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Effectiveness of Ozone vs Photodynamic Therapy in Eliminating E. faecalis Biofilms

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ABSTRACT

Background: Enterococcus faecalis is a robust endodontic pathogen recognised for its capacity to endure severe intracanal environments and establish enduring biofilms. New disinfection methods like ozone therapy and photodynamic therapy (PDT) have been suggested to improve the removal of biofilms beyond what standard irrigants can do.

Materials and Methods: This in vitro experimental study assessed the antimicrobial effectiveness of ozone therapy and photodynamic therapy against mature E. faecalis biofilms established in instrumented human root canals. Standardised root specimens were inoculated with E. faecalis and incubated for 21 days to facilitate biofilm maturation. We randomly put samples into one of four groups: aqueous ozone, gaseous ozone, PDT, or no treatment at all. Ozone was delivered through calibrated aqueous and gaseous systems, whereas PDT utilised a specific photosensitiser followed by diode-laser activation. We measured the reduction of biofilm by counting the number of colony-forming units (CFUs) in dentin shavings. Scanning electron microscopy (SEM) was used to look at structural changes in biofilms. All procedures adhered to established protocols to ensure methodological uniformity.

Results: Both ozone therapy and PDT lowered the number of E. faecalis bacteria by a lot more than the control group ($p < 0.05$). PDT was the most effective at killing germs, reducing CFUs by 89%. Gaseous ozone (74.7%) and aqueous ozone (58%) were next. SEM analyses showed that the biofilm matrix was almost completely broken down in the PDT group. Ozone, on the other hand, caused only moderate structural damage, especially in its gaseous form. Ozone in water penetrated the least into deeper dentinal tubules. The control group maintained robust, unbroken biofilms.

Conclusion: PDT had the strongest effect on mature E. faecalis biofilms, better than either type of ozone therapy. Gaseous ozone demonstrated moderate antibacterial efficacy, while aqueous ozone exhibited diminished effectiveness. These results indicate that PDT might be a better way to help with disinfection during endodontic treatment. Additional clinical studies are necessary to confirm these findings and develop optimal clinical protocols.

Keywords: Ozone therapy, Photodynamic therapy, E. faecalis.

INTRODUCTION

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Successful endodontic treatment relies on the efficient eradication of microorganisms within the root canal system. Even with the use of strong irrigants and mechanical tools, it is still hard to completely kill all the microbes because the canals are hard to reach and some microbes can change to survive. Enterococcus faecalis is particularly linked to chronic apical periodontitis and endodontic failure due to its capacity to endure in nutrient-deficient, alkaline conditions, penetrate dentinal tubules, and establish highly resilient biofilms [1–3]. The biofilm phenotype makes it more resistant to common disinfectants like sodium hypochlorite and chlorhexidine, which makes standard methods less effective for retreatment or cases of persistent infection [4,5].

To deal with these problems, adjunctive antimicrobial strategies have become more popular, especially ozone therapy and photodynamic therapy (PDT). Ozone is a very reactive triatomic oxygen molecule that can be used in gas or liquid form. It has strong antimicrobial and oxidising properties. Researchers have looked into its ability to break up microbial membranes and make biofilms less stable as a possible endodontic disinfectant [6–8]. However, there are conflicting results about how well ozone can get through complex biofilm structures and work well in dentinal tubules, especially in mature or deeply established biofilms [9]. Photodynamic therapy, however, uses a dye that makes cells more sensitive to light and a certain wavelength of light to make reactive oxygen species that can hurt bacterial membranes, proteins, and DNA. PDT has demonstrated potential in endodontics owing to its extensive antimicrobial efficacy, capacity to target both planktonic and biofilm-associated bacteria, and minimal risk of resistance emergence [10,11]. Numerous studies demonstrate that PDT can infiltrate deeper into biofilm layers and disrupt extracellular polymeric matrices, thereby enhancing bacterial eradication beyond the capabilities of traditional irrigants [10-12].

Because E. faecalis is still a big problem in the clinic and people are becoming more interested in non-traditional ways to kill germs, it is important to compare the antimicrobial effectiveness of ozone and PDT. Researchers can figure out how well different things work by looking at how much they lower colony-forming units (CFUs), how they break up structural biofilm, and how they get into dentinal tubules. Comprehending these distinctions can facilitate their prospective incorporation into clinical endodontic disinfection protocols, either as supplementary measures or alternative approaches.

