

Evaluation of the Antimicrobial Efficacy of Fullerene Nanoparticle Irrigant with Diode Laser Mediated Photodynamic Therapy Against *Enterococcus faecalis* in Root Canals – An In Vitro Study

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ABSTRACT

Objective: To evaluate the antimicrobial activity of a fullerene (C60) nanoparticle irrigant alone and in combination with diode laser-mediated photodynamic therapy (PDT) against *Enterococcus faecalis* biofilm in root canals.

Methods: Sixty single-rooted extracted natural mandibular premolar teeth were calibrated to a workable length, sterilised, and injected with *E. faecalis* for a duration of 14 days to facilitate mature biofilm development. Samples were randomly distributed into three categories (n = 20): 3% sodium hypochlorite (NaOCl, control); 20% w/v fullerene nanoparticle solution; and fullerene nanoparticle solution triggered by a 450 nm diode laser (0.2 W, 40 seconds). Intracanal sample specimens were cultivated on brain heart infusion agar, both prior to and after to irrigation, and colony-forming units (CFU) were measured. The data were examined using the paired t-test, one-way analysis of variance, and subsequent post hoc comparison (p < 0.05).

Results: Mean post-treatment counts were 3.58 log₁₀ CFU/mL for NaOCl, 3.07 log₁₀ CFU/mL for fullerene, and 2.87 log₁₀ CFU/mL for fullerene with laser activation. There was a decrease from baseline of 68.08% for fullerene and 79.64% for fullerene with laser activation (p < 0.001) while NaOCl showed no significant decrease (p = 0.57). Significant intergroup differences were observed (F = 211.92, p < 0.001). The lowest residual microbial load was observed in the laser-activated group.

Conclusion: The fullerene nanoparticle irrigant activated by PDT using a diode laser showed significant antimicrobial activity against *E. faecalis* in root canals in vitro and needs further investigation before clinical application.

Keywords: *Enterococcus faecalis*; Fullerenes; Lasers, semiconductor; Photochemotherapy; Root canal irrigants

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INTRODUCTION

Despite considerable technological advances in dentistry, persistent root canal infection remains a challenge in endodontics. *Enterococcus faecium* is among the species mostly implicated in failed root canal treatment, being recovered in approximately 70%–77% of such cases [1]. The species tolerates conditions that most bacteria cannot, persisting under high alkalinity, oxidative stress, and

starvation. Much of its pathogenic behavior traces back to a combination of virulence factors and the adaptive responses it mounts when stressed. The ability of *E. faecalis* to travel as deep as 300 µm along the dentinal tubules is arguably its defining trait, since neither an irrigant nor a hand instrument can be guaranteed to reach that far in every case [2]. The organism also forms well-structured and highly organized biofilms that confer marked resistance to antimicrobial agents compared with planktonic

