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Original Article

Indocyanine green-activated photodynamic therapy with diode laser eradicates *Enterococcus faecalis* in infected root canals: An *in vitro* study

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ABSTRACT

Background: The current endodontic disinfection techniques may be supplemented with photodynamic therapy (PDT), which is believed to eliminate intracanal bacteria more efficiently. This *in vitro* study aimed to assess the antimicrobial effectiveness of PDT using various photosensitizers, in conjunction with a near-infrared diode laser (810 nm wavelength), in the root canals of teeth infected with *Enterococcus faecalis*. This research is crucial for determining the optimal PDT method for eliminating *E. faecalis*, thereby enhancing the effectiveness of endodontic disinfection techniques.
Materials and Methods: This *in vitro* experimental study was conducted on 50 intact human maxillary first molars, which were decoronated, and palatal roots were instrumented to ISO size X5. After autoclaving, roots were inoculated with *E. faecalis* (ATCC 29212; 10^8 CFU/mL) and incubated anaerobically for 72 h. The roots were randomly divided into five experimental groups: control (C), laser alone (L), PDT with indocyanine green (ICG), PDT with methylene blue (MB), and PDT with cetrimide (CT) 2%. Root canals were flushed with phosphate-buffered saline, and serial dilutions were plated on agar. Colony-forming units were counted after 48-h incubation. Data were analyzed using one-way analysis of variance followed by Tukey's *post hoc* test for pairwise comparisons. A significance level of $P < 0.05$ was considered.
Results: PDT with ICG resulted in complete eradication of *E. faecalis* (0 CFU/mL), whereas PDT with MB and CT produced significant reductions (5.08 ± 0.2 and 5.55 ± 0.1 $\log_{10}$ CFU/mL, respectively; $P < 0.05$). Laser alone reduced CFU/mL to 5.94 ± 0.07.
Conclusion: PDT with ICG and an 810 nm diode laser achieved complete eradication of *E. faecalis*, outperforming MB, CT, and laser alone. These results advocate for ICG-PDT as a potent adjunct in endodontic disinfection protocols.

Key Words: Anti-infective agents, diode laser, drug combination, endodontics, photodynamic therapies, photosensitizing agents, root canal preparation

INTRODUCTION

Periapical lesions and endodontic treatment failures are frequently attributed to persistent microbial infections, with *Enterococcus faecalis* emerging as a predominant pathogen due to its ability

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to colonize dentinal tubules and form resilient biofilms.[1] Conventional disinfection relies on sodium hypochlorite (NaOCl), yet its cytotoxicity, limited penetration into anatomical complexities, and incomplete biofilm eradication underscore the need for safer, more effective adjunctive therapies.[2]

Photodynamic therapy (PDT) presents a promising solution by combining light-sensitive agents (photosensitizers; PS) with laser irradiation to generate reactive oxygen species (ROS), selectively targeting pathogens without thermal damage.[3-5] Unlike traditional methods, PDT's mechanism_oxidative destruction of bacterial membranes, proteins, and DNA_addresses biofilm resistance while preserving host tissues, making it ideal for intricate root canal systems.[6,7]

Recent advancements highlight near-infrared (NIR) lasers (e.g. 810 nm) for deeper tissue penetration and reduced scattering. Indocyanine green (ICG), an FDA-approved NIR-absorbing dye, excels in ROS generation and biocompatibility, whereas methylene blue (MB), a phenothiazine dye, offers broad-spectrum antimicrobial activity.[8,9] Cetrimide (CT), a quaternary ammonium compound, enhances PDT efficacy through surfactant-mediated biofilm disruption and intrinsic antibacterial properties.[10]

Despite promising findings, comparative studies on PS efficacy in endodontic PDT remain limited. Existing research predominantly focuses on visible-light-activated agents, with sparse data on NIR-compatible PS like ICG. Furthermore, the synergistic potential of antimicrobial surfactants like CT in PDT protocols warrants exploration to optimize bacterial eradication.

This *in vitro* study evaluates the antimicrobial efficacy of PDT using ICG, MB, and CT activated by an 810 nm diode laser against *E. faecalis* in infected root canals. By addressing gaps in PS selection and laser parameters, this work aims to advance PDT's clinical translation as a standardized adjunct in endodontic disinfection.

MATERIALS AND METHODS

Study design

This study was conducted as an *in vitro* experiment.

Bacterial culture

E. faecalis (ATCC 29212) was cultured on esculin bile agar to confirm species identity, then subcultured in brain–heart infusion broth under anaerobic conditions (85% N_2, 10% H_2, and 5% CO_2) at 37°C for 72 h. Bacterial cells were pelleted through centrifugation (1000 rpm, 5 min), washed twice, and resuspended in phosphate-buffered saline (PBS). Density was adjusted to 10^8 CFU/mL using spectrophotometry (OD_{600} = 0.1), validated through colony counts.[7]

