

Antibacterial Efficacy of Nanocarrier-Assisted Antimicrobial Photodynamic Therapy Against *Enterococcus faecalis* in Root Canals: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: *Enterococcus faecalis* has emerged as a predominant endodontic pathogen due to its high isolation rate in endodontic failure cases, remarkable biofilm-forming capacity, and inherent resistance to conventional therapies. Recent advances in nanocarrier-assisted antimicrobial photodynamic therapy (Nano-aPDT) have introduced a novel disinfection paradigm, demonstrating promising potential in eradicating persistent root canal infections through three key mechanisms: enhanced photosensitizer stability, improved targeted delivery, and optimized reactive oxygen species (ROS) generation.

Objectives: This systematic review and meta-analysis was designed to: comprehensively evaluate the antimicrobial efficacy of nanocarrier-assisted photodynamic therapy (Nano-aPDT) against *Enterococcus faecalis* biofilms; conduct comparative effectiveness analyses between Nano-aPDT and standard interventions including sodium hypochlorite (NaOCl) irrigation and conventional photodynamic therapy (PDT); critically appraise the therapeutic advantages and technical limitations of Nano-aPDT as an endodontic disinfection strategy; and synthesize evidence-based recommendations to facilitate clinical translation of this emerging technology.

Methods: The study protocol received prospective registration in PROSPERO (CRD420251068155) and was executed in strict compliance with PRISMA reporting standards. An exhaustive systematic search was conducted spanning PubMed, Embase, Web of Science, Scopus, and the Cochrane Library through June 1, 2025. Methodological rigor was evaluated utilizing the Cochrane Risk of Bias assessment instrument. Quantitative synthesis was undertaken employing RevMan 5.4 statistical software, utilizing risk ratios (RR) accompanied by 95% confidence intervals (CI) as principal effect size metrics, implemented via the Mantel-Haenszel random-effects modeling approach. Between-study heterogeneity was rigorously assessed through I^2 statistical measures.

Results: The final analysis included nine rigorously screened studies, comprising eleven independent datasets comparing Nano-aPDT versus NaOCl and nine datasets evaluating Nano-aPDT against conventional PDT. Meta-analysis revealed two key findings: First, Nano-aPDT showed statistically significant but modestly reduced antibacterial efficacy relative to NaOCl (pooled RR = 0.85, 95% CI: 0.74–0.97, $p = 0.01$). Second, Nano-aPDT demonstrated substantially greater bactericidal activity compared to conventional PDT (pooled RR = 1.33, 95% CI: 1.11–1.78, $p = 0.004$).

Both comparisons exhibited substantial heterogeneity ($I^2=99\%$), indicating notable variations across studies in experimental design, treatment parameters, and outcome assessment. Most studies carried moderate risk of bias, primarily due to: Suboptimal randomization methodologies; Inadequate allocation concealment; Lack of blinding implementation; Incomplete reporting of analytical approaches.

Conclusions: In vitro evidence indicates that nanocarrier-assisted antimicrobial photodynamic therapy (Nano-aPDT) achieves significantly greater bactericidal activity against *Enterococcus faecalis* than conventional PDT, although its efficacy remains slightly lower than that of NaOCl.

Keywords

Nano-Photodynamic Therapy, Antimicrobial Photodynamic Therapy, Enterococcus faecalis, Endodontic Disinfection, Sodium Hypochlorite, Nanoparticles, Biofilms, Reactive Oxygen Species, Drug Delivery Systems.

Introduction

The primary objective of root canal therapy is complete eradication of microorganisms within the root canal system, particularly the highly resistant *Enterococcus faecalis*, which is recognized as a predominant pathogen in persistent and secondary endodontic infections [1]. Contemporary consensus guidelines recommend 2.5%-5.25% sodium hypochlorite (NaOCl) as the standard irrigation solution [2]. Evidence demonstrates that NaOCl concentrations exceeding 2.5% exhibit superior antimicrobial activity and effective tissue dissolution, with efficacy dependent on concentration, temperature, exposure duration, and irrigation volume.

Despite being the gold standard for root canal irrigation, NaOCl at higher concentrations is associated with significant adverse effects including dentinal weakening, cytotoxic effects, and potential procedural complications. These limitations have driven the exploration of safer and more effective alternatives, such as photodynamic therapy and nanoparticle-based systems.

Antimicrobial photodynamic therapy (aPDT) has emerged as a novel disinfection strategy, utilizing photosensitizers activated by specific wavelength lasers to generate reactive oxygen species (ROS) with microbicidal effects. Compared to NaOCl, aPDT offers distinct advantages: a multi-target antimicrobial mechanism with low resistance potential [3], demonstrated efficacy against mature biofilms and multidrug-resistant strains [4], localized action with minimal collateral tissue damage, and potential synergistic effects when combined with conventional irrigation protocols.