forms. Within biofilms, *E. faecalis* maintains a metabolic plasticity that allows it to persist in an inhospitable environment and to recolonize rapidly when favorable conditions arise [3]. These features contribute to the persistence of periapical inflammation and affect long-term success. Chemomechanical preparation is the basis for root canal disinfection, but anatomical complexity limits accessibility, particularly for irrigants. For many years, sodium hypochlorite (NaOCl) the irrigant of choice, appreciated for its ability to kill bacteria and dissolve organic tissue in biofilms. Its drawback is well known. Due to its cytotoxicity, inadvertent extrusion of the material past the apex can result in a significant inflammatory response of the periapical tissues. Another commonly used irrigant is Ethylenediaminetetraacetic acid (EDTA) which is used for smear layer removal but has limited antimicrobial activity [4]. These disadvantages emphasise the necessity of developing sophisticated disinfection methods that can effectively penetrate biofilms, improve the elimination of microbes and maintain biocompatibility. Photodynamic therapy (PDT) is an adjuvant that has garnered heightened interest in the field of endodontics. The concept is straightforward: a photosensitising compound captures light at a specific wavelength, leading to the production of reactive oxygen species in the presence of molecular oxygen. The offerings encompass singlet oxygen and a range of free radicals. These substances oxidise the lipids, proteins, and nucleic acids of the microbial cell to an extent that the organism is unable to repair itself and ultimately perishes. PDT demonstrates efficacy against both planktonic and biofilm-associated microorganisms, while its localised action minimises harm to adjacent host tissues [5]. Fullerene (C60) nanoparticles are a new class of photosensitisers for PDT. Fullerenes are a type of nanostructures composed of carbon with a unique spherical structure that provides special photophysical and photochemical properties, such as a large absorption of light, high photostability and an excellent ability to generate singlet oxygen upon activation, especially under blue light at a wavelength of approximately 450 nm [6]. They can act as photosensitisers on their own, reducing the potential toxicity and simplifying the clinical application, and also present antioxidant, anti-inflammatory, and cytoprotective properties, which support their biocompatibility in biomedical applications [7]. The integration of fullerene nanoparticles with diode laser-mediated PDT therefore represents a novel and minimally invasive strategy for root canal disinfection. Previous work has demonstrated the efficacy of PDT against *E. faecalis*: a clinical

and molecular microbiological study identified PDT as an effective adjunct against biofilm-resistant organisms, with a significant reduction in *E. faecalis* [8], and a recent systematic review reported that PDT penetrates biofilm matrices more effectively than conventional irrigants [9]. Although PDT and nanoparticle-based disinfection strategies have each demonstrated significant antimicrobial efficacy against *E. faecalis*, their combined application has rarely been examined, and studies evaluating fullerene nanoparticle-mediated PDT within the root canal system are scarce. In light of this context, the current in vitro investigation aimed to assess the efficacy of a fullerene nanoparticle irrigant, both with and without diode laser-assisted PDT, in diminishing *E. faecalis* biofilm in root canals. The null hypothesis posited that there would be no variance in the decrease of *E. faecalis* among the three irrigation regimens.

METHODS

Study design and setting

The work was carried out over six months at the Department of Conservative Dentistry and Endodontics, Bengaluru, India. Microbiological analysis and quantification of colony-forming units (CFU) were carried out at CREDORA Life Sciences, Bengaluru, India.

Ethics statement

The research protocol received evaluation and endorsement by the IEC of GDCRI, Bengaluru (approval No. GDCRI/IEC-ACM (2)/09/2025-26). Informed consent in writing for the utilisation of the extracted teeth was secured from all contributors.

Sample size estimation and grouping

The sample size was estimated using G*Power software (version 3.1.9.7) with an effect size of 0.5 (Cohen d), a significance level (α) of 0.05, and a power ($1 - \beta$) of 0.90, which yielded a total of 60 specimens. The specimens were randomly allocated to three separate groups of 20 each: group I, 3% NaOCl (control); group II, fullerene nanoparticle irrigant; and group III, fullerene nanoparticle irrigant with diode laser activation.

Specimen selection

Human mandibular premolars with a single root, extracted for orthodontic indications, were from the Department of Oral and Maxillofacial Surgery at GDCRI, Bengaluru. Teeth selected were those with completely formed apices, straight canals with an open apical foramen and were free of caries, cracks or resorptive defects. Teeth with curved/calcified canals, fractures, immature apices or previous endodontic treatment were excluded.

