positively charged chitosan gets attached to the bacterial membrane, thereby altering the membrane's permeability, which causes the leakage of intracellular components resulting in cell death.[23,24] With regard to the light source used, a diode laser at a wavelength of 665–810 nm was commonly used.[15-18] Diode laser penetrates well into complex anatomies, and it is portable, easy to handle, and inexpensive.[3]

Table 3: Meta-regression of bacterial reduction log control versus nanoparticle-mediated photodynamic therapy group

Confounding variable	Coefficient	SE	Z	P >[z]	95% CI
Nanoparticles	13.58015	3.461872	3.92	0.000	6.795004–20.36529
Photosensitizer	−0.8406725	0.6645105	−1.27	0.206	−2.143089–0.4617442
Incubation time	0	Omitted			
Light source	−12.37716	2.117912	−5.84	0.000	−16.52819–−8.22613
Exposure time	−3.827071	2.481241	−1.54	0.123	−8.690214–1.036072
_cons	−5.491041	1.984384	−2.77	0.006	−9.380362–−1.60172

Test of residual homogeneity=I^2 (%)=0.00, Q_res=χ^2 (3)=2.33, Probability>Q_res=0.5074. SE=Standard error, CI=Confidence interval

Meta-regression of bacterial reduction log photodynamic therapy versus nanoparticle-mediated photodynamic therapy group

Confounding variable	Coefficient	SE	Z	P >[z]	95% CI
Photosensitizer	4.152761	2.316868	1.79	0.073	−0.3882173–8.69374
Nanoparticles	−2.639488	1.196455	−2.21		−4.984497–−0.2944785
Incubation time	0.5297289	0.8683494	0.61	0.542	−1.172205–2.231662
Light source	0	Omitted			
Exposure time	−2.016971	0.9539038	−2.11	0.034	−3.886589–−0.1473543
_cons	−0.0526889	0.7832578	−0.07	0.946	−1.587846–1.482468

Test of residual homogeneity=I^2 (%)=0.00, Q_res=χ^2 (1)=0.03, Probability>Q_res=0.8694. SE=Standard error, CI=Confidence interval

Table 4: Meta-regression of percentage reduction of control versus nanoparticle-mediated photodynamic therapy group

Confounding variable	Coefficient	SE	Z	P >[z]	95% CI
Nanoparticles	4.250009	18.90072	0.22	0.822	−32.79472–41.29474
Photosensitizer	24.18181	9.306058	2.60	0.009	5.942271–42.42135
Incubation time	−96.15909	45.23263	−2.13	0.034	−184.8134–−7.504771
Light source	179.0682	63.37348	2.83	0.005	54.85842–303.2779
Exposure time	−44.49998	21.82463	−2.04	0.041	−87.27547–−1.724493
_cons	32.1591	29.24382	1.10	0.271	−25.15774–89.47594

Test of residual homogeneity=I^2 (%)=100.00, Q_res=χ^2 (5)=6.9e+06, Probability>Q_res=0.000. SE=Standard error, CI=Confidence interval

Meta-regression of percentage reduction of bacteria photodynamic therapy versus nanoparticle-mediated photodynamic therapy group

Confounding variable	Coefficient	SE	Z	P >[z]	95% CI
Photosensitizer	−hotosens	1.051977	−.051	0.468	−.468977s–1.297701
Nanoparticles	−anopartic	0.4777423	−.477	0.365	−.3657423–0.5038144
Incubation time	−ncubatio	2.398835	−.398	0.000	−.000835o–−.000835o
_cons	29.28846	4.535605	6.46	0.000	20.29884–38.17808

Test of residual homogeneity=I^2 (%)=0.00, Q_res=χ^2 (1) =0.01, Probability>Q_res=0.9361. SE=Standard error, CI=Confidence interval