However, conventional PDT faces inherent limitations. Most photosensitizers are hydrophobic molecules prone to aqueous aggregation, resulting in poor stability and limited tissue penetration that compromises their antimicrobial efficacy in complex root canal systems and deep biofilm layers [5,6]. To address these challenges, researchers have incorporated nanocarriers (liposomes, polymeric nanoparticles, metallic nanoparticles) into PDT systems to enhance photosensitizer solubility, stability, and targeted delivery. Substantial evidence confirms that nanocarrier systems significantly improve cellular uptake and distribution of photosensitizers [7,8]. Garapati et al. demonstrated that nanocarriers facilitate photosensitizer translocation across microbial cell walls, while subsequent reviews have highlighted their protective effects against photosensitizer degradation and enhanced penetration at infection sites [9]. The antibacterial mechanism of Nano-PDT against *Enterococcus faecalis* in root canals is schematically illustrated in Figure 1.

Although numerous *in vitro* studies have established the antibacterial potential of Nano-aPDT, existing research exhibits

methodological heterogeneity and limited sample sizes. A systematic quantitative comparison between Nano-aPDT and conventional NaOCl for *E. faecalis* eradication remains lacking. This systematic review and meta-analysis aims to evaluate the efficacy of Nano-aPDT against *E. faecalis* in root canal treatment, providing higher-level evidence to inform clinical translation and guide future research directions.

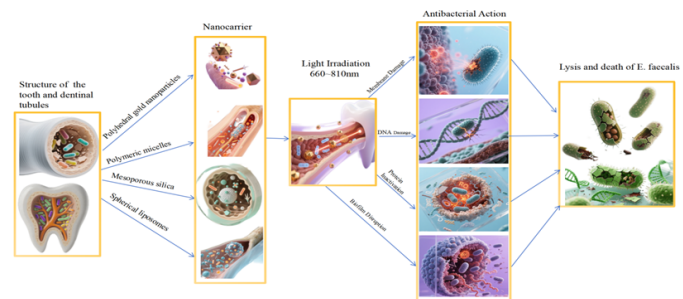


Figure 1: Antibacterial action of nano-PDT against *Enterococcus faecalis* in root canal systems.

Materials and Methods

Protocol and Registration

This study was designed and conducted in strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [10] and the latest methodological recommendations from the Cochrane Handbook for Systematic Reviews of Interventions. The protocol was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number: CRD420251068155). To enhance scientific rigor and consistency in the selection process, the protocol incorporated contemporary methodological frameworks employed in *in vitro* studies and related systematic reviews [11,12].

Research Question (PICO Framework)

Population (P): *In vitro* models simulating root canal infections, including artificial root canals, extracted human teeth, or biofilm-based infection models, with microbial challenges restricted to bacteria relevant to pulp reinfection or apical periodontitis, particularly *Enterococcus faecalis*.

Intervention (I): Nanocarrier-assisted photodynamic therapy (Nano-PDT), defined as photosensitizers encapsulated in nanomaterials (e.g., liposomes, polymeric nanoparticles, metallic nanoparticles) and activated by laser irradiation at specific wavelengths to exert antimicrobial effects within the root canal system.

Comparison (C): Either conventional photodynamic therapy (using non-nanocarrier photosensitizers under comparable irradiation parameters) or sodium hypochlorite (NaOCl) irrigation at defined concentrations and exposure times.

Outcomes (O): Primary outcome: Reduction in bacterial load (CFU/mL), Secondary outcomes: ROS production, photosensitizer penetration depth, and potential effects on dentine properties (e.g., microhardness changes, elemental composition).

Search Strategy

A comprehensive, systematic search strategy was developed to identify studies evaluating the antibacterial efficacy of Nano-PDT against *E. faecalis* in *in vitro* root canal infection models. Five electronic databases (PubMed, Web of Science, Embase, Scopus, CNKI and Cochrane Library) were searched from inception to June 1, 2025, with no language restrictions except for Chinese and English publications.

The search combined controlled vocabulary (MeSH/EMTREE) and free-text terms in titles/abstracts, including: "Photodynamic Therapy," "PDT," "Antimicrobial Photodynamic Therapy," "aPDT," "Root Canal Therapy," "Dental Pulp Cavity," "Root Canal," "Endodontics," "Pulpitis," "Apical Periodontitis," "Nanocarrier," "Nanoparticle," "Nanocomposite," "Nanoliposome," and "Enterococcus faecalis."

Study Selection and Data Extraction

Two independent reviewers screened all retrieved records against predefined inclusion/exclusion criteria. Initial screening was based on titles/abstracts, followed by full-text assessment of potentially eligible studies. Disagreements were resolved through discussion with a third reviewer.