Specimen preparation and biofilm formation

The 60 selected teeth were cleaned and disinfected to eliminate calculus and organic matter, subsequently preserved in a 0.1% thymol solution

and washed with distilled water before to utilisation. Standard endodontic access canals were created using high-speed diamond burs with a continuous water coolant. The working length was established 1 mm proximal to the location where a size 20 K-file initially was observed at the apical foramen. The canals were equipped with the ProTaper Universal rotary system (Dentsply Maillefer, Ballaigues, Switzerland) utilising the F3 finishing file (size 30, 0.09 taper). In the course of preparation, 2 mL of 3% NaOCl was utilised as an irrigant between files to facilitate debris elimination and temporary disinfection. Subsequent to the shaping process, each canal was flushed with 5 mL of distilled water to eliminate the NaOCl remnants, and the specimens underwent sterilisation via autoclaving at 121°C for 15 minutes. The canals were subsequently inoculated with a standardised suspension of *E. faecalis* (MTCC 9845; Microbial Type Culture Collection, Chandigarh, India) calibrated to 1.5×10^8 CFU/mL, corresponding to a 0.5 McFarland standard, and incubated at 37°C for 14 days to facilitate the development of mature biofilm within the root canals. Baseline intracanal specimens were obtained before irrigation to assess microbial counts before and after treatment.

Preparation of the fullerene nanoparticle irrigant.

To prepare the fullerene nanoparticle irrigant, an aqueous nanosuspension of polyvinylpyrrolidone was used as a solubilising and stabilising agent. Fullerene (C₆₀) powder (Stereo Rasayan, Nashik, Maharashtra, India) was slowly added to 0.5% aqueous polyvinylpyrrolidone solution with magnetic stirring. The mixture was ultrasonically dispersed with a probe sonicator (200 W, 20 kHz; Electrosonic Industries, Mumbai, Maharashtra, India) for 30 minutes to achieve uniform nanoparticle dispersion and to prevent aggregation. The prepared irrigant (20% w/v) was stored in amber glass bottles at room temperature to prevent photodegradation.

Irrigation protocols

Group I (NaOCl, control): all the canals were irrigated with 5 mL of 3% NaOCl for 5 minutes.

Group II (fullerene): the canals were irrigated with 5 mL of the fullerene (C₆₀) nanoparticle irrigant (20% w/v), prepared as an aqueous dispersion, which was left in the canal for 5 minutes without activation.

Group III (fullerene + laser): the canals were irrigated with 5 mL of the fullerene nanoparticle irrigant, followed by activation with a 450 nm diode laser (SOLASE Pro; LAZON Medical Laser, Shenyang, Liaoning, China) at a power of 0.2 W for an exposure time of 40 seconds per canal, delivered through a 200 µm optical fiber tip. The tip was inserted 1 mm short of the working length and

moved in a slow helical motion to allow uniform activation of the irrigant. Laser exposure activates the fullerene nanoparticles and induces the photodynamic production of ROS, thereby enhancing microbial killing.

Data collection and microbiological sampling

After irrigation procedures, the antimicrobial efficacy was microbiologically checked after a final flush with physiological saline. Samples of the canal contents were obtained by placing a sterile paper point in the canal for 1 minute and immediately transferred to a sterile vial containing 1 mL of saline. The vials were vortexed for 30 s to dislodge bacteria, and serial dilutions were plated on brain heart infusion agar. Colonies were counted after 48 hours of incubation at 37°C and expressed as CFU/mL, which is the bacterial reduction in each group.

Statistical analysis

Analysis was performed in IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), with significance accepted at $p < 0.05$. For each group, the mean and standard deviation (SD) were calculated. Baseline and post-treatment counts within a group were compared with the paired t-test; differences across the three groups were tested by one-way analysis of variance, with the Tukey honestly significant difference test used for pairwise post hoc comparison.

RESULTS

Post-treatment microbial counts

The NaOCl group showed the highest post-treatment microbial count among all groups (3.58 log₁₀ CFU/mL), with limited dispersion (SD 0.06) (Figure 1A). This was followed by the fullerene group, which showed a lower mean value (3.07 log₁₀ CFU/mL) with very low dispersion (SD 0.03) (Figure 1B). The fullerene + laser group showed the lowest microbial count (2.87 log₁₀ CFU/mL) but a slightly wider dispersion than the other groups (SD 0.11) (Figure 1C). Quantification of CFU indicated that laser-activated irrigation produced the greatest antimicrobial effect against *E. faecalis* (Table 1).