Out of the nine articles in this systematic review, only five were included for meta-analysis.[8,15-18] Due to the lack of a common methodological approach and diversity in the studied parameters, the meta-analysis could be performed only for two parameters: bacterial reduction log and percentage reduction. *E. faecalis* being a Gram-positive bacterium is much more sensitive to photodynamic therapy than Gram-negative bacteria.[25] The photosensitizer molecule efficiently binds to the bacteria, causing lethal damage at the nucleic acid level or the cytoplasmic membrane, or both sites. This is associated with structural and dynamic changes resulting in the inactivation of the bacteria. The results of the meta-analysis showed a substantial reduction of the *E. faecalis* CFUs in the N-PDT group when compared to the control group. Pourhajibagher and Bahador conducted a systematic review and meta-analysis and reported that there was a statistically significant microbial reduction in the infected root canal systems when PDT was used as an adjunct to chemo-mechanical debridement when compared to other disinfection protocols.[26] However, in the current meta-analysis, PDT performed better than N-PDT when compared to the bacterial reduction log [Figure 2].

The meta-regression analysis showed that the type of nanoparticle and light source were covariates influencing the bacterial reduction log, whereas the type of photosensitizer and incubation time were significant covariates influencing the percentage reduction owing to heterogeneity [Tables 3 and 4]. The subgroup analysis showed that the diode laser at 810 nm (light source) and MB-loaded PLGA and AN/ICG (nanoparticle) had better antibacterial efficacy. PLGA in the form of microspheres, enclosing the nanoparticle, can degrade and cause a slow release of the encapsulated nanoparticle, which can account for its better performance.[27] However, the superiority of MB-loaded PLGA is debatable as it is based on one study only.[18] Previous studies have been inconclusive in the comparison of MB and RB, with few reporting the superiorities of MB and others reporting the superiority of RB in the presence of tissue inhibitors.[19,20] In the current review, both TBO and ICG were similar in their antimicrobial efficacy when used with N-PDT. A comparative study of MB and TBO by Usacheva *et al*. concluded that both were efficient in reducing the microbial load, although TBO was more efficient than MB.[28] TBO is less hydrophilic which increases the interaction with constituents of bacterial cell walls resulting in damage to the lipids and proteins.[29] Individual comparison of nanoparticles for their antimicrobial activity through a network meta-analysis was not possible due to the lack of a common control group.

Balakrishna *et al*. in an *in vitro* study evaluated the effect of conventional irrigation with 2.25% NaOCl and PAD with toluidine blue and diode laser on *Enterococcus faecalis* in root canals and reported that PDT when used as an adjunct to irrigation with NaOCl was significantly more effective in removing *E. faecalis* from the root canals.[30] In another study by Hegde *et al*., triple antibiotic paste (TAP) combined with chitosan nanoparticles combined with diode laser showed better results compared to TAP.[31] A systematic review by Chrepa *et al*. stated that when PDT was used in the presence of tissue inhibitors, there is a significant decrease in antimicrobial efficacy.[32] This might be due to the weaker chemical interaction of the nanoparticle with the dentin matrix components and reduced uptake of photosensitizers by the bacterial cells which diminishes the antimicrobial activity of N-PDT.[19]

There is a lack of clinical studies assessing the antimicrobial effect of N-PDT. Case reports employing the use of PDT in the management of anterior teeth with chronic dentoalveolar abscess, radicular cyst, and posttreatment apical periodontitis with MB as the photosensitizer have reported successful results.[33-35] Suresh *et al*. in a case report used chitosan nanoparticles conjugated with RB (CSRB) along with PDT for the treatment of extensive root resorption and reported clinical success with wound healing.[36]

Guimarães *et al*. assessed the effect of photobiomodulation (PDT + low-level LASER therapy) on postoperative symptoms after single-visit endodontic treatment of single-rooted teeth with symptomatic apical periodontitis and reported that there was no significant difference in the postoperative pain and tenderness when compared to the control group.[37]