Supplementary search methods included: Backward citation tracking (reference lists of included studies); Forward citation tracking (Google Scholar); Manual searches of relevant journals

Eligibility criteria were established by a multidisciplinary team comprising two endodontic specialists, one oral mucosal disease specialist, and one basic science researcher to ensure scientific robustness.

Inclusion Criteria

- Study design: *In vitro* experimental studies (extracted tooth models, artificial root canals, or biofilm models) with appropriate experimental/control groups
- Specimens: Artificial root canals, extracted human teeth, or *E.*

- faecalis* biofilm models
- Intervention: Nano-PDT with specified nanocarrier type, photosensitizer, and irradiation parameters
- Comparison: Conventional PDT or NaOCl irrigation with defined parameters
- Outcomes: At least one primary outcome (bacterial reduction) and optionally secondary outcomes
- Language: English or Chinese
- Data: Complete and extractable for quantitative synthesis

Exclusion Criteria

- Animal studies, clinical trials, reviews, meta-analyses, conference abstracts
- Models unrelated to root canal infections
- Unclear nanocarrier/photosensitizer composition or irradiation parameters
- Combined antimicrobial interventions without separable data
- Missing primary/secondary outcomes
- Non-English/Chinese publications
- Incomplete or erroneous data

Risk of Bias Assessment

Methodological quality was assessed using the Cochrane Risk of Bias Tool (Version 6.4), evaluating seven domains: Random sequence generation; Allocation concealment; Blinding of personnel; Blinding of outcome assessment; Incomplete outcome data; Selective reporting; Other biases.

Most studies reported random sequence generation but lacked details on allocation concealment ("unclear risk"). Due to the inherent nature of *in vitro* experiments, blinding was deemed impractical ("unclear risk"), potentially introducing performance and detection bias. Most studies showed low risk for incomplete outcome data and selective reporting. Other biases (e.g., inconsistent reporting of photosensitizer concentrations, irradiation parameters) were also rated as "unclear risk".

While most studies adequately reported outcomes, methodological limitations particularly regarding allocation concealment and blinding may compromise the reliability of conclusions, warranting cautious interpretation. The risk of bias assessment is illustrated in Figure 2.

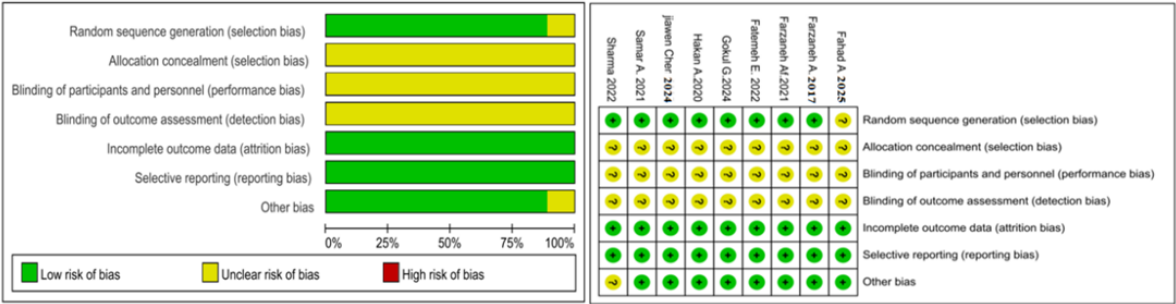


Figure 2: Risk of Bias Assessment Based on the Cochrane Tool.

Table 1: Summary of included in vitro studies.

