EUROPEAN ENDODONTIC JOURNAL

ORIGINAL ARTICLE

Effectiveness of Laser-activated Irrigation Modalities on Intracanal Bacterial Elimination and Apical Extrusion

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ABSTRACT

Objective: This study aimed to compare photon-induced photoacoustic streaming (PIPS) and diode laser with passive ultrasonic irrigation (PUI) in activating intracanal irrigants for bacterial elimination from the root canal and apical extrusion.

Methods: Sixty extracted single-canal human teeth were chemo-mechanically prepared and placed in 3 ml glass vials with sterile 0.9 % sodium chloride solution. The root canals were inoculated with *Enterococcus faecalis* and incubated for 24 hours at 37°C. The samples were divided into three experimental groups (PIPS, diode laser, and PUI) and a control group (n=15 each). The experimental groups had root canals filled with saline and activated according to the manufacturer's instructions, while the control group received saline without activation. Bacterial samples were collected from the canals and outside the apex for quantification, cultured on nutrient agar for 24 hours at 37°C, and counted as colony-forming units. Mean values were compared using one-way ANOVA and Bonferroni tests at 5 % significance.

Results: All activation protocols significantly eliminated intracanal *E. faecalis* compared to the negative control group ($p<0.05$). PUI and the diode lasers were significantly more effective than PIPS ($p<0.05$). Extruded bacteria were higher in PUI than in PIPS and diode lasers.

Conclusion: Within the limitations of this study, the tested techniques extruded bacteria and did not completely eliminate intracanal bacteria. The diode laser showed the best bacterial elimination and extrusion outcome.

Keywords: Diode laser, laser-activated irrigation, passive ultrasonic irrigation, photon-induced photoacoustic streaming, root canal disinfection

Please cite this article as:
Abbas MM, Yadadi SS, Sulaiman A, Mahmoud O, Jamleh A, Al Shehadat S. Effectiveness of Laser-activated Irrigation Modalities on Intracanal Bacterial Elimination and Apical Extrusion. Eur Endod J 2025; 10: 35-40

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Received : July 26, 2024,
Revised : September 16, 2024,
Accepted : October 07, 2024

Published online: February 19, 2025
DOI 10.14744/eej.2024.36450

HIGHLIGHTS

- This study compared the effectiveness of PIPS, diode laser, and PUI in eliminating bacteria and preventing extrusion.
- Laser-activated irrigation offers promising results in root canal disinfection.
- PUI and diode laser showed superior disinfection performance compared to PIPS.
- Diode laser and PIPS caused less bacterial extrusion compared to PUI.

INTRODUCTION

Effective irrigation of the root canal system is essential for the success of root canal treatment. However, traditional irrigation techniques often fail to adequately deliver irrigation throughout the canal system, even after sufficient canal shaping, which can lead to a significant proportion of untouched root canal surface area (1, 2). To overcome this shortcoming, activating the irrigant might facilitate the cleanliness of the

root canals by using sonic devices, ultrasonic devices, and lasers (3). Additional methods are therefore required to enhance irrigant penetration into the root canal system, enabling them to reach previously inaccessible regions. Several methods of agitating endodontic irrigants are available to enhance root canal disinfection and smear layer removal. Among the most well-documented are manual dynamic activation, sonic activation, passive ultrasonic irrigation (PUI), and laser-activated irrigation (LAI) (4, 5). An optimal irrigation protocol should chemically and physically disinfect the root canal system by eradicating the planktonic bacteria and biofilm (6).

PUI was introduced in endodontics by Weller et al. (7), who utilised a metal instrument at a 30 kHz ultrasonic frequency. The instrument is passively placed inside the root canal filled with irrigant and activated to agitate the irrigant, leading to acoustic streaming and cavitation (8). It has demonstrated superior effectiveness compared to many other techniques and has consequently gained widespread adoption in endodontic practice (9, 10). However, it has potential adverse effects on the structure, particularly when it touches dentine and is used for prolonged periods or with excessive power (11). Furthermore, PUI is more technique-sensitive than other irrigation techniques, requiring careful handling to prevent complications such as instrument deformation, instrument separation, or extrusion of irrigants into periapical tissues (12).