The results of the present review suggest that N-PDT is more effective in reducing *E. faecalis* than the control group. Rios *et al*. in a clinical trial assessed the antibacterial effect of PDT using TBO following disinfection with 6% NaOCl and reported that the root canals treated with NaOCl alone showed a 0.66% survival rate of *E. faecalis* when compared to the combination of both NaOCl and PDT, where the survival rate was lowered to 0.1%.[38] In contrast, Aydin *et al.* reported that N-PDT was effective in reducing *E. faecalis* biofilm but was not as effective as the standard irrigant 2.5% NaOCl. This could be either due to incomplete penetration of the photosensitizer into the dentinal tubules and the biofilm or rapid depletion of molecular oxygen in the dentinal tubules minimizing the singlet oxygen-mediated damage.[8]

Systematic reviews have reported that there is a higher reduction in the *E. faecalis* load in the PDT group when compared to the control group, but no consensus was reached due to the lack of evidence in the literature available.[7,39]

Strengths

Strict inclusion and exclusion criteria were followed for conducting this systematic review and meta-analysis. Two

independent reviewers carried out the data extraction and quality assessment. In this systematic review, the efficacy of the nanoparticle, photosensitizer, and light source in the presence of tooth substrate was analyzed, whereas in the previous reviews, only one of the abovementioned factors was considered.

Limitations

A high degree of heterogeneity was observed across the studies due to the lack of a standardized methodological approach, which hinders the comparison of the results based on the use of nanoparticles, photosensitizer, light source, duration of exposure, and outcome measures. This systematic review included articles published in the English language only and *in vitro* studies that may not be accurately replicable in *in vivo* conditions. However, the limited evidence based on case reports advocates the successful reduction of microbial load using PDT in clinical scenarios.

Future perspectives

Future studies with robust study designs should be carried out to establish a standardized protocol with regard to the presence of tissue inhibitors, photosensitizer concentration, irradiation time, energy dosage, type of nanoparticle, and method of assessing antimicrobial efficacy which will give consistent and effective outcomes. To evaluate the role of nanoparticles in PDT, studies comparing PDT and N-PDT are needed. Further standardized laboratory and clinical studies are needed to obtain a reliable conclusion.

CONCLUSION

The currently available evidence is low and inconclusive with regard to the superior efficacy of PDT with nanoparticles due to the vast amount of heterogeneity and variable outcomes measured among the studies. The meta-regression analysis found two covariates, namely the type of nanoparticle and light source to influence the bacterial reduction log. Further clinical, animal, and laboratory studies with uniform methodology are necessary to attain a better conclusion.

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Conflicts of interest

There are no conflicts of interest.