Author/Year	Nanocarrier	Nanocarrier concentration and particle size	Infection model	Photosensitizer	aPDT parameters: wavelength; power density; irradiation time	Fluence (J/cm ²)	Main outcome
Farzaneh A., [13]	Silver nanoparticles (AgNPs)	100 ppm; mean particle size: 30 nm	<i>E. faecalis</i> biofilm in root canals	Indocyanine green (ICG)	810 nm; 200 mW/cm ² ; 30 s	—	CFU count
Hakan A., [14]	Silver nanoparticles (AgNPs)	10 ppm (combined with TBO); mean particle size: 52 nm	<i>E. faecalis</i> biofilm in root canals	Toluidine blue O (TBO)	620–640 nm (LED); 2000–4000 mW/cm ² ; 60 s	—	CFU count
Farzaneh Af., [15]	Silver nanoparticles (AgNPs)	100 ppm; mean particle size: 30 nm	<i>E. faecalis</i> biofilm in root canals	Indocyanine green (ICG)	808 nm; 250 mW/cm ² ; 60 s	—	CFU count
Samar A., [16]	Quartz particles (QP)	Concentration: NR; QP: 0.35–3.5 µm (unloaded MB); MB@QP: 3.0–4.5 µm	<i>E. faecalis</i> biofilm in root canals	Methylene blue (MB)	660 nm (diode laser); 40 mW; 60 s	60	Viability
Fatemeh E., [17]	Nanocurcumin (nano-Cur)	5 mg/mL; particle size: NR	<i>E. faecalis</i> biofilm in root canals	Curcumin (Cur)	390–480 nm (LED); 1000 ± 100 mW/cm ² ; 60 s	—	Viability
Sharma, [18]	Silver nanoparticles (AgNPs)	100 ppm; particle size: NR	<i>E. faecalis</i> biofilm in root canals	Indocyanine green (ICG)	940 nm (diode laser, 200 mW, continuous wave); 30 s	—	CFU count
Gokul G., [19]	Polydopamine nanoparticles (nPd)	Concentration: NR; particle size: 240 nm	<i>E. faecalis</i> biofilm in root canals	Curcumin (Cur)	Diode laser: 405 nm and 808 nm; 200 mW and 100 mW; 60 s	31.58	Viability
Jiawen Chen, [20]	Ce6/ZIF-8@PEI nanoparticles	1 mL; Ce6 concentration: 18 µg/mL; mean particle size: 136.3 nm	<i>E. faecalis</i> biofilm in root canals	Chlorin e6 (Ce6)	660 nm (red light); 150 mW/cm ² ; 5 min	45	CFU count
Fahad A., [21]	MB@AgNP and MB@QP composites	Concentration: NR; QP: 0.35–3.5 µm (unloaded MB); MB@QP: 3.0–4.5 µm; AgNPs: non-uniform nanoparticles	<i>E. faecalis</i> biofilm in root canals	Methylene blue (MB)	665 nm (diode laser); 1 W; 60 s	60	Viability

Abbreviations: aPDT, antimicrobial photodynamic therapy; AgNPs, silver nanoparticles; Ce6, chlorin e6; CFU, colony-forming units; Cur, curcumin; ICG, indocyanine green; LED, light-emitting diode; MB, methylene blue; NR, not reported; QP, quartz particles; TBO, toluidine blue O; ZIF-8, zeolitic imidazolate framework-8; PEI, polyethyleneimine.

Results

Search Results

The systematic search identified 968 records from multiple databases: PubMed (n=413), Web of Science (n=412), Scopus (n=58), Embase (n=37), Cochrane Library (n=47), and CNKI (n=1). After deduplication, 458 unique records underwent title/abstract screening, excluding 431 studies. Full-text review was conducted for 27 articles, with one excluded due to inaccessibility. Among the remaining 26 studies, 17 were excluded for lacking control groups or inappropriate study designs. Ultimately, nine in vitro studies [13–21] met all inclusion criteria for qualitative and quantitative synthesis (Table 1). Two independent reviewers achieved perfect agreement ($\kappa=1.00$) in study selection. The PRISMA flow diagram illustrates the screening process (Figure 3).

Meta-analysis of Antibacterial Efficacy

Nano-aPDT versus NaOCl

Eleven *in vitro* studies [13–21] comparing Nano-aPDT with sodium hypochlorite (NaOCl) were included (Figure 4). The Mantel-Haenszel random-effects model yielded a pooled risk ratio (RR) of 0.85 (95% CI: 0.74–0.96, $p=0.01$), indicating statistically superior bacterial eradication by NaOCl. Substantial heterogeneity

($I^2=99\%$) reflected variations in experimental conditions, photosensitizer types, and nanocarrier materials.

Nano-aPDT versus Conventional PDT

Nine studies [13,14,16–21] demonstrated Nano-aPDT's significant advantage over conventional PDT (pooled RR=1.33, 95% CI: 1.11–1.61, $p=0.004$) (Figure 5). This supports nanocarriers' role in enhancing photosensitizer delivery, stability, and antimicrobial activity. However, high heterogeneity ($I^2=99\%$) warrants cautious interpretation.

Subgroup Analysis

This meta-analysis assessed the antimicrobial efficacy of nano-assisted photodynamic therapy (Nano-aPDT) versus sodium hypochlorite (NaOCl), with subgroup analyses for three photosensitizers: curcumin, methylene blue, and indocyanine green. Findings are as follows (Figure 6):

Each subgroup included 2 studies. For curcumin, the pooled risk ratio (RR) was 0.53 (95% CI: 0.39–0.72), $Z=4.12$ ($P<0.0001$), conclusively demonstrating that Nano-aPDT combined with curcumin exerted significantly superior efficacy to NaOCl.