Sample collection and preparation

Fifty freshly extracted human maxillary first molars, obtained from patients aged 18 to 35 and stored in 0.5% NaOCl for 2–4 weeks, were selected based on strict criteria: mature palatal roots with a single, straight canal, free of resorption, fractures, or prior endodontic treatment. Radiographic and microscopic evaluations confirmed a solitary canal configuration. The teeth were decoronated with a diamond disk under water cooling, and the root canals were negotiated using a size 10 K-file. Canal preparation was performed to an ISO size X5 using NiTi rotary files (ProTaper Next System), with intermittent irrigations using 2.5% NaOCl. Following mechanical preparation, canals were irrigated sequentially with 1 mL of 17% ethylenediaminetetraacetic acid (EDTA) (applied for 3 min) and 1 mL of Chex 2% combined with NaOCl (also for 3 min), with saline rinses before and after each irrigant. The specimens were then transferred into sterile microcentrifuge tubes containing 1 mL PBS and autoclaved at 121°C for 20 min.

After sterilization, each canal was inoculated with 1 mL of BHI broth containing approximately 10^9 *E. faecalis* cells (1 OD unit) using a 27G Endo-Eze needle. The samples were fully immersed in the bacterial suspension and incubated anaerobically at 37°C for 72 h to ensure the establishment of infection.

Laser parameters

An 810 nm diode laser (0.5 W, continuous wave) delivered light through a 400-µm optical fiber.[3] The fiber was moved in a helical motion to ensure uniform irradiation across the canal surface (total fluence: 143 J/cm²). Power density (2.38 W/cm²) was calculated based on the irradiated canal surface area (0.21 cm²).

Experimental groups and treatment protocols

Following confirmation of infection, the 50 samples were randomly divided into five groups (n = 10 per group):

1. Control (C): No treatment
2. Laser Alone (L): Canals were irradiated with an 810 nm diode laser at 0.5 W

3. PDT with ICG (ICG + Laser): Canals were filled with 1 mL of freshly prepared ICG solution (100 µg/mL in PBS) and allowed to incubate in the dark for 15 min before laser activation[5]
4. PDT with MB (MB + Laser): Canals received 0.5 mL of a 0.01% MB solution, applied for 5 min before laser irradiation
5. PDT with CT (CT + Laser): Canals were treated with 0.5 mL of a 2% CT solution for 5 min before laser exposure.

For laser treatments, an 810 nm diode laser (Quicklase Dental Laser, China) operating in continuous wave mode at 0.5 W was used. The laser light was delivered through a 400-µm optical fiber, which was maneuvered in a spiral motion from the apical to the cervical end to ensure even light distribution. Excluding the control group, each specimen was irradiated for a total of 60 s, administered in three 20-s cycles with 20-s intervals between exposures. This protocol resulted in a power density of 2.38 W/cm² and an energy fluence of 143 J/cm².

Microbiological analysis

After treatment, each canal was flushed with 1 mL of PBS using a 27-G Endo-Eze irrigation needle. The collected fluid was transferred to sterile 1.5-mL Eppendorf tubes and serially diluted (neat to 10^{-7}) using tenfold dilutions.[6] Aliquots of 0.1 mL from each dilution were plated on agar and incubated at 35°C ± 2°C for 48 h. Colony-forming units (CFUs) were then counted to assess the antibacterial efficacy.

Statistical analysis

Data analysis was performed using SPSS (IBM SPSS Statistics 26, IBM Corp., Armonk, NY, USA). The normality of the data was verified with the Shapiro–Wilk test. Group comparisons were made using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test for pairwise comparisons. A $P < 0.05$ was considered statistically significant.

RESULTS

All experimental groups showed significant reductions in bacterial load compared to the untreated control group ($P < 0.05$) [Figure 1]. In untreated canals (control), a robust biofilm was established, with a mean bacterial load of 6.59 ± 0.08 $\log_{10}$ CFU/mL, confirming the persistence of infection without intervention. In contrast, irradiation with the 810 nm diode laser alone reduced the CFU count to

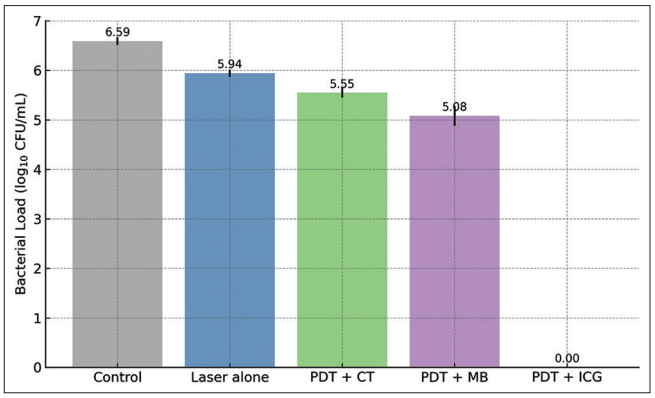

Figure 1: Comparative efficacy of treatment groups against *Enterococcus faecalis* biofilm.

5.94 ± 0.07 $\log_{10}$ CFU/mL, demonstrating the intrinsic antibacterial activity of the laser.