Laser-activated irrigation (LAI) has been proposed as an adjunct in canal disinfection to maximise smear layer removal and improve canal disinfection (13, 14). It may also be an effective adjunct in reducing postoperative pain compared to needle irrigation (15). LAI includes erbium-doped yttrium aluminium garnet laser (Er:YAG) lasers and diode lasers. Er:YAG lasers, particularly through photon-induced photoacoustic streaming (PIPS), use laser energy to generate acoustic waves that agitate the irrigant, improving its flow within the root canal system. This technique, utilising low-energy (20 mJ) and short-pulse durations (50 µs), enhances disinfection and debris removal (16). Studies have shown that the PIPS allows the irrigant to reach challenging areas more effectively, resulting in fewer apical bacteria and biofilm than ultrasonic activation (17).

Similarly, diode lasers generate acoustic waves through laser energy to promote fluid agitation, aiding in the disinfection and cleaning of the root canal system. Diode lasers have shown promising results in improving root canal disinfection and reducing bacterial load (18). However, the available evidence has not adequately addressed the efficacy and safety of PIPS and diode lasers. Thus, this study investigated the effectiveness of PIPS and diode lasers used in endodontic irrigation activation in terms of bacterial elimination from the root canal and bacterial extrusion. The null hypothesis is that PIPS, diode lasers, and PUI have comparable effectiveness as irrigation activation devices.

MATERIALS AND METHODS

Sample Preparation

This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee at the University of Sharjah (REC-21-10-12-S). Sound teeth with single, straight, and round canals were obtained from a pool of teeth extracted at the University Dental Hospital Sharjah for unknown reasons. The teeth were cleaned of debris and subjected to buccal and proximal periapical radiographs to confirm their suitability for the study. A stereomicroscope (Leica Wild M420, Bensheim, Germany) at 10× magnification was used to ensure further that the inclusion criteria were met. Only intact, single-rooted lower premolar teeth with straight, round roots were included in this study. Teeth with multiple canals, curved roots (>30°), caries, restorations, root resorption, cracks, calcified canals, or large foramina were excluded. The selected samples were then stored in physiological buffered saline solution (PBS) at +4°C.

The sample size was calculated based on Cochran's formula for sample size determination to achieve 80% power for the study, with the level of significance set at 5%. Mittal et al. (19) were used as the reference study, and the calculations determined that 60 samples should be included.

Endodontic access cavities were made using the Dentsply access cavity set (Dentsply Maillefer, Ballaigues, Switzerland). The sample crowns were trimmed to a length of 17 mm to standardise the length and create a stable reference point. Chemo-mechanical preparation was performed using the ProTaper Gold system (Dentsply Sirona, Ballaigues, Switzerland) up to the F4 file following the manufacturer's instructions. Each tooth was irrigated with 5 ml of 5.25% sodium hypochlorite during the preparation. To minimise microleakage via the lateral canals during the experiment, the external root surfaces were coated with two layers of nail varnish (Rimmel, London, UK). A size 15 K-file was extended 1 mm beyond the apex to penetrate the varnish layer and maintain apical patency. A single operator performed all chemical and mechanical preparation steps for all groups.

The samples were individually placed in glass vials (Global Shopping LLC, New Jersey, USA), with the cementoenamel junction level fixed using light-cured flowable composite (Aelite Flo, Illinois, USA) through holes in a rubber lid (Fig. 1). The entire setup was then sterilised using an autoclave device (Hiclave HV-50, Saitama, Japan) at 121°C for 30 min at a pressure of 0.1 MPa.

Root Canal Contamination

The bacterial suspension was prepared by culturing 1 ml of a pure culture of (*Enterococcus faecalis*) *E. faecalis* (ATCC 29212, American Type Culture Collection, University Boulevard, Manassas, USA) in brain-heart infusion broth for 24 hours. Subsequently, 0.5 ml of this culture was diluted into brain-heart infusion broth to achieve a McFarland concentration of 0.5.