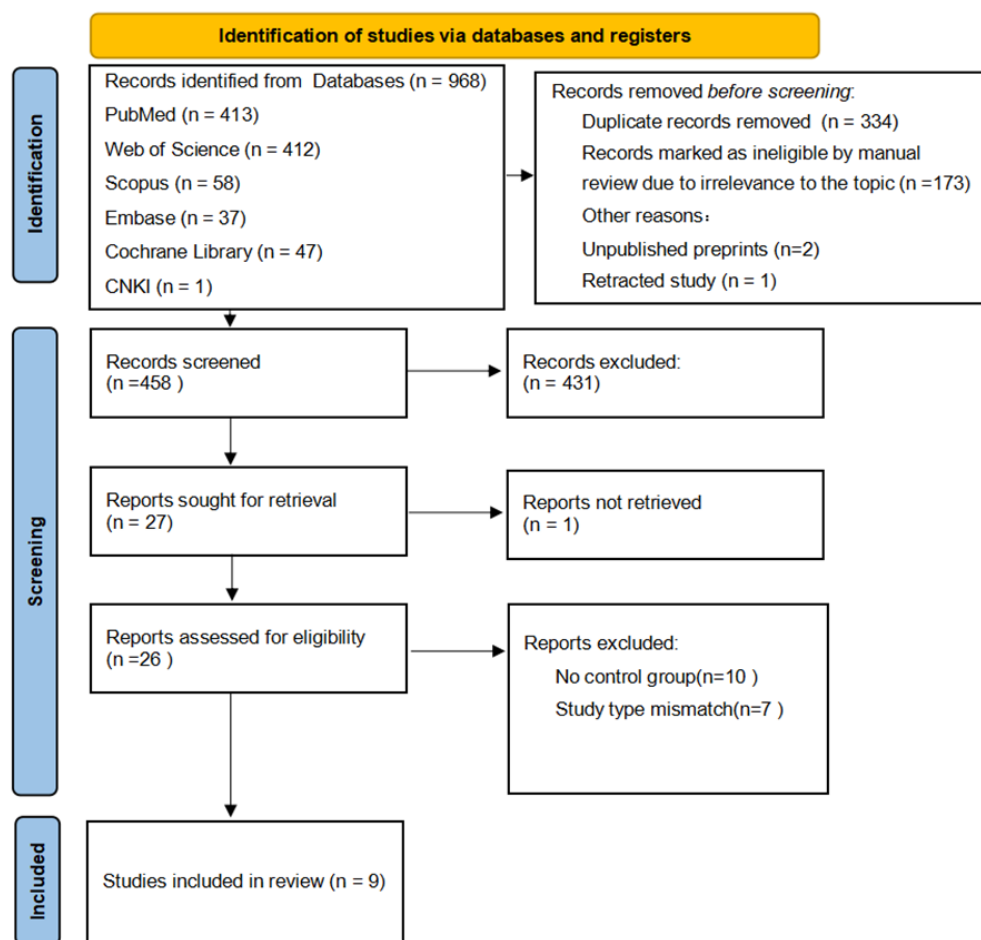


Figure 3: PRISMA flow diagram of study selection.

Moderate heterogeneity ($I^2=72\%$) did not compromise the conclusion. For methylene blue, pooled $RR=1.47$ (95% CI: 0.16–13.31), $Z=0.34$ ($P=0.73$), with markedly high heterogeneity ($I^2=99\%$); no significant efficacy disparity was noted, and results were unstable, notably swayed by a single study. For indocyanine green, pooled $RR=0.86$ (95% CI: 0.51–1.46), $Z=0.55$ ($P=0.58$); due to slightly conflicting outcomes and high heterogeneity ($I^2=98\%$), no significant efficacy difference was observed.

Pooled analysis of all 6 studies yielded $RR=0.85$ (95% CI: 0.58–1.25), $Z=0.82$ ($P=0.41$), with no significant efficacy difference. Extremely high overall heterogeneity ($I^2=98\%$) stemmed from methylene blue and indocyanine green subgroups. Subgroup heterogeneity tests ($\chi^2=3.09$, $df=2$, $P=0.21$) showed no significant efficacy variations across subgroups. However, the curcumin subgroup yielded relatively definitive results, necessitating further exploration into heterogeneity sources to refine research.

In summary, curcumin combined with Nano-aPDT may have distinct advantages but requires validation via more homogeneous studies. Due to substantial heterogeneity, methylene blue and indocyanine green subgroups have yet to exhibit consistent superiority of Nano-aPDT over NaOCl. Future studies should

prioritize optimizing photosensitizer selection and standardizing experimental protocols.

Risk of Bias and Heterogeneity Analysis

Cochrane's risk-of-bias assessment indicated a moderate overall risk, primarily attributed to unreported allocation concealment and inadequate blinding procedures. Methodological variations in detection approaches likely introduced performance and detection biases.