Comparison of microbial counts before and after treatment

When the mean CFU/mL values before and after the experiment were compared, the NaOCl group showed a marginal increase in bacterial counts after treatment, indicating an absence of antimicrobial activity; this change was not statistically significant ($p = 0.57$). The fullerene group showed a substantial reduction in CFU/mL of 68.08%, confirming its effectiveness in reducing *E. faecalis*. The greatest effect was observed in the fullerene + laser group, with a 79.64% reduction in microbial counts after treatment. The reductions in the fullerene and fullerene + laser groups were both

statistically significant ($p < 0.001$) (Table 1, Figures 2 and 3).

Comparison among the groups

On one-way analysis of variance, the three groups differed significantly in post-treatment $\log_{10}$ CFU/mL ($F = 211.92$, $p < 0.001$). Residual load was lowest in the fullerene + laser group and highest in the NaOCl group, with the fullerene group in between (Table 2).

Tukey post hoc testing showed that every pairwise difference in post-treatment $\log_{10}$ CFU/mL was significant ($p < 0.001$). The fullerene group showed significantly lower post-treatment values than the NaOCl group, indicating a superior antimicrobial effect of the fullerene irrigant over NaOCl. The fullerene + laser group showed significantly lower values than both the NaOCl and the fullerene groups, reflecting the greatest reduction in microbial load. The difference was most pronounced between the fullerene + laser and NaOCl groups, followed by the difference between the fullerene + laser and fullerene groups. Overall, these findings confirm that laser-activated irrigation achieved the lowest residual microbial counts, followed by the fullerene irrigant, whereas NaOCl showed the least antimicrobial efficacy among the tested interventions (Table 3).

DISCUSSION

This study was designed to establish how well a fullerene nanoparticle irrigant, applied with and without diode laser-mediated PDT, clears *E. faecalis* colonies in root canals. Laser-activated irrigation produced a superior outcome compared with the nanoparticle irrigant alone and with NaOCl, and the null hypothesis was therefore rejected. The fullerene nanoparticle irrigant also demonstrated a better antimicrobial effect than conventional NaOCl, which did not produce a satisfactory outcome and was associated with a marginal increase in microbial counts in the present study.

The absence of an antimicrobial effect in the NaOCl group merits comment. The specimens used here harbored 14-day mature biofilms, and the concentration (3%), volume (5 mL), and contact time (5 minutes) employed may have been insufficient to disrupt a biofilm of this maturity. The extracellular polymeric matrix of a mature *E. faecalis* biofilm consumes free available chlorine at its surface and limits penetration of NaOCl into the deeper layers, and the buffering capacity of dentin further reduces the availability of active chlorine. Passive irrigation without adjunctive agitation, as used in the control group, may therefore have delivered an insufficient concentration of active chlorine to the biofilm–dentin interface [4]. The marginal increase in counts recorded after NaOCl irrigation lies within the range of sampling and

plating variability and should not be interpreted as bacterial proliferation.