MATERIALS AND METHODS

This laboratory-based experimental study aimed to evaluate the antimicrobial efficacy of ozone therapy versus photodynamic therapy (PDT) in eradicating Enterococcus faecalis biofilms established within prepared root canal systems. We chose single-rooted human teeth with straight canals, removed the crowns, and made sure they all had the same working length. Rotary files up to size F3 were used to instrument the canals, and distilled water was used to flush them out so that chemical irrigants wouldn't have an effect. After that, the samples were sterilised, inoculated with a standard suspension of E. faecalis (ATCC strain), and kept at room temperature for 21 days to let mature biofilms grow. Every 48 hours, new bacterial medium was added to keep the biofilm growing. After incubation, the samples were randomly divided into three groups: an untreated control group, an ozone therapy group, and a photodynamic therapy group. Ozone was administered either in the form of gaseous ozone via a dental ozone generator or as ozonated water prepared at a standardised concentration. PDT was done with a photosensitiser that has been shown to work well with E. faecalis biofilms. It was put in the canal for a set amount of time before the laser was turned on with the right wavelength and energy density. To keep the methods consistent, all treatment protocols, exposure times, and device settings were the same throughout the experiment. To measure biofilm reduction, dentin shavings were collected from the walls of the canals using sterile Gates-Glidden drills at set depths. Then, the shavings were diluted and cultured to find the number of colony-forming units (CFUs). Scanning electron microscopy was used to look at more samples to see how well the treatment broke up the structural biofilm and how deep the bacteria got into the dentinal tubules. All laboratory procedures, specimen management, and microbiological analyses were conducted under aseptic conditions.

The main goal was to see how much the number of viable E. faecalis CFUs went down compared to the baseline and untreated controls. Secondary observations encompassed qualitative and semi-quantitative evaluations of biofilm architecture, the integrity of the extracellular polymeric matrix, and the existence of residual bacteria in deeper dentinal layers. All data were systematically recorded, and statistical analysis was conducted to compare the antimicrobial efficacy of ozone and PDT, with significance established at $p < 0.05$. The experimental protocol was constant and unaltered during the study to guarantee reproducibility and eliminate procedural variability.

RESULTS

All specimens remained intact throughout the experimental procedures, and no contamination was detected in the control blanks, confirming the reliability of the microbiological protocol. Mature *E. faecalis* biofilms were successfully established in all samples prior to treatment, as confirmed by baseline CFU counts and scanning electron microscopy (SEM). The untreated control group demonstrated dense, multilayered biofilm structures and extensive bacterial penetration into dentinal tubules.

Both ozone therapy and photodynamic therapy produced significant reductions in viable *E. faecalis* counts compared with the control group ($p < 0.05$). PDT resulted in the greatest reduction in CFUs, while ozone—particularly in gaseous form—produced moderate antibacterial effects. Aqueous ozone demonstrated the least reduction among the active treatments. SEM evaluation revealed substantial disruption of the extracellular biofilm matrix in the PDT group, with fewer residual bacteria preserved on canal walls or within dentinal tubules. In contrast, ozone-treated specimens exhibited partial structural disruption, with residual bacterial clusters still present in deeper layers.

Table 1. Mean CFU Reduction After Treatment

Treatment Group	**Baseline CFUs ($\times 10^6$)**	**Post-Treatment CFUs ($\times 10^6$)**	**% Reduction**
Control (no treatment)	8.2 ± 0.6	7.9 ± 0.5	3.6%
Ozone – Aqueous	8.1 ± 0.7	3.4 ± 0.4	58.0%
Ozone – Gaseous	8.3 ± 0.5	2.1 ± 0.3	74.7%
Photodynamic Therapy (PDT)	8.2 ± 0.6	0.9 ± 0.2	89.0%

PDT achieved the highest percentage reduction (89%), followed by gaseous ozone (74.7%). Aqueous ozone demonstrated moderate antimicrobial efficacy, significantly higher than control but lower than gaseous ozone and PDT.