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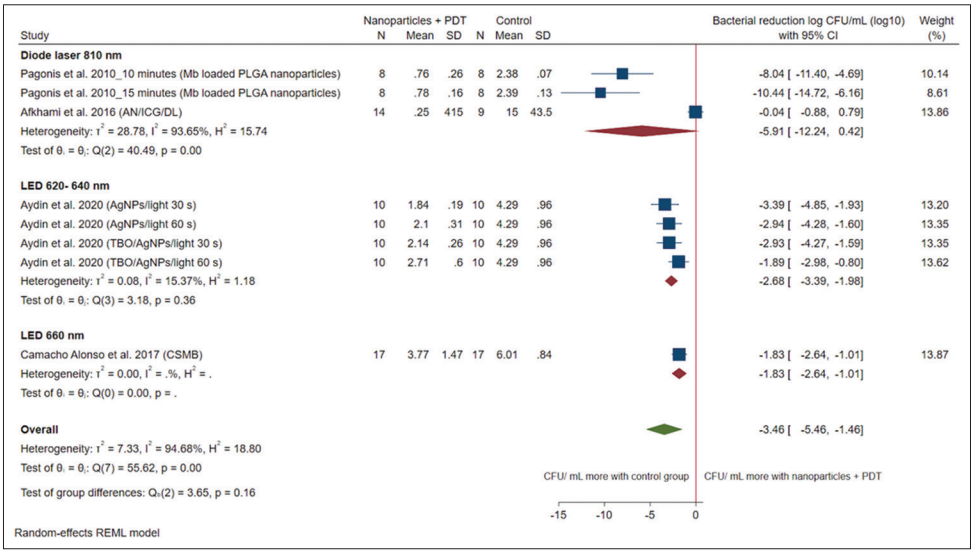
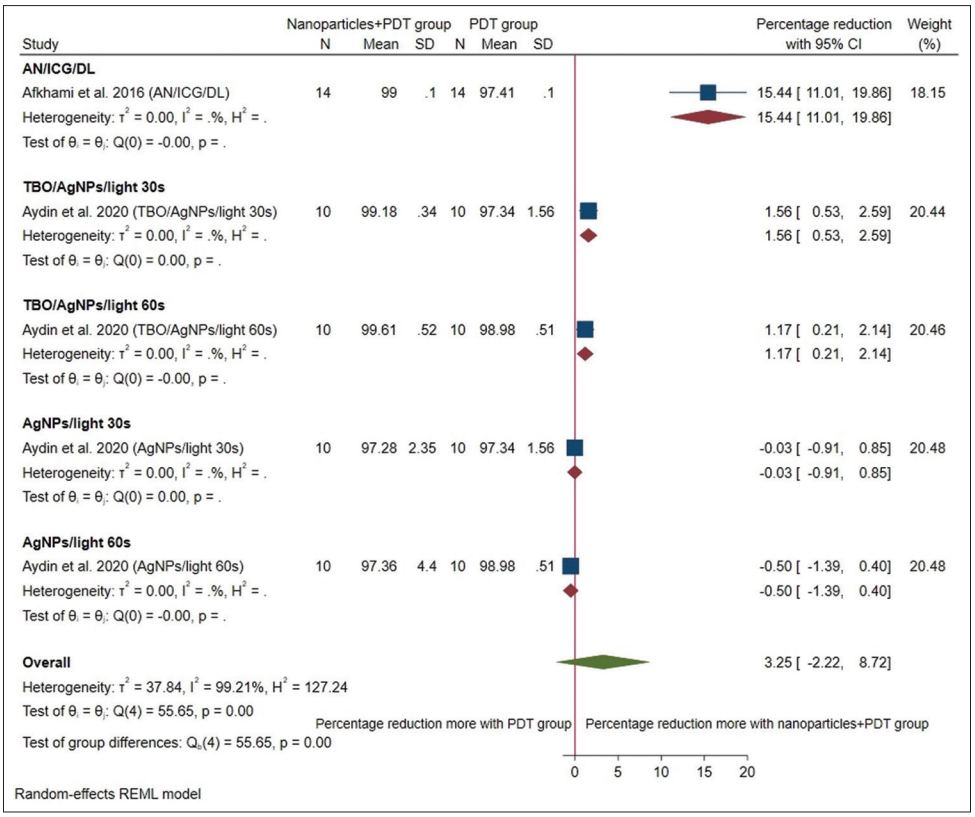
Interrater agreement — Number of subjects = 81; Ratings per subject = 2; Number of rating categories = 2

	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
Percent Agreement	0.9259	0.0293	31.62	0.000	0.8677	0.9842
Brennan and Prediger	0.8519	0.0586	14.55	0.000	0.7353	0.9684
Cohen/Conger's Kappa	0.8266	0.0683	12.10	0.000	0.6906	0.9625
Scott/Fleiss' Pi	0.8264	0.0684	12.07	0.000	0.6902	0.9626
Gwet's AC	0.8708	0.0528	16.49	0.000	0.7657	0.9759
Krippendorff's Alpha	0.8275	0.0684	12.09	0.000	0.6913	0.9637