The results on laser-activated disinfection aligned with the current literature on the effectiveness of laser-mediated disinfection against *E. faecalis* in post RC treatments. Gutknecht et al. examined the antimicrobial activity of a 980 nm diode laser in root dentin and found a favourable result in diminishing *E. faecalis* [10]. Nagayoshi et al. examined the antibacterial capacity of diode lasers and discovered that the presence of a photosensitiser alongside laser irradiation lowered the survival of *E. faecalis* [11]. Hendi et al. indicated that diode lasers shown sufficient effectiveness against *E. faecalis* in root canals; however, the incorporation of silver nanoparticles with diode lasers resulted in a more significant elimination of *E. faecalis* [12]. da Silva et al. assessed the efficacy of antimicrobial photodynamic therapy utilising a low-level laser (indium gallium aluminium phosphide, 660 nm; 100 mW; 40 seconds) administered via a fiber-coupled optical system as a supplementary measure in endodontic procedures, noting its effectiveness against *E. faecalis* [8]. Schoop et al. employed a potassium titanyl phosphate laser to target *E. faecalis* within the root canal and discovered significant elimination, even in the deeper dentin layers [13]. The enhancement of endodontic results thru laser-assisted disinfection is attributed to multiple concurrent mechanisms employed by the laser - photothermal, photoacoustic, and photomechanical - all exhibiting antimicrobial properties; the heat generated denatures microbial proteins, disrupts membranes, and inactivates enzymes. Various laser modalities have been employed for the elimination of debris and smear layer from the canals, such as carbon dioxide (CO₂), neodymium-doped yttrium aluminium garnet (Nd:YAG), erbium-doped yttrium aluminium garnet (Er:YAG), and erbium, chromium-doped yttrium scandium gallium garnet (Er,Cr:YSGG) lasers. [15]. Diode lasers can, however, be considered ideal for laser-mediated root canal disinfection because their wavelengths of 810–980 nm penetrate deeper into the dentinal tubules [16]. They are semiconductor lasers that produce light when electrical energy passes through a diode made of gallium aluminum arsenide (GaAlAs), and they show a high affinity for pigmented and bacterial tissues but very low absorption in water [16]. They can also pass effectively through narrow and curved root canals by means of a flexible fiber-optic delivery system, which remains one of the challenges in endodontics [17]. Diode lasers are therefore clinically advantageous compared with other lasers in terms

of cost-effectiveness, ease of use, and antimicrobial efficacy.

The fullerene (C₆₀) nanoparticle irrigant exhibited satisfactory antimicrobial activity against *E. faecalis* post RCT, but the efficacy was more marked when the nanoparticles acted synergistically with the diode laser. Fullerenes are carbon-based nanoparticles that act as photosensitizers through light-activated ROS generation, leading to oxidative damage, membrane disruption, and effective biofilm penetration [7,18]. Nanoparticles in general damage the bacterial wall and penetrate the cell membrane, stimulating RO production and interrupting the replication of DNA through the controlled release of ions [19]. The distinctive mechanism of fullerenes as photosensitizers confers antimicrobial activity with a minimal risk of resistance; they have high chemical stability and are lipophilic, which favors effective penetration into biofilms. Fullerenes also provide a controlled antimicrobial effect that limits the toxicity index, and may therefore be regarded as superior to other nanoparticles, although they are less cost-effective [7]. Nanoparticles other than fullerenes that have been investigated include chitosan, silver, graphene, poly(lactic-co-glycolic acid), bioactive glass, mesoporous calcium silicate, hydroxyapatite, zirconia, glucose oxidase magnetic nanoparticles, copper, and zinc oxide [20].

Comparison with the literature

The present results, compared to recent studies of photodynamic root canal disinfection, clarify both their contribution and their limits (Table 4). Rfysh et al. inoculated palatal roots for 72 hours and reported complete eradication of *E. faecalis* (0 CFU/mL) with indocyanine green-mediated PDT and an 810 nm diode laser, an outcome better than that obtained with methylene blue, cetrimide or laser irradiation alone [21]. Higuchi *et al.*, using chitosan-coated indocyanine green-loaded nanospheres with a diode laser (810nm) in a porcine root canal model carrying a 21-day biofilm, recorded a maximum reduction of 1.89 log₁₀ [22]. Rostami *et al.* combined indocyanine green with an 808 nm diode laser and shockwave-enhanced emission photoacoustic streaming against a 2-week biofilm and found the combined protocol more effective than either modality used alone [23]. Among carbon-based nanomaterials, which are the closest structural analogues of fullerene, Jouhare *et al.* evaluated methylene blue-functionalized reduced graphene oxide against 21-day biofilms on 105 single-rooted teeth and reported that sonic and laser activation of the functionalized nanomaterial produced the greatest decrease in bacterial load, exceeding that obtained with methylene blue alone or with 5% NaOCl [24]. In a model closely comparable to the present one, Alkudairy and

AlRefeai used chitosan nanoparticles and Rose Bengal-chitosan activated by a diode laser as a final irrigant in 75 single-rooted mandibular premolars and reported favorable antibacterial results together with improved smear layer removal and push-out bond strength [25].