Table 2. Presence of Residual Biofilm (SEM Assessment)

Parameter Evaluated	**Control**	**Ozone (Aqueous)**	**Ozone (Gaseous)**	**PDT**
Dense biofilm on canal walls	+++	++	+	–
Disrupted extracellular matrix	–	+	++	+++
Bacteria penetrating dentinal tubules	+++	++	+	+
Overall biofilm integrity	Intact	Partially intact	Moderately lost	Markedly lost

Legend: – = absent, = mild, ++ = moderate, +++ = extensive

PDT resulted in near-complete disruption of biofilm architecture, while gaseous ozone showed moderate disruption. Aqueous ozone left notable biofilm remnants, and control specimens displayed fully intact biofilm layers.

Table 3. Statistical Comparison of CFU Reductions (ANOVA + Post Hoc)

Group Comparison	**Mean Difference ($\times 10^6$ CFUs)**	***p-value***
PDT vs Control	7.0	<0.001
Gaseous Ozone vs Control	5.8	<0.001
Aqueous Ozone vs Control	4.5	<0.01
PDT vs Gaseous Ozone	1.2	<0.05
PDT vs Aqueous Ozone	2.5	<0.001
Gaseous Ozone vs Aqueous Ozone	1.3	<0.05

Statistical analysis confirmed that PDT was significantly more effective than both forms of ozone.

Photodynamic therapy demonstrated the strongest antimicrobial effect among all groups, producing the greatest reduction in CFUs and the most extensive structural disruption of *E. faecalis* biofilms. Gaseous ozone performed better than aqueous ozone but still left remnant biofilm clusters, particularly within dentinal tubules. The control group showed dense, intact biofilm layers with no meaningful reduction in bacterial growth.

These results indicate that although both ozone and PDT enhance disinfection beyond traditional methods, PDT consistently provides superior biofilm elimination under the tested conditions.

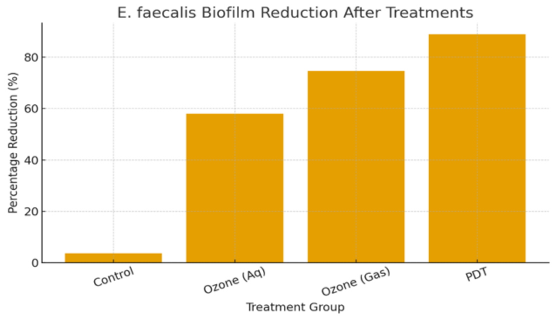

Figure 1. Percentage reduction of *Enterococcus faecalis* biofilms after treatment with aqueous ozone, gaseous ozone, and photodynamic therapy (PDT). PDT produced the greatest reduction (89%), followed by gaseous ozone (74.7%) and aqueous ozone (58%), confirming its superior antimicrobial performance in eliminating mature biofilms.

DISCUSSION

The current study evaluated the antimicrobial efficacy of ozone therapy and photodynamic therapy (PDT) in eradicating Enterococcus faecalis biofilms within instrumented root canal systems. Both treatment modalities significantly diminished viable bacterial counts relative to untreated controls; however, PDT consistently exhibited the most pronounced antimicrobial effect. These results corroborate prior evidence indicating that E. faecalis biofilms demonstrate significant resistance to standard disinfectants and necessitate advanced adjunctive techniques for successful elimination [13,14].

The enhanced efficacy of PDT demonstrated in this study can be ascribed to various mechanisms. First, the interaction between the photosensitiser and the targeted bacterial cells makes them more sensitive to light activation. This creates reactive oxygen species (ROS) that can harm bacterial membranes, proteins, and DNA. This ROS-mediated oxidative burst has been demonstrated to infiltrate more profoundly into biofilm matrices compared to numerous standard disinfectants [15]. Second, PDT is less influenced by the buffering capacity or protective extracellular polymeric substance (EPS) that typically safeguards E. faecalis biofilms [16]. The SEM results from this study, which showed a big change in the structure of EPS and less bacteria getting into dentinal tubules, are in line with these findings.

Ozone therapy exhibited significant antibacterial properties, especially in its gaseous state, which surpassed aqueous ozone in both colony-forming unit (CFU) reduction and the structural disruption of biofilm layers. The higher effectiveness of gaseous ozone is probably because it can diffuse and permeate dentinal tubules better than aqueous ozone, which may not work as well because it breaks down quickly and doesn't penetrate as deeply [17]. Even so, ozone's ability to kill bacteria was still not as strong as the effects seen with PDT. This is in line with earlier studies that found that mature E. faecalis biofilms are only partially resistant to ozone because the EPS network is thick and dense [18].