Subsequently, 15 µl of the *E. faecalis* suspension was carefully introduced into the root canal using a sterile pipette. To ensure the uniform distribution of bacteria along the canal, a size 10 K-file was used to gently agitate the suspension throughout the entire length of the canal (16). The contaminated samples were then aerobically incubated at 37°C for 24 hours to promote bacterial growth and colonisation.

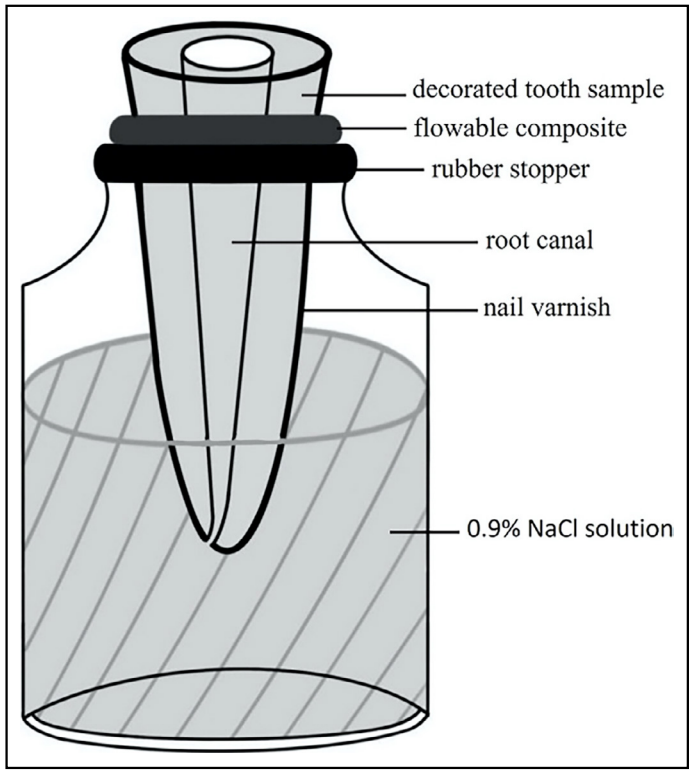

Figure 1. Diagram for the setup of the test apparatus. Sample teeth were inserted into the glass containers through the rubber stopper and fixed using a flowable composite. Each glass container was filled with 1.2 ml of 0.9 % NaCl solution before conducting the experiments

Experimental Irrigation Protocols

The samples were randomly and equally divided into three experimental groups (n=15) based on the irrigant activation protocol: the PIPS, diode, and PUI groups. Additionally, 15 infected teeth, which did not undergo any irrigation activation, served as the negative control group. On the day of the experiment, each glass vial was filled with 1.2 ml of normal saline (Fig. 1). Each sample in the experimental groups was irrigated with 3 ml of normal saline and then activated using PIPS, diode laser, or PUI. The specific parameters for all activation protocols are detailed in Table 1.

The PIPS protocol was performed using a Fotona laser machine (2940 nm wavelength) with a dedicated fibre optic tip (size 600/9) placed at the pulp chamber level. The settings were adjusted to 0.3 W, 15 Hz, and 20 mJ, and the procedure was run without water- or air-cooling for two 30-second cycles following the manufacturer's guidelines.

The novel diode LAI protocol utilised an 810 nm Claros nano diode laser (Elexxion, Singen Hohentwiel, Germany), set to 1 Watt in continuous wave firing mode. A 200 µm fibre optic tip was placed 4 mm short of the working length and retracted in a spiralling motion for three 10-second cycles following the manufacturer's guidelines. The procedure was run without water or air cooling.

The PUI activation utilised a 45 kHz Ultra X cordless ultrasonic device (Eighteeth, Changzhou, China). The ultrasonic tip was inserted 2 mm short of the working length and moved up and down to allow it to vibrate freely for three 30-second cycles. Following each cycle, the irrigant was refreshed to maintain effectiveness. Samples in the control group served as negative controls receiving the *E. faecalis*, but no irrigation was made.