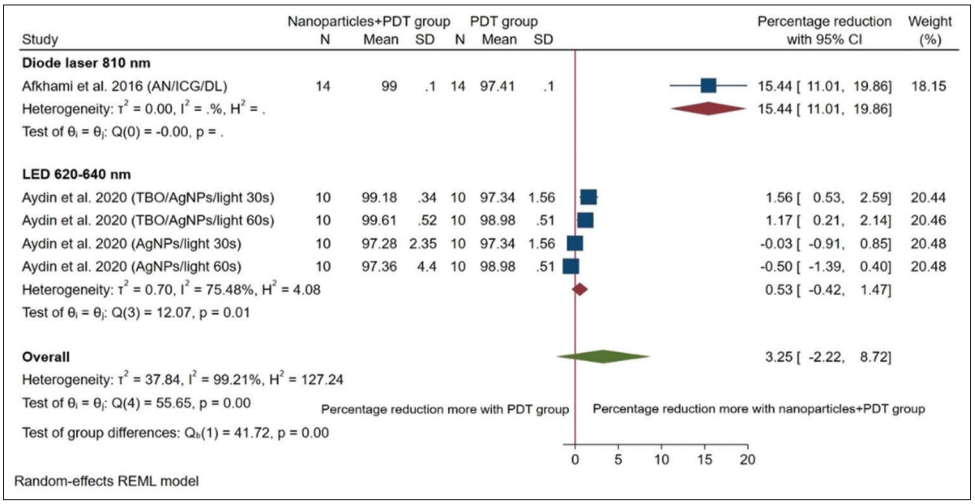
Supplementary Figure 1: Cohen's kappa inter-examiner reliability



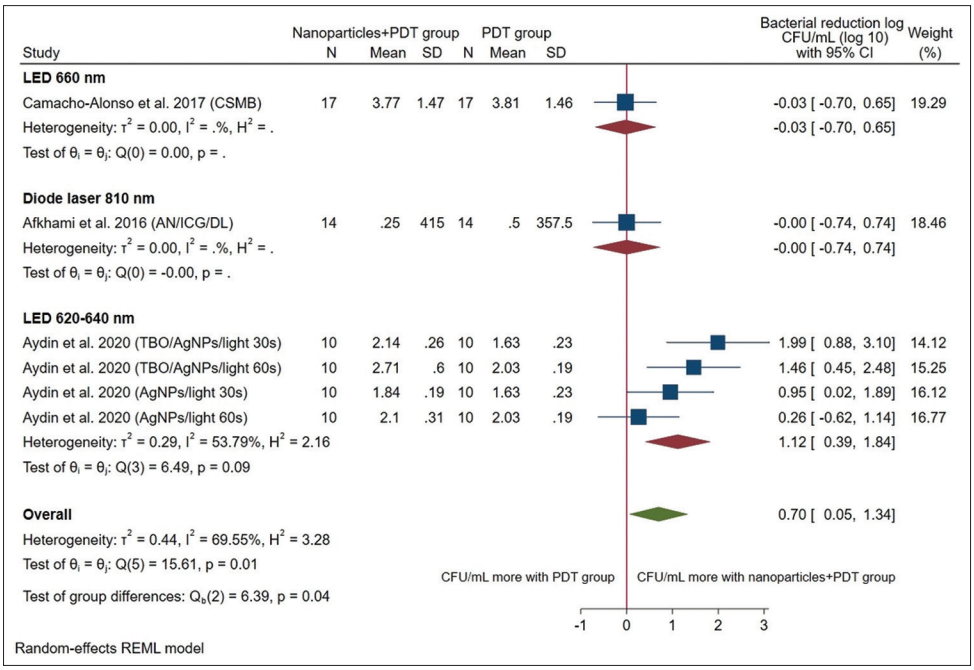
Supplementary Figure 2: Meta-analysis forest plot for random-effects maximum likelihood model of bacterial reduction log comparing nanoparticle-mediated photodynamic therapy and control groups for subgroup light source. PDT = Photodynamic therapy, SD = Standard deviation, CFU = Colony-forming unit, CI = Confidence interval, PLGA = Polylactic glycolic acid, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood, LED = Light-emitting diode