The reduction achieved in the present study (0.70 log₁₀; 79.64%) was accordingly smaller than that reported for the indocyanine green protocols, and several factors may account for the difference. The wavelength used here (450 nm) lies in the blue region of the spectrum, which is absorbed and scattered by dentin considerably more than the near-infrared wavelengths (808–810 nm) employed in the comparator studies, so the depth to which light reached fullerene molecules within the dentinal tubules was correspondingly limited. The irradiation time of 40 seconds per canal was also much shorter than the 5-minute exposure used by Higuchi *et al.* [22]. In addition, the biofilms in the present study were matured for 14 days, against 72 hours in the study of Rfyshet *al.* [21], and susceptibility to photodynamic inactivation decreases as biofilm architecture matures [3]. Finally, the concentration of the fullerene irrigant and its irradiation parameters have not yet been optimized, whereas indocyanine green protocols have been refined across many studies. Taken together, these differences in wavelength, irradiation time, and biofilm maturity, rather than an inherent limitation of fullerene as a photosensitizer, most plausibly explain why the present protocol did not match the complete eradication reported by Rfyshet *al.* [21] with indocyanine green, and they indicate that systematic optimization of the fullerene-mediated protocol is warranted before a direct comparison between the two approaches can be drawn.

Set against this, fullerene offers properties that dye-based photosensitizers do not. Fullerene (C₆₀) is substantially more photostable than indocyanine green or methylene blue, both of which undergo rapid photobleaching during irradiation, and it acts as an intrinsic photosensitizer with a high singlet oxygen quantum yield and low dark toxicity [6,18]. Whether these advantages translate into a clinical benefit will require comparisons matched for wavelength penetration, irradiation time, and photosensitizer concentration. Dentin discoloration, a recognized drawback of dye-based photosensitizers, was not assessed here and warrants evaluation for fullerene formulations.

Furthermore, conventional disinfectants such as NaOCl do not penetrate the dentinal tubules beyond approximately 100–200 µm, whereas laser irradiation has been reported to affect dentin to a depth of up to 1,000 µm [16]. Targeting microorganisms at that depth reduces the likelihood

of endodontic failure. Many nanoparticles have been used as effective disinfectants in dentistry, but the use of fullerene nanoparticles as a root canal irrigant remains novel.

Given the diverse mechanisms by which microorganisms resist disinfection, the need for advanced technologies has increased. Pairing fullerenes with a diode laser appears to act synergistically against *E. faecalis*: the laser photoactivates the nanoparticles, while its photothermal contribution reinforces the antibacterial action of the irrigant itself. This synergism may provide a more targeted and broad-spectrum antimicrobial action with promising applications in endodontics. The protocol, safety measures, and optimum irradiation parameters nevertheless require further standardization before widespread use. Fullerene nanoparticle-mediated PDT used in conjunction with diode lasers may serve as a promising adjunct to conventional root canal disinfection protocols, improving the disinfection of complex canal systems and potentially minimizing failures and improving clinical outcomes.

This study has several limitations. As an in vitro investigation using a monospecies biofilm, it may not reflect the polymicrobial nature of clinical endodontic infections. The control group was irrigated passively with NaOCl and no activation, which might result in an underestimate of the antimicrobial efficacy of current irrigation activation techniques. Further limitations to the clinical extrapolation of the results are variations in laser parameters and in the concentration of fullerene nanoparticles, the lack of evaluation of the penetration of dentinal tubules and of cytotoxicity, and the lack of long-term evaluation. Future studies should evaluate in vitro and clinical outcomes in parallel to facilitate assessment of clinical applicability. Reproducibility demands standardisation of laser parameters, nanoparticle concentrations and irradiation protocols. Employing multi-species biofilm models and cytotoxicity testing would improve the evidence base for clinical translation.