Bacterial Sampling

After completing the experimental irrigation protocols, bacterial samples were collected from intra-canal and extra-canal sources for all groups. To collect intra-canal bacteria, a sterile size 20 paper point was inserted into each root canal to the working length and left in place for 5 minutes. The paper point was then aseptically removed and placed into an Eppendorf tube containing 50 µl of PBS. For the extra-canal bacterial sampling, 5 µl of the normal saline in the glass vials was transferred to an Eppendorf tube containing 45 µl of PBS. The samples were then vortexed for 30 seconds and serially diluted to 10–2. Next, 5 µl aliquots of each diluted sample were plated onto a nutrient agar medium. The plates were incubated for 24 hours at 37°C, and bacterial colonies were counted using a colony counter. The results were reported as colony-forming units (CFU). All procedures were performed in an aseptic environment.

Statistical Analysis

The CFU data were converted to base-10 logarithms. Since the Shapiro–Wilk test showed the data were normally distributed, a one-way analysis of variance and post hoc Bonferroni tests were conducted at a 5% significance level using SPSS version 24.0 (IBM, Armonk, NY, USA).

RESULTS

All methods significantly reduced the intra-canal counts of *E. faecalis* compared with the negative control; however, none achieved complete eradication (Table 2). PUI and the diode laser showed superior disinfection performance over PIPS ($p<0.05$). However, there were no significant differences in bacterial reduction between the PUI and Diode lasers ($p>0.05$), as shown in (Table 3).

Bacterial extrusion was observed in all samples from the experimental groups, with significantly higher levels than in the control group ($p<0.05$). Post hoc analysis revealed that the PIPS and diode laser groups exhibited significantly low-

TABLE 1. Protocols for each activation system used in the present study

Protocol	No. of cycles (time in seconds)	Tip placement	Power (Watt)	Frequency (Hz)	Wavelength (nm)
PIPS	2 (30)	Coronal access cavity	0.3	15	2940
Diode	3 (10)	4 mm short of the apex	1	0 (continuous)	810
PUI	3 (30)	2 mm short of the apex	N/A	45×10^3	N/A

PIPS: Photon-induced photoacoustic streaming (9), PUI: Passive ultrasonic irrigation (14)

TABLE 2. Result of the Log CFU comparison using One-way ANOVA

	Activation technique	Mean (SD)	F (df)	p
Intra-canal	Negative control	6.1 (0.5)	107.001	
	PUI	2.75 (0.41)	(3, 56)	<0.001*
	Diode	2.1 (0.37)		
	PIPS	3.81 (0.54)		
Extra-canal	Negative control	0.05 (0.05)	94.599	
	PUI	3.835 (0.81)	(3, 56)	<0.001*
	Diode	1.692 (0.7)		
	PIPS	2.245 (0.63)		

*: Significant at the level 0.05. Normality assumption is fulfilled. CFU: Colony-forming units, SD: Standard deviation, PIPS: Photon-induced photoacoustic streaming (9), PUI: Passive ultrasonic irrigation (14)

TABLE 3. Result of the Post Hoc Bonferroni multiple comparisons of the Log CFU

	(I) Activation technique	(J) Activation technique	MD (I-J) (95 %CI)	p
Intra-canal	Negative control	PUI	3.39	<0.001*
		Diode	4.04	<0.001*
		PIPS	2.33	<0.001*
	PUI	Negative control	-3.39	<0.001*
		Diode	0.65	0.06
		PIPS	-1.06	<0.001*
	Diode	Negative control	-4.03	<0.001*
		PUI	-0.65	0.06
		PIPS	-1.71	<0.001*
	PIPS	Negative control	-2.33	<0.001*
		PUI	1.06	<0.001*
		Diode	1.71	<0.001*
Extra-canal	Negative control	PUI	-3.79	<0.001*
		Diode	-1.65	<0.001*
		PIPS	-2.21	<0.001*
	PUI	Negative control	3.79	<0.001*
		Diode	2.14	<0.001*
		PIPS	1.59	<0.001*
	Diode	Negative control	1.65	<0.001*
		PUI	-2.14	<0.001*
		PIPS	-0.55	0.11
	PIPS	Negative control	2.21	<0.001*
		PUI	-1.59	<0.001*
		Diode	0.55	0.11

*: Significant at the level 0.05. Normality assumption is fulfilled. CFU: Colony-forming units, MD: Mean difference. CI: Confidence interval, PIPS: Photon-Induced