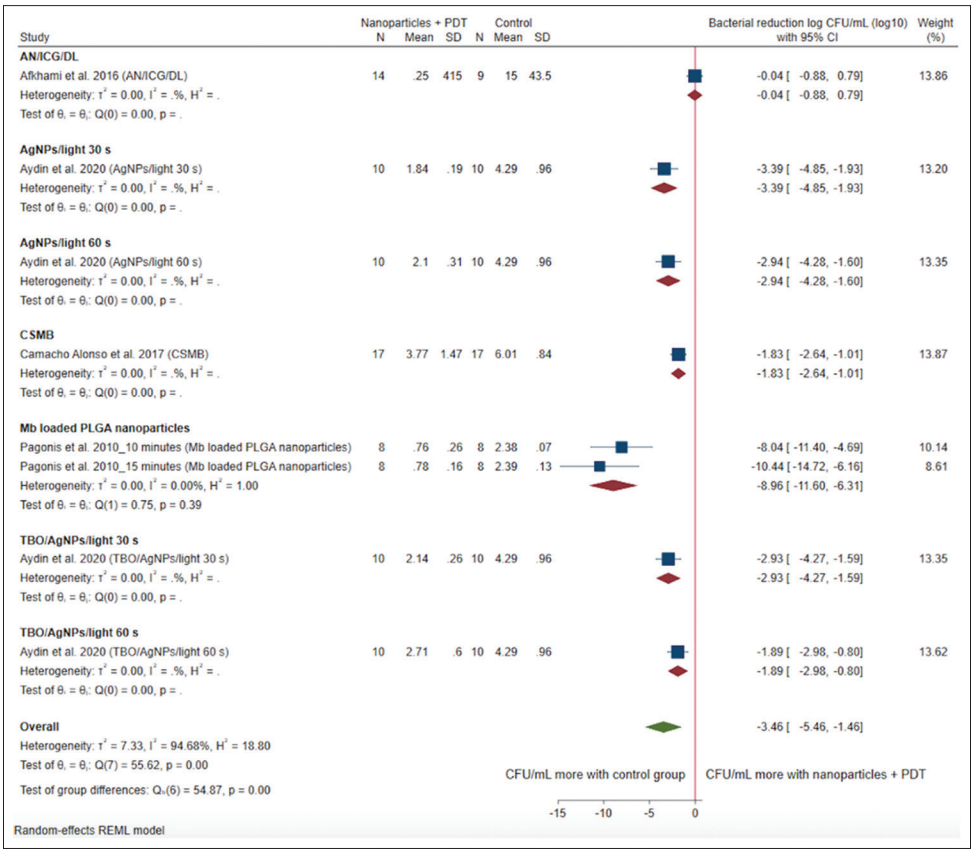
Supplementary Figure 3: Meta-analysis forest plot for random-effects maximum likelihood model of percentage reduction comparing nanoparticle-mediated photodynamic therapy and control groups for subgroup light source. PDT = Photodynamic therapy, SD = Standard deviation, CI = Confidence interval, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood



