

ORIGINAL ARTICLE

Zinc oxide nanoparticles and blue light enhance sodium hypochlorite disinfection against *Enterococcus faecalis*: an in vitro study

ABSTRACT

Aim: This study aimed to evaluate the antimicrobial efficacy of zinc oxide nanoparticles (ZnO NPs) and blue light, both individually and in combination, against *Enterococcus faecalis* (*E. faecalis*) in vitro. Their performance was compared to 1% sodium hypochlorite (NaOCl) to determine whether these adjuncts could enhance root canal disinfection.

Methodology: A laboratory investigation was conducted using eight experimental groups: *E. faecalis* with phosphate-buffered saline (control), 1% NaOCl, ZnO NPs, blue light, 1% NaOCl/ZnO NPs, 1% NaOCl/blue light, ZnO NPs/blue light, and 1% NaOCl/ZnO NPs/blue light. Standardized *E. faecalis* inocula were exposed to each treatment under controlled conditions. Bacterial cells were then recovered, serially diluted, and plated on agar to determine colony-forming units (CFU). Reductions in CFU, compared to the control, were analyzed using Mann-Whitney tests. Statistical significance was set at $p < 0.05$.

Results: Most experimental groups exhibited significantly lower bacterial counts than the control ($p = 0.000$), except for the blue light-only group, which was not statistically different. Among the single-agent treatments that reached significance, 1% NaOCl produced the greatest bacterial reduction, followed by ZnO NPs. When combined, dual treatments reduced bacterial counts more than single agents. The triple combination achieved the highest reduction overall.

Conclusions: This study highlights the potential of ZnO NPs and blue light as adjuncts to 1% NaOCl for more effective elimination of *E. faecalis* in planktonic cultures. The enhanced antimicrobial activity observed with combined treatments suggests that incorporating nanoparticles and photodisinfection could reduce the need for higher NaOCl concentrations while maintaining robust antibacterial efficacy.

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Introduction

Effective root canal disinfection is critical for the success of endodontic treatments, particularly in eradicating *Enterococcus faecalis* (*E. faecalis*), a resilient pathogen associated with persistent infections. Sodium hypochlorite (NaOCl) has long been considered the gold standard irrigant due to its robust antimicrobial activity and ability to dissolve organic tissues, making it the most popular choice in endodontic therapy (1). Due to its cytotoxicity and potential to irritate periapical tissues, clinicians often opt for concentrations of NaOCl lower than the maximum 5.25%. However, the optimal concentration that balances efficacy and safety has yet to be determined, highlighting the need for alternative or adjunctive irrigation solutions that mitigate these side effects (2). Although highly effective, NaOCl presents several challenges, including reduced efficacy with prolonged exposure due to protein interaction, unpleasant odor and taste, and risks of severe tissue damage upon extravasation into periapical areas (3).

Recent advancements in nanotechnology and photodynamic therapy have opened new possibilities for enhancing root canal disinfection. Zinc oxide nanoparticles (ZnO NPs) exhibit potent antimicrobial activity, attributed to their size-dependent effects, disruption of bacterial membranes, and induction of reactive oxygen species (4,5). Meanwhile, blue light within the 400–500 nm spectrum is widely employed in dentistry for caries management, resin curing, and biofilm reduction, offering a safer alternative to ultraviolet radiation (6–8). Beyond its standalone antibacterial properties, blue light can augment nanoparticle-induced reactive oxygen species production, thereby amplifying bacterial elimination (9–11).

Despite these advancements, there is limited evidence on the combined application of ZnO NPs and blue light as adjuncts to NaOCl in root canal disinfection. To address this gap, this study evaluates the antimicrobial efficacy of ZnO nanoparti-

cles and blue light, both individually and in combination, against *Enterococcus faecalis*, and benchmarks their performance against sodium hypochlorite to propose enhanced disinfection protocols for clinical endodontics.

Materials and Methods

Ethical approval was obtained from the institutional ethics committee of Oman Dental College (Ref: ERIC-GS-2023-216), confirming that no human or animal subjects were involved. The study was conducted in accordance with the Declaration of Helsinki, ensuring adherence to recognized ethical standards. This laboratory investigation assessed the antibacterial properties of ZnO NPs and blue light under controlled experimental conditions. These were tested both individually and in combination against *E. faecalis*. Additionally, their efficacy was benchmarked against 1% NaOCl to determine any significant differences in bacterial reduction.