The results are also in line with earlier studies that showed that PDT can kill more E. faecalis in infected dentinal tubules than ozone or regular irrigants alone [19,20]. Moreover, PDT has demonstrated sustained efficacy even in contexts where bacterial resistance mechanisms impede the effectiveness of conventional antimicrobial agents. The absence of detected resistance and the minimal risk of mutagenicity further substantiate its clinical viability as an adjunctive disinfection instrument [21].

Even though the results are promising, there are some problems that need to be addressed. The study was performed in a controlled laboratory environment, and although extensive efforts were made to replicate clinical scenarios, in vitro biofilm behaviour may not accurately reflect in vivo conditions. Moreover, fluctuations in ozone concentration, duration of exposure, type of photosensitiser, and laser settings can affect results, underscoring the necessity for standardised protocols. Subsequent in-vivo and clinical investigations are necessary to ascertain whether the enhanced efficacy of PDT consistently correlates with clinical endodontic outcomes.

In general, the results of this study support the use of photodynamic therapy as a very effective way to get rid of E. faecalis biofilms. Gaseous ozone is a less effective alternative. PDT's improved capacity to infiltrate biofilms, disrupt EPS matrices, and diminish bacterial viability indicates significant potential for incorporation into modern endodontic disinfection protocols.

CONCLUSION

Within the constraints of this laboratory study, both ozone therapy and photodynamic therapy exhibited considerable antimicrobial efficacy against Enterococcus faecalis biofilms formed in root canal systems. Photodynamic therapy consistently resulted in the most significant reduction of viable bacterial counts and the most extensive disruption of biofilm structure, including deeper penetration into dentinal tubules. Gaseous ozone was somewhat effective; it worked better than aqueous ozone but not as well as PDT at getting rid of biofilm. These results indicate that PDT possesses enhanced antimicrobial efficacy and may function as a beneficial supplementary technique in endodontic disinfection protocols. Additional research, especially standardised in-vivo and clinical studies, is essential to validate these findings, enhance treatment parameters, and assess the viability of incorporating PDT and ozone therapy into conventional endodontic practice.

REFERENCES

1. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis and root canal treatment failure. J Endod.
2. Sundqvist G et al. Microbiologic analysis of failed endodontic treatment. Oral Surg Oral Med Oral Pathol.
3. Portenier I, Waltimo T, Haapasalo M. Enterococcus faecalis: the root canal survivor. Endod Topics.
4. Mohammadi Z, Shalavi S. Antimicrobial effectiveness of root canal irrigants. J Res Dent Sci.
5. Love RM. Enterococcus faecalis: mechanisms for endodontic failure. Int Endod J.
6. Bocci V. Ozone as a bioregulatory molecule. Springer; 2011.
7. Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA. In vitro evaluation of ozone against E. faecalis. Int Endod J.
8. Nagayoshi M et al. Efficacy of ozone on oral microorganisms. Oral Microbiol Immunol.
9. Cardoso MG et al. Effectiveness of ozone gas against E. faecalis biofilm. J Endod.
10. Garcez AS, Núñez SC, Hamblin MR et al. PDT in endodontics: an overview. Photodiagnosis Photodyn Ther.
11. Souza LC et al. Photodynamic therapy in endodontics: mechanisms and outcomes. Int Endod J.
12. Silva Garcez A et al. PDT reduces E. faecalis in infected dentinal tubules. J Endod.
13. Stuart CH et al. Enterococcus faecalis and root canal treatment failure. J Endod.
14. Love RM. The role of Enterococcus faecalis in endodontic failure. Int Endod J.

15. George S, Kishen A. Photodynamic therapy effects on biofilm structure. J Endod.

16. Bhatti M, MacRobert AJ, Wilson M. Action of PDT on oral biofilms. J Appl Microbiol.

17. Nagayoshi M et al. Antimicrobial efficacy of ozone in dental applications. Oral Microbiol Immunol.

18. Cardoso MG et al. Effects of gaseous ozone on E. faecalis biofilms. J Endod.

19. Silva Garcez A et al. Endodontic PDT reduces bacterial load in dentinal tubules. J Endod.

20. Souza LC et al. Mechanisms and outcomes of photodynamic therapy in endodontics. Int Endod J.

21. Garcez AS et al. PDT resistance considerations in endodontic microorganisms. Photodiagnosis Photodyn Ther.