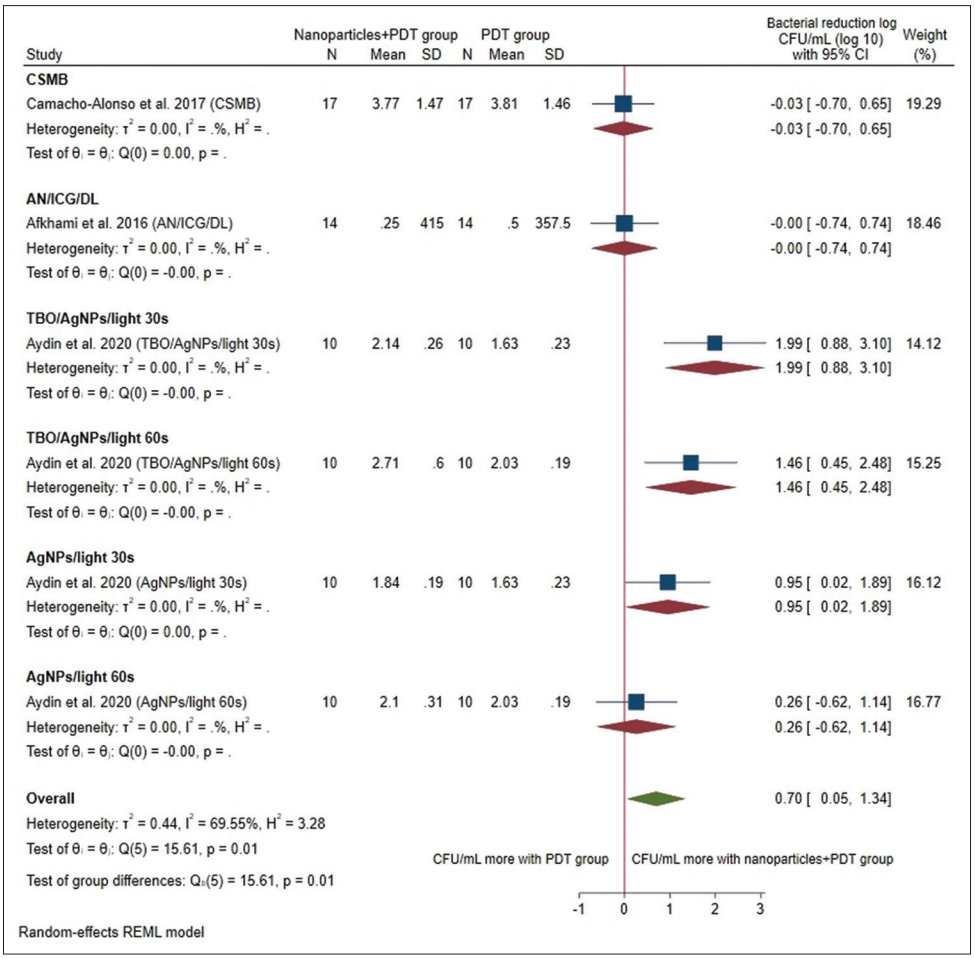
Supplementary Figure 4: Meta-analysis forest plot for random-effects maximum likelihood model of percentage reduction comparing photodynamic therapy (PDT) and nanoparticle-mediated PDT groups for subgroup light source. PDT = Photodynamic therapy, SD = Standard deviation, CI = Confidence interval, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood



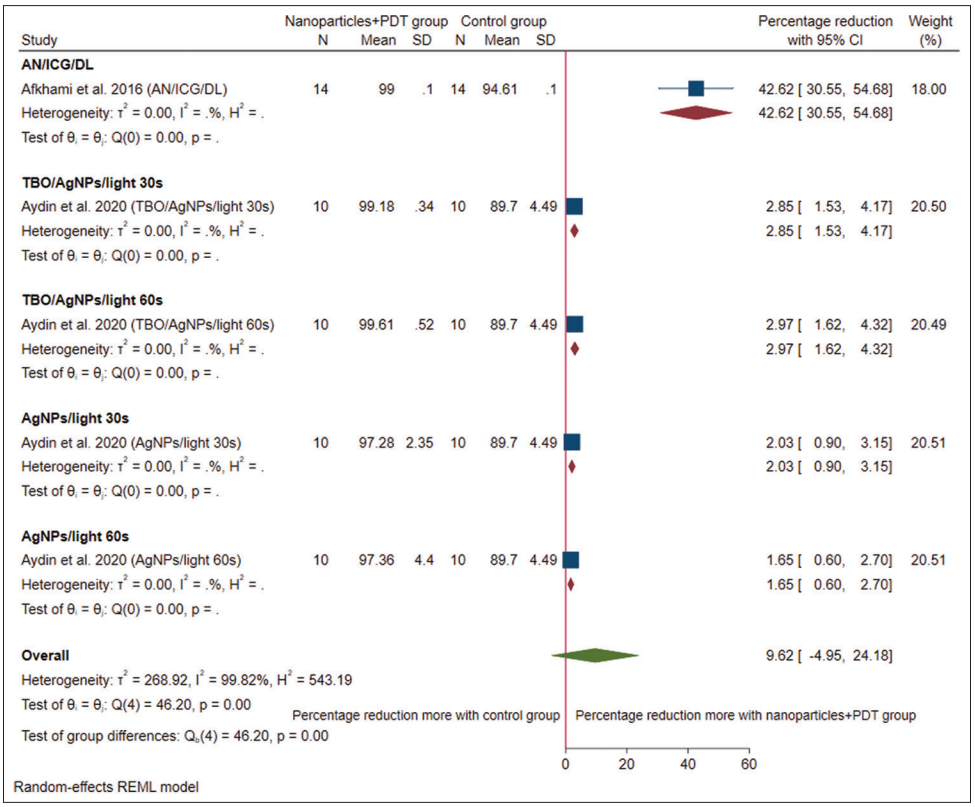
Supplementary Figure 5: Meta-analysis forest plot for random-effects maximum likelihood model of bacterial reduction log comparing photodynamic therapy (PDT) and nanoparticle-mediated PDT groups for subgroup light source. PDT = Photodynamic therapy, SD = Standard deviation, CFU = Colony-forming unit, CI = Confidence interval, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood, LED = Light-emitting diode