The study included eight experimental groups, detailed as follows:

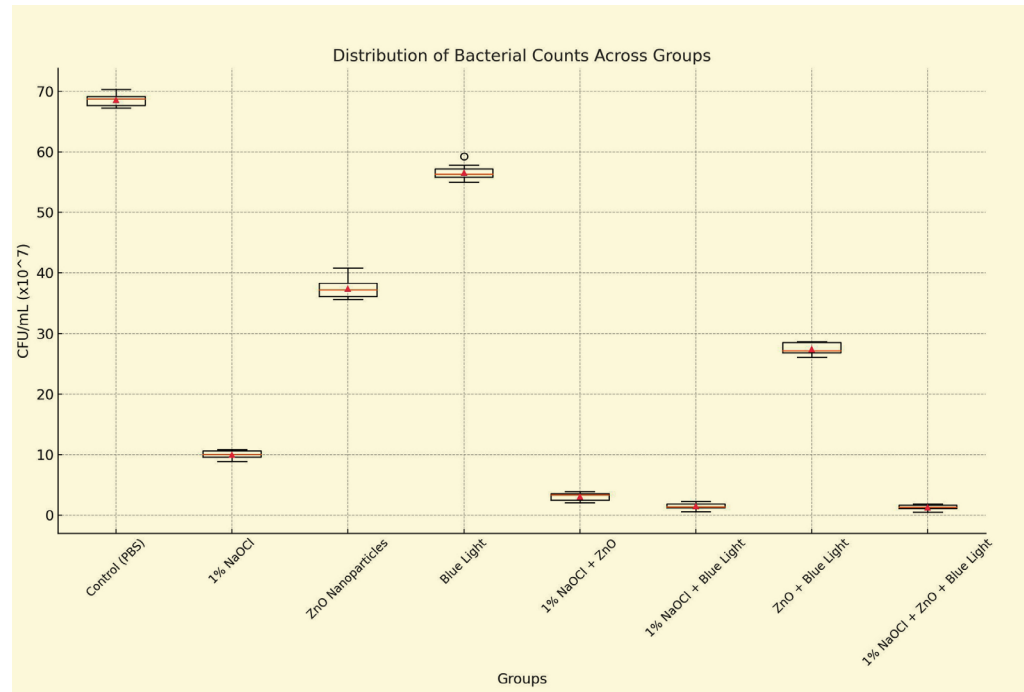
- Group 1: Control (*E. faecalis* with phosphate-buffered saline (PBS))
- Group 2: *E. faecalis* with 1% NaOCl
- Group 3: *E. faecalis* with ZnO NPs
- Group 4: *E. faecalis* with blue light
- Group 5: *E. faecalis* with 1% NaOCl and ZnO NPs
- Group 6: *E. faecalis* with 1% NaOCl and blue light
- Group 7: *E. faecalis* with ZnO NPs and blue light
- Group 8: *E. faecalis* with 1% NaOCl, ZnO NPs, and blue light

Cultivation and Inoculation of *E. faecalis*

Strains of *E. faecalis* NCTC 12697 / ATCC® 29212 (TCS Biosciences, Buckingham, UK) were cultivated using broth overnight. Cultures were maintained on agar plates supplemented with 5% defibrinated horse blood (TCS Biosciences, Buckingham, UK) and sub-cultured weekly. For experimental purposes, single colonies were transferred to tryptone soy broth (Fisher Scientific, Loughborough, UK) and incubated

Figure 1.

Bacterial counts - Box plot showing the distribution of bacterial counts (CFU/mL) across experimental groups. The central line in each box represents the median, while the box bounds depict the interquartile range (IQR). Whiskers extend to the minimum and maximum non-outlier values, and individual data points indicate outliers. The mean is marked as a diamond in each group.



overnight at 37°C. Cell concentrations were standardized using McFarland turbidity standards and adjusted to an optical density (OD) of 0.2 using a diluphotometer.

Preparation and Application of Experimental Solutions

Sodium hypochlorite (5%, Fisher Scientific, Loughborough, UK) was diluted to 1%. A fresh working solution was prepared for each experiment. Each test involved mixing 1 mL of bacterial solution with 1 mL of 1% NaOCl for 10 seconds. The mixture was incubated for five minutes in a 6-well tissue culture plate before serial dilution and plating on blood agar.

Zinc oxide nanoparticles (particle size: <100 nm; Sigma-Aldrich, Poole, UK) were dispersed in water. A 1 mL dispersion of ZnO NPs was added to 1 mL of bacterial solution. The mixture was vortexed for 10 seconds and treated similarly to the NaOCl group.

Bacterial solutions were exposed to blue light emitted by a Bivar UV5TZ-405-30 LED (wavelength: 390-407 nm; irradiance: 120 mW/cm²; Digikey Electronics, Slough, UK) for 300 seconds. The light source was positioned at a fixed distance of 50 mm from the solutions. Serial dilutions were pre-

pared immediately after exposure.