CONCLUSIONS

Despite the limitations of this in vitro study, application of fullerene nanoparticles combined with diode laser-mediated photodynamic therapy had a positive antimicrobial effect on *E. faecalis* in RC treated teeth as compared with the use of conventional sodium hypochlorite irrigation. The fullerene irrigant with the diode laser showed remarkable synergistic disinfecting action combining photodynamic and thermal effects. The synergistic action may, in turn, improve the outcome of endodontic treatment by more thoroughly clearing organisms from the canal

system. Following further longitudinal studies and standardization of the protocol, this photodynamic approach may be considered for implementation in routine day to day clinical practice.

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FIGURE

LEGENDS

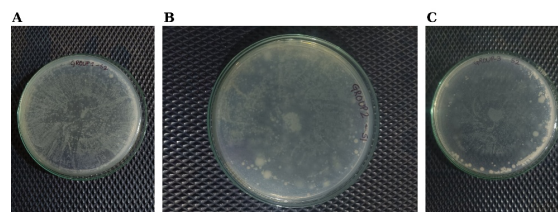


Figure 1. Culture plates showing post-treatment growth of *Enterococcus faecalis*. (A) Sodium hypochlorite group, with the highest post-treatment microbial count (3.58 log₁₀ CFU/mL). (B) Fullerene nanoparticle group (3.07 log₁₀ CFU/mL). (C) Fullerene nanoparticle group with diode laser activation, with the lowest post-treatment microbial count (2.87 log₁₀ CFU/mL). CFU, colony-forming

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units.

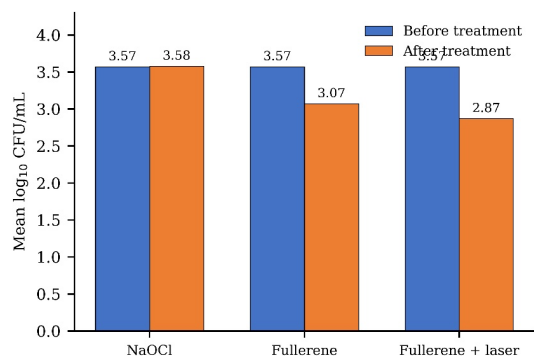


Figure 2. Mean log₁₀ CFU/mL before and after treatment in the 3 experimental groups. Blue bars, before treatment; orange bars, after treatment. CFU, colony-forming units; NaOCl, sodium hypochlorite.

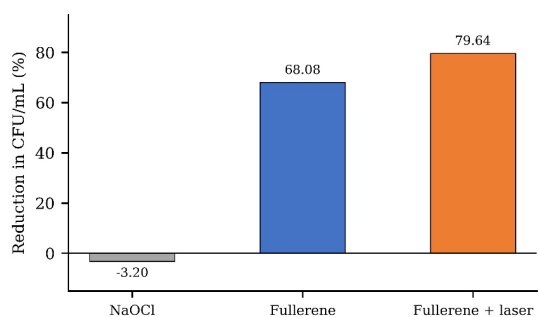


Figure 3. Percentage reduction in CFU/mL after treatment in the 3 experimental groups. A negative value indicates an increase in microbial count. CFU, colony-forming units; NaOCl, sodium hypochlorite.

TABLES

Table 1. Baseline and post-treatment log₁₀ CFU/mL, percentage reduction, and paired t-test comparison by group.