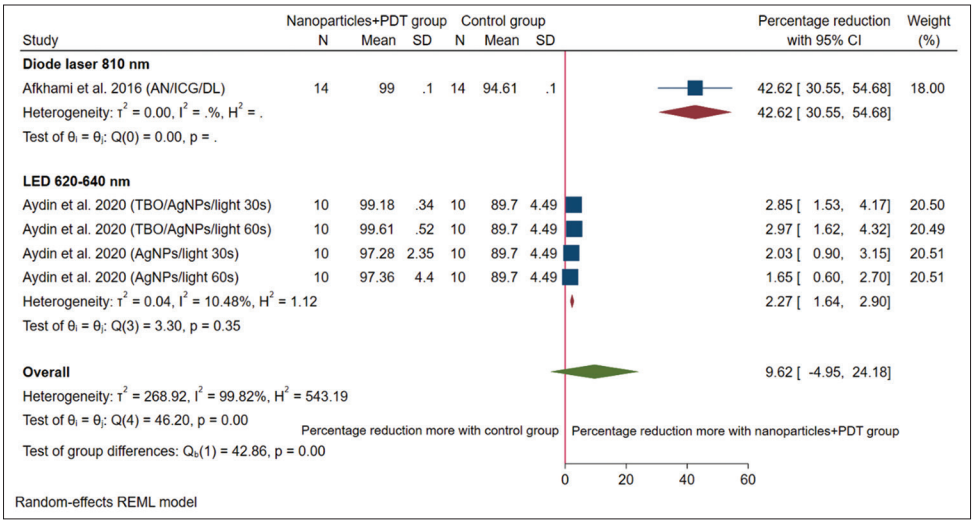
Supplementary Figure 6: Meta-analysis forest plot for random-effects maximum likelihood model of bacterial reduction log comparing nanoparticle-mediated photodynamic therapy and control groups for subgroup nanoparticles. PDT = Photodynamic therapy, SD = Standard deviation, CFU = Colony-forming unit, CI = Confidence interval, PLGA = Polylactic glycolic acid, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood, LED = Light-emitting diode, MB = Methylene blue



Supplementary Figure 7: Meta-analysis forest plot for random-effects maximum likelihood model of bacterial reduction log comparing photodynamic therapy (PDT) and nanoparticle-mediated PDT groups for subgroup nanoparticles. PDT = Photodynamic therapy, SD = Standard deviation, CFU = Colony-forming unit, CI = Confidence interval, PLGA = Polylactic glycolic acid, ICG = Indocyanine green, TBO = Toluidine blue O, LED = Light-emitting diode, REML = Random-effects maximum likelihood



Supplementary Figure 8: Meta-analysis forest plot for random-effects maximum likelihood model of percentage reduction comparing nanoparticle-mediated photodynamic therapy and control groups for subgroup nanoparticles. PDT = Photodynamic therapy, SD = Standard deviation, CI = Confidence interval, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood



Supplementary Figure 9: Meta-analysis forest plot for random-effects maximum likelihood model of percentage reduction comparing photodynamic therapy (PDT) and nanoparticle-mediated PDT groups for subgroup nanoparticles. PDT = Photodynamic therapy, SD = Standard deviation, CI = Confidence interval, ICG = Indocyanine green, TBO = Toluidine blue O, REML = Random-effects maximum likelihood