Despite statistically significant outcomes, extreme heterogeneity ($I^2=99\%$) substantially compromises result reliability. Key heterogeneity sources include:

Nanocarrier diversity (PLGA, liposomes, metal oxides) with divergent physicochemical properties; Photosensitizer heterogeneity (methylene blue, Ce6, curcumin) and concentration variations; Non-standardized laser parameters (wavelength: 630–810 nm; power density: 50–300 mW/cm²; exposure: 30–180 s); Variable NaOCl protocols in controls (concentration: 1–5.25%; exposure: 1–10 min); *E. faecalis* strain discrepancies (ATCC reference vs. clinical isolates); Model inconsistencies (suspensions vs. biofilms) and unreported experimental details (inoculum size, irrigation volume, treatment duration).

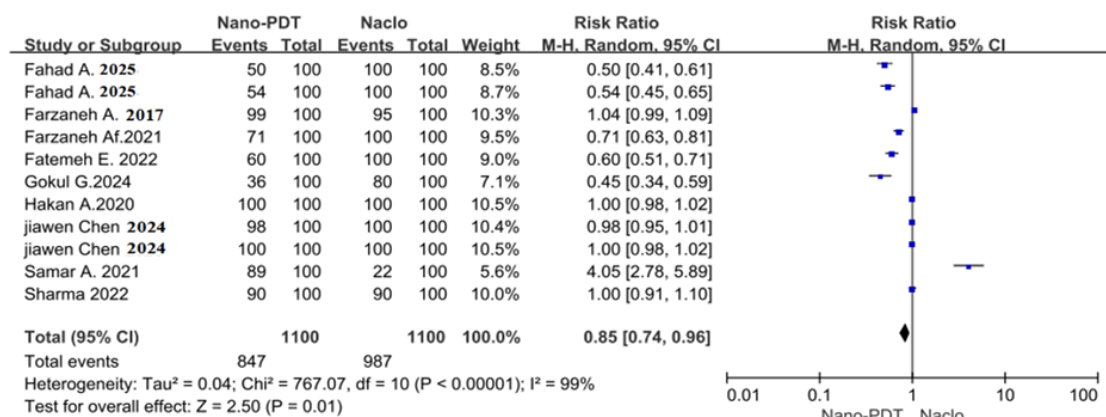


Figure 4: Forest plot of Nano-aPDT vs. NaOCl against *E. faecalis*.

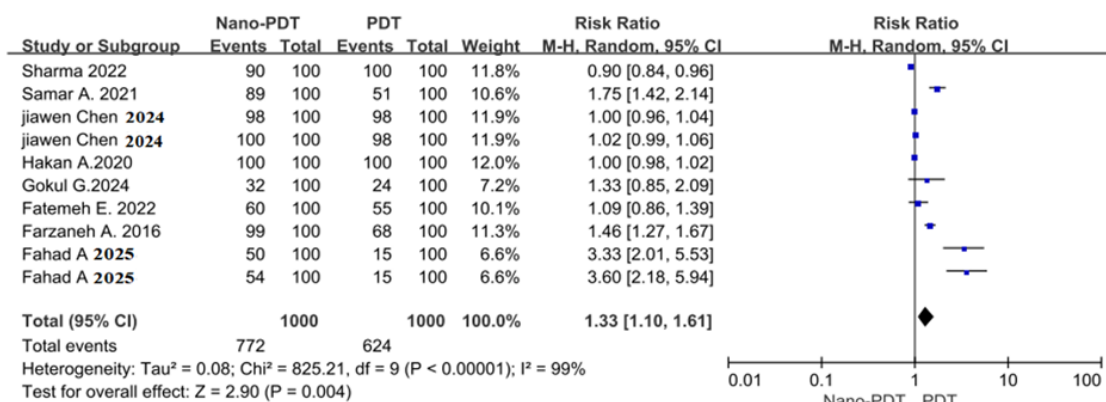


Figure 5: Forest plot of Nano-aPDT vs. PDT against *E. faecalis*.