PDT further enhanced bacterial eradication. Canals treated with CT-mediated PDT exhibited a reduction to 5.55 ± 0.10 $\log_{10}$ CFU/mL, significantly lower than both the control and laser-alone groups. MB-PDT achieved an even more marked reduction, with bacterial counts dropping to 5.08 ± 0.20 $\log_{10}$ CFU/mL, thereby outperforming CT-mediated PDT. Remarkably, ICG-activated PDT achieved complete eradication of *E. faecalis*, with no detectable CFUs observed posttreatment, highlighting its superior efficacy.

Statistical analysis supported these observations. Shapiro–Wilk testing confirmed the normal distribution of the data ($P > 0.05$), and one-way ANOVA revealed significant differences among the groups ($F = 1204.6$, $P < 0.001$). Tukey's *post hoc* analysis further delineated these differences, showing that all treatment groups differed significantly from the control ($P < 0.001$). Comparisons between the laser-alone group and the PDT groups yielded statistically significant differences, with PDT + CT ($P = 0.003$), PDT + MB ($P < 0.001$), and PDT + ICG ($P < 0.001$) all showing superior performance. In addition, the PDT + ICG group differed significantly from both the PDT + MB and PDT + CT groups ($P < 0.001$), underscoring the enhanced bactericidal potential of ICG-activated PDT.

DISCUSSION

The findings of this study underscore the exceptional efficacy of ICG-mediated PDT in eradicating *E. faecalis* from infected root canals, achieving complete bacterial elimination (0 CFU/mL). This

aligns with its recognized role as a resilient pathogen in chronic endodontic infections, often evading conventional disinfection due to its biofilm-forming ability and dentinal tubule colonization.[11] The superiority of ICG-PDT over the MB and CT groups highlights the critical influence of PS properties and laser parameters. ICG's NIR absorption (810 nm) enables deeper tissue penetration (4–6 mm) compared to MB's visible-light activation (665 nm), effectively targeting bacteria in anatomically complex regions, such as isthmuses and apical deltas.[8,12]

CT's dual role as a surfactant and antimicrobial agent likely enhanced PDT efficacy by disrupting biofilm matrices and increasing bacterial membrane permeability, consistent with prior studies demonstrating its substantivity and biofilm clearance.[13] However, its lower reduction (5.55 ± 0.10 log~10~CFU/mL) compared to ICG emphasizes the need for PS-specific optimization. Importantly, PDT's safety advantage over traditional laser protocols _avoiding thermal risks such as dentin carbonization or root resorption_ was evident. While diode lasers alone (5.94 ± 0.07 log~10~CFU/mL) can generate cytotoxic heat, PDT's oscillatory fiber motion and intermittent irradiation (three 20-s cycles) mitigated temperature rises, as validated by Alfredo *et al*.[14] Furthermore, cytotoxicity studies corroborate PDT's biocompatibility: Kashef *et al*. reported no fibroblast toxicity, whereas George and Kishen noted 97.7% bacterial kill versus only 30% fibroblast damage, contrasting sharply with NaOCl's neurotoxic risks.[15,16]

Despite robust outcomes, discrepancies with earlier studies warrant consideration. For instance, Souza *et al*. observed statistically insignificant PDT effects, potentially due to inadequate oxygen levels in root canals or suboptimal PS diffusion.[17] This study's rigorous PS incubation times (15 min for ICG) and standardized laser parameters (0.5 W, 143 J/cm²) may explain the superior results, aligning with protocols by Garcez *et al*. and Foschi *et al*.[6,18]

Future research should prioritize clinical validation through long-term trials to assess PDT's durability *in vivo*, such as the 6-month follow-up protocol by Garcez *et al*.,[18] which could confirm therapeutic consistency in dynamic clinical environments. Combination therapies integrating ICG-PDT with conventional agents, such as EDTA or NaOCl, warrant exploration to dismantle residual biofilm matrices and enhance synergistic antimicrobial outcomes. Finally, cost-effectiveness analyses are essential to evaluate PDT's economic viability, particularly in resource-limited settings, ensuring equitable access to this advanced disinfection modality without compromising clinical standards. These steps will bridge translational gaps, optimizing PDT for widespread adoption in endodontic practice.

While promising, this *in vitro* model simplifies clinical realities. Natural infections involve polymicrobial consortia, and variations in root canal anatomy (e.g. curvature, accessory canals) may alter PDT efficacy.[19] In addition, the single-strain focus on *E. faecalis* excludes interactions with fungi or resistant Gram-negative species.

CONCLUSION

ICG-PDT emerges as a paradigm shift in endodontic disinfection, combining unparalleled antimicrobial efficacy with minimal cytotoxicity. By addressing anatomical and microbial complexities in future studies, this modality could bridge the gap between laboratory success and clinical adoption, revolutionizing standards in root canal therapy.

Ethical approval

This study was approved by the Health Research Committee of the Baghdad College of Dentistry (Approval Code: Ref No. 492, dated January 19, 2022).

Authors' contribution

Conceptualization, methodology, supervision, writing – review and editing: M. R. H; Data curation, investigation, visualization, writing – original draft: A. F. R; Formal analysis, methodology, project administration, resources: M. S; Validation, writing – original draft, writing – review and editing: S. A. All authors have read and approved the final version of the manuscript.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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