Serial Dilution and Colony Counting

Serial dilutions were performed to achieve countable colony numbers (30-300 colonies). Agar plates were prepared using trypticase soy agar (Oxoid Ltd., Cheshire, UK) and Iso-Sensitest agar (Oxoid Ltd., Cheshire, UK). Plates were incubated at 37°C for 24 hours. Bacterial colonies were manually counted using a Stuart® colony counter, with adjustments made based on the dilution factor to calculate colony-forming units (CFU) per milliliter.

Data Analysis

Bacterial counts were tabulated, and error bar graphs were generated to illustrate group comparisons. Statistical analyses were conducted using R (Version 4.4.0, R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Mann-Whitney tests were employed to compare the control and experimental groups.

Results

The intensity of blue light at a distance of 50 mm was measured using a spectrophotometer. The measured irradiance values

Table 1.

Summary of bacterial colony-forming units (CFU/mL), reductions compared to control, and statistical significance for each experimental group.

Group	Treatment	Mean CFU/mL (x10 ⁷) (SD)	Median CFU/mL (x10 ⁷)	Reduction vs Control (x10 ⁷)	P-value*
1	Control (PBS)	68.0 (1.5)	68.0	0	-
2	1% NaOCl	10.4 (0.8)	11.0	57.6	0.000
3	ZnO NPs	38.2 (1.8)	40.0	30.0	0.000
4	Blue Light	56.7 (1.4)	58.0	12.0	NS
5	1% NaOCl+ZnO NPs	3.4 (0.7)	2.0	64.6	0.000
6	1% NaOCl+Blue Light	1.6 (0.6)	1.0	66.4	0.000
7	ZnO NPs+Blue Light	27.4 (1.2)	28.0	41.0	0.000
8	1% NaOCl+ZnO NPs+BL	1.1 (0.5)	0.0	66.9	0.000

*Mann-Whitney U-tests indicates that median is significantly different to median of the control (group 1) ($p=0.000$). BL: blue light; CFU: colony-forming units; NaOCl: sodium hypochlorite; PBS: phosphate-buffered saline; ZnO NPs: zinc oxide nanoparticles; NS: not significant.

were as follows: visible light, 3.17688 mW/cm²; total UV, 1.41556 mW/cm²; blue light, 1.14664 mW/cm²; and total output, 4.59244 mW/cm². The antibacterial efficacy of the experimental groups is summarized in Table 1, which presents the mean and median CFU/mL, reductions compared to the control group, and associated p-values for each treatment group.

The bacterial reductions across groups were statistically analyzed using Mann-Whitney U-tests, comparing each experimental group to the control. All experimental groups exhibited significantly lower bacterial counts than the control ($p=0.000$), with the combination treatments demonstrating the highest efficacy. Group 8, combining 1% NaOCl, ZnO NPs, and blue light, achieved the greatest bacterial reduction. Among single-agent treatments, 1% NaOCl was the most effective, followed by ZnO NPs and blue light, respectively. Dual and triple combinations significantly enhanced bacterial reduction.

Discussion

This study investigated the antimicrobial efficacy of ZnO NPs and blue light photo-

disinfection, both individually and in combination, against *E. faecalis* in planktonic cultures. The findings confirmed that 1% NaOCl alone is highly effective, consistent with its longstanding status as the gold standard in root canal irrigation (12,13). However, bacterial reduction was further enhanced when ZnO NPs and blue light were applied, either independently or along with NaOCl. These results highlight that while NaOCl remains indispensable, complementary methods can improve its antimicrobial activity and possibly minimize some of its drawbacks (14). NaOCl's established role in endodontic disinfection arises from its robust antimicrobial properties and ability to dissolve organic tissue. Yet, it also presents significant limitations, such as restricted penetration into dentinal tubules and cytotoxic effects, which justify the need for adjuncts (12,14). Because no single agent has replaced NaOCl, current research focuses on identifying therapies that augment NaOCl and lower concentration-dependent side effects (9-11,15,16). The synergistic bacterial reduction noted in this study, particularly when NaOCl was combined with ZnO NPs or blue light, supports the



wider trend in endodontics to incorporate nanotechnology and photodynamic therapy.

Evidence shows that nanoparticles can enhance antimicrobial efficacy by disrupting bacterial membranes and generating reactive oxygen species (16-18). ZnO NPs, in particular, have low toxicity, favorable cost-effectiveness, and size-dependent antimicrobial properties against a diverse range of bacteria (4). Djearamane et al. (2022) reported that ZnO NPs inhibit *E. faecalis* by disrupting cell membranes and distorting bacterial cell structures (5). Other studies have shown that incorporating ZnO NPs into dental materials reduces bacterial adhesion and biofilm formation (19,20). The current findings, which demonstrated reduced bacterial counts following treatment with ZnO NPs, support prior reports that ZnO NP solutions can prevent *E. faecalis* adhesion and help disaggregate biofilm structures (21,22).