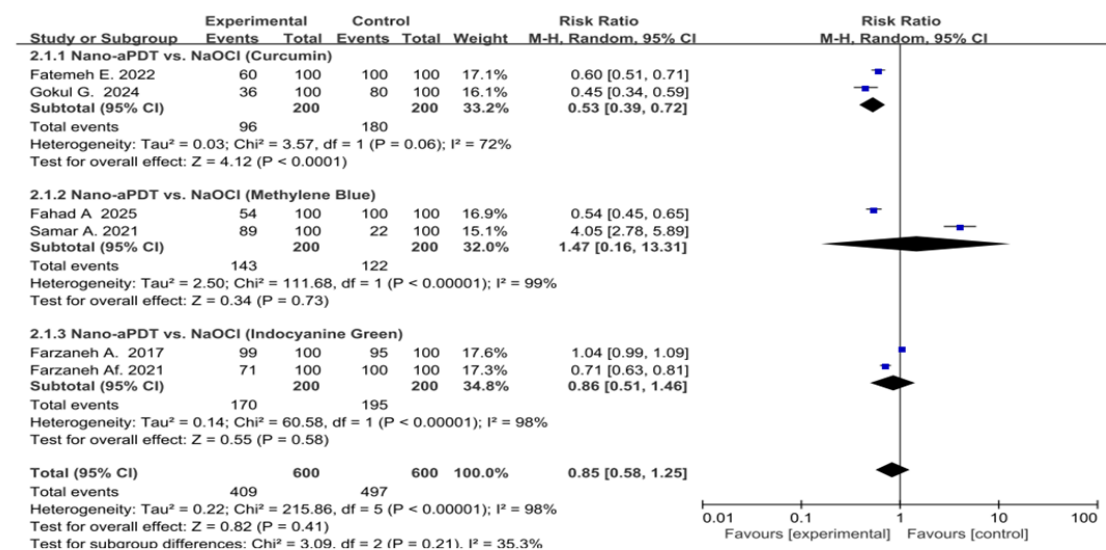


Figure 6: Forest plot of subgroup analysis (different photosensitizers) for Nano-aPDT vs. PDT against *E. faecalis*.

To assess the robustness of the primary findings in the context of this substantial heterogeneity and experimental variability, a leave-one-out sensitivity analysis was performed. This analysis confirmed that the overall direction and statistical significance of the pooled estimates remained stable ($*p < 0.05$ for all comparisons), regardless of which individual study was sequentially excluded. This indicates that the core finding of a statistically significant effect is not unduly driven by any single study, despite the significant differences in experimental conditions.

Quality of Evidence (GRADE)

The overall evidence quality was moderate, downgraded due to: risk of bias (incomplete randomization/blinding reporting); inconsistency (substantial heterogeneity); imprecision (wide confidence intervals); indirectness (exclusively *in vitro* evidence).

While the true effect likely approximates our estimates, these limitations underscore the need for standardized protocols. Future studies should prioritize the standardization of intervention parameters and reporting practices, while enhancing the reproducibility and transparency of experimental designs, in order to improve the generalizability and reliability of systematic review findings in this field.

Discussion

Advancing the clinical translation of Nano-aPDT requires addressing several critical challenges rooted in the biological and anatomical complexity of root canal infections. The persistence and reinfection of microorganisms remain the principal cause of endodontic treatment failure, largely due to the intricate microanatomy of the root canal system. Studies have shown that isthmuses are present in up to 55.5–95.48% of maxillary first molars [22,23] and accessory canals, lateral canals, apical bifurcations, and ramifications are also common. These narrow, irregular structures are inaccessible to conventional mechanical preparation and chemical irrigation, creating protected niches for microorganisms that contribute to microleakage, reinfection, and persistent periapical inflammation.

Enterococcus faecalis is the most clinically significant resistant pathogen in endodontic failure. It survives under nutrient-deprived, hypoxic, and anatomically challenging conditions by forming robust biofilms. Resistance to standard irrigants, including sodium hypochlorite (NaOCl) and chlorhexidine (CHX), is well documented. Although higher NaOCl concentrations (2.5–5.25%) improve antimicrobial and tissue-dissolving efficacy, concentrations above 3% may adversely affect dentin by reducing its microhardness and fracture resistance [24,25], increasing the risk of structural damage. Moreover, NaOCl efficacy depends on contact time and replenishment frequency. *In vitro* studies have shown near-complete eradication of *E. faecalis* after 40 minutes of exposure [26], highlighting the time dependency of its antibacterial action.

Antimicrobial photodynamic therapy (aPDT) is a promising adjunct to conventional irrigation due to its multi-target mechanism,

broad-spectrum activity, and low potential for inducing resistance [27]. However, conventional aPDT remains limited by the physicochemical properties of common photosensitizers such as curcumin, methylene blue, indocyanine green, and chlorin e6. Their high molecular weight and hydrophilicity limit penetration into dentinal tubules, isthmuses, and biofilms. At low concentrations, their efficacy is insufficient, whereas at higher concentrations, they tend to aggregate on canal surfaces, creating a “self-shielding” effect that impairs light penetration and reduces ROS production [28].

Recent advancements in nanotechnology have yielded significant improvements in antimicrobial photodynamic therapy through three key mechanisms. First, nanocarrier systems - particularly silver nanoparticles and liposomes - have demonstrated enhanced photosensitizer delivery by simultaneously improving tissue penetration depth, molecular stability, and target specificity. Second, physical activation techniques including ultrasound and shockwave-enhanced emission photoacoustic streaming (SWEEPS) have been shown to optimize photosensitizer distribution within complex root canal anatomy while amplifying reactive oxygen species (ROS) generation.