Group	Mea	Mea					
	n	n	Mean				
	log ₁₀	log ₁₀	differ	Redu	t	d	p
	CFU	CFU	ence	ction	val	f	val
	/mL	/mL	(log ₁₀	(%)	ue		ue
	(befo	(afte	)				
	re)	r)					
NaO							
Cl							
(cont	3.57	3.58	-0.01	-3.20	-0.1	1	0.5
rol)					59	9	7

Group	Mea	Mea					
	n	n	Mean				
	log ₁₀	log ₁₀	differ	Redu	t	d	p
	CFU	CFU	ence	ction	val	f	val
	/mL	/mL	(log ₁₀	(%)	ue		ue
	(befo	(afte	)				
	re)	r)					
Fullerene	3.57	3.07	0.50	68.08	42.30	19	<0.001 ^a
Fullerene + laser	3.57	2.87	0.70	79.64	20.80	19	<0.001 ^a

CFU, colony-forming units; NaOCl, sodium hypochlorite; df, degrees of freedom. A negative value for reduction indicates an increase in microbial count.

^a)Statistically significant (p < 0.05).

Table 2. Comparison of post-treatment log₁₀ CFU/mL among the 3 groups (one-way analysis of variance).

Group	Mean					
	log ₁₀	SD	95% CI	F value	p value	
	CFU/m					
	L					
NaOCl (control)	3.58	0.06	3.53 – 3.63	211.92	<0.001 ^a	
Fullerene	3.07	0.03	3.05 – 3.09			
Fullerene + laser	2.87	0.11	2.79 – 2.94			

CFU, colony-forming units; SD, standard deviation; CI, confidence interval; NaOCl, sodium hypochlorite.

^a)Statistically significant (p < 0.05).

Table 3. Post hoc pairwise comparison of post-treatment log₁₀ CFU/mL (Tukey honestly significant difference test).

Group comparison	Mean difference (log ₁₀)	95% CI	p value
NaOCl vs. fullerene	0.51	0.45–0.57	< 0.001 ^a
NaOCl vs. fullerene + laser	0.71	0.61–0.81	< 0.001 ^a
Fullerene vs. fullerene + laser	0.20	0.13–0.27	< 0.001 ^a

CFU, colony-forming units; CI, confidence interval; NaOCl, sodium hypochlorite.

^aStatistically significant (p < 0.05).

Table 4. Comparison of the present findings with recent studies of photodynamic root canal disinfection against *Enterococcus faecalis*.

Study (year)	Photosensitizer or nanoparticle	Light source	Biofilm model	Main antimicrobial outcome
Rfyshet <i>et al.</i> (2025) [21]	Indocyanine green	810 nm diode laser	72 hours; human palatal roots	Complete eradication (0 CFU/mL)
Higuchi <i>et al.</i> (2021) [22]	Chitosan-coated indocyanine green-loaded nanospheres	810 nm diode laser, 2.1 W, 5 min	21 days; porcine root canal model	Maximum reduction of 1.89 log ₁₀
Rostami <i>et al.</i> (2023) [23]	Indocyanine green, with or without	808 nm diode laser	2 weeks; human single-	Combined protocol more

Study (year)	Photosensitizer or nanoparticle	Light source	Biofilm model	Main antimicrobial outcome
			SWEEPS	canal teeth effective than either modality alone
Jouharet <i>al.</i> (2025) [24]	Methylene blue-functionalized reduced graphene oxide	Laser with sonic activation	21 days; 105 single-rooted teeth	Greatest reduction with sonic plus laser activation, exceeding 5% NaOCl Favorable
Alkhudairy and AlRefeai (2024) [25]	Chitosan nanoparticles and Rose Bengal-chitosan	Diode laser	75 single-rooted mandibular premolars	antibacterial effect with improved smear layer removal
Present study	Fullerene (C ₆₀) nanoparticles, 20% w/v	450 nm diode laser, 0.2 W, 40 s	14 days; 60 human mandibular premolars	Reduction of 0.70 log ₁₀ (79.64%)

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CFU, colony-forming units; NaOCl, sodium hypochlorite; SWEEPS, shockwave-enhanced emission photoacoustic streaming.