Our findings support previous evidence that blue light is effective against *E. faecalis*, reinforcing its potential for endodontic disinfection when combined with ZnO nanoparticles (6,8). Other studies have also demonstrated that blue light can inhibit a broader range of pathogens, including *Staphylococcus aureus* and *Prevotella intermedia* (23,24). This effect arises from the excitation of microbial chromophores, such as flavins and porphyrins, leading to reactive oxygen species generation (25). Microorganisms do not develop resistance to blue light, making it a sustainable antimicrobial approach (26). Given its documented safety profile (27), blue light is a promising tool for root canal disinfection. The improved bacterial reduction noted in this study reflects its synergistic potential with other antimicrobials, as also reported previously in the literature (9-11, 28-30).

Recent investigations into light-activated nanoparticles affirm the synergistic effects observed in this study. Minhaco et al. (2023) found that curcumin-loaded polymeric nanoparticles activated by blue light significantly reduced bacterial viability in biofilm models (31). Afkhami et al. (2021) similarly reported that silver nanoparticles

activated through passive ultrasonic irrigation and photon-induced photoacoustic streaming achieved over 90% bacterial reduction (32). These advances support the concept that combining nanoparticles with light-based activation methods can address limitations associated with conventional irrigation.

E. faecalis was selected due to its clinical importance in persistent endodontic infections and its ability to penetrate deeply into dentinal tubules (33). Its capacity for biofilm formation further complicates disinfection, which underscores the need to test novel antimicrobial strategies. Although using planktonic cultures facilitated a clear assessment of direct antimicrobial activity, this model does not fully capture the complexity of root canal biofilms (34). Future research should therefore employ biofilm and *ex vivo* models to better approximate clinical environments, particularly given that the dense extracellular matrix in biofilms can reduce antimicrobial penetration.

Clinically, integrating ZnO NPs and blue light into root canal disinfection protocols can enhance the penetration and effectiveness of antimicrobial agents in complex canal anatomies. This combination may be especially valuable in retreatment cases or infections involving resistant organisms such as *E. faecalis*. Lowering reliance on higher concentrations of NaOCl also offers a potential reduction in cytotoxicity to periapical tissues, which can increase patient safety and comfort. The broader shift in dentistry toward minimally invasive, targeted therapies aligns well with the development of light-activated antimicrobial protocols, which aim to optimize bacterial reduction while maintaining biocompatibility. Despite these promising results, a few limitations of this study must be acknowledged. Planktonic models do not replicate the protective matrix of biofilms or the variability of clinical conditions, including organic debris and irregular canal anatomy. Long-term biocompatibility and cytotoxicity data on ZnO NPs and blue light are also essential. Further research should evaluate these therapies using biofilm and *ex vivo* models and es-

establish their safety profiles before widespread clinical adoption. Additional studies on alternative photosensitizers and activation methods may further enhance the efficacy of this approach. By addressing these gaps, future investigations will provide deeper insights into the clinical potential of combining nanoparticles and photodisinfection for improved endodontic outcomes.

Conclusion

This work highlights the potential of combining ZnO nanoparticles and blue light as a promising adjunct to sodium hypochlorite for root canal disinfection. The findings demonstrate that this combination enhances bacterial reduction and addresses some of the limitations of conventional irrigation methods. ZnO NPs offer effective antimicrobial and antibiofilm properties, while blue light adds a safe and widely available activation method. Together, they provide a synergistic approach that could improve the success rates of endodontic treatments, particularly in challenging cases involving resistant bacteria like *E. faecalis*. However, the translation of these results into clinical practice requires further validation in biofilm models, ex vivo systems, and safety studies. By bridging these gaps, this innovative approach has the potential to redefine modern endodontic therapy, aligning with the broader trend of minimally invasive and targeted dental treatments.

Clinical relevance

Combining zinc oxide nanoparticles, blue light, and sodium hypochlorite significantly improved the elimination of *Enterococcus faecalis* in a laboratory model of root canal disinfection. This synergy may allow lower concentrations of irrigants, potentially reducing tissue toxicity and improving patient safety in endodontic therapy.

Disclosures

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- Ethical Approval: The study was approved by the Ethics, Research, and Innovation Committee ERIC (Ref: ERIC-GS-2023-216).
- Authorship Contributions: Concept – G.S.; Design – G.S; Materials – G.S.; Data collection and/or processing – G.S., A.Q.; Data analysis and interpretation – G.S., A.Q.; Literature search – A.Q.; Writing – G.S., A.Q.; Critical review – G.S, A.Q.

Conflict of Interest

All authors declared no conflict of interest.

Financial Disclosure

Nothing to disclose.

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