Notably, oxygen-releasing nanocomposites represent a breakthrough for overcoming hypoxia-limited PDT efficacy. The Ce6/CaO₂/ZIF-8@PEI system [29] exemplifies this approach through sustained oxygen release, thereby maintaining therapeutic ROS production even in low-oxygen root canal environments. Complementing this, magnetically guided nanoparticles [30] utilize external magnetic fields to achieve unprecedented penetration into dentinal tubules, effectively disrupting established biofilms.

Furthermore, multifunctional platforms like the CGP-based nanocarrier [31] demonstrate the expanding therapeutic scope of Nano-aPDT by combining nitric oxide (NO)-mediated antibacterial action with osteogenic potential - particularly valuable for periapical lesion management. Equally promising, natural compound delivery systems such as curcumin-loaded solid lipid nanoparticles (CUR-SLN) [32] successfully balance antimicrobial efficacy with tissue biocompatibility, overcoming a major limitation of conventional photosensitizers.

Importantly, parameter optimization has substantially enhanced treatment outcomes. The AgNPs/ICG-PDT system activated at 910 nm achieved bacterial reduction comparable to 5.25% NaOCl, while nano-curcumin with SWEEPS activation yielded near-sterile conditions (mean count: 0.22 vs 0 for NaOCl) [17,18]. Additionally, auxiliary activation methods including sonic and ultrasonic irradiation have proven effective for increasing both photosensitizer penetration depth and antimicrobial coverage [33,34], suggesting their potential as force multipliers in clinical protocols.

This progression of innovations demonstrates how nanotechnology has transformed aPDT from a promising concept to a potentially viable clinical solution, addressing multiple limitations of conventional endodontic disinfection methods through: Enhanced

physical drug delivery properties; Overcoming microenvironmental limitations (e.g., hypoxia); Enabling multifunctional therapeutic approaches; Optimizing activation parameters for maximal efficacy;

Collectively, these advances establish Nano-aPDT as a promising and adaptable platform with the potential to address a broad range of clinical challenges in endodontic disinfection and related fields.

Although Nano-aPDT exhibits a slower bactericidal rate than NaOCl, it offers synergistic advantages in targeted delivery, tissue penetration, controlled ROS generation, and biocompatibility [35]. These features support its potential as a precision disinfection strategy in complex root canal systems. Beyond endodontic infections, Nano-aPDT shows promise in managing periodontal disease, dental caries, oral precancerous lesions, and peri-implantitis, reflecting its versatility and cross-indication potential [27]. Based on the complementary mechanisms of the two technologies, we propose an innovative strategy combining reduced-concentration NaOCl (1–2%) with Nano-aPDT: Leveraging NaOCl's ability to rapidly degrade the biofilm extracellular matrix, which significantly enhances nanocarrier penetration into deep dentinal tubules, while utilizing Nano-aPDT to generate localized bursts of reactive oxygen species (ROS) for precise eradication of NaOCl-tolerant residual microorganisms [36]. This synergistic protocol not only reduces NaOCl concentration by 60–80% (compared to conventional 5.25% applications), effectively mitigating its collateral cytotoxicity, but also overcomes the therapeutic limitations of monotherapies against mature biofilms. Moreover, both photodynamic therapy and nanoparticle-augmented photodynamic therapy in endodontic treatment have demonstrated significant antimicrobial efficacy by disrupting quorum sensing pathways, downregulating relevant gene expression, suppressing microbial metabolic activity, and compromising biofilm integrity. In addition, these approaches have shown preliminary immunomodulatory potential, as evidenced by macrophage activation and attenuation of proinflammatory cytokine expression [33].

Limitations and Future Directions

Despite the promising potential of Nano-aPDT demonstrated in this systematic review and meta-analysis, several challenges and limitations must be addressed before widespread clinical adoption.

Firstly, the overall antibacterial efficacy of Nano-aPDT remains inferior to that of NaOCl, which is the current gold standard. Additionally, the high heterogeneity among included studies—stemming from differences in experimental models, nanocarrier materials, photosensitizer types, irradiation parameters, and evaluation methods—limits the generalizability and comparability of results.

Secondly, standardized intervention protocols are lacking. Variability in photosensitizer concentration, carrier design, laser wavelength, power density, and activation techniques complicates replication and optimization efforts. Furthermore, long-term biocompatibility, safety, and effects on periapical tissue healing

require further elucidation through well-designed animal and clinical studies.

Future research should prioritize: Establishing standardized and optimized treatment parameters; Conducting rigorously designed, adequately powered animal and multicenter clinical trials; Developing unified and clinically relevant outcome measures for disinfection efficacy and tissue regeneration; Exploring synergistic treatment protocols combining Nano-aPDT with conventional irrigation methods such as reduced-concentration NaOCl; Investigating immunomodulatory effects and potential benefits for tissue healing and inflammation control.

Addressing these gaps will be critical to translating Nano-aPDT from an innovative experimental approach into a practical, effective clinical tool for managing complex root canal infections and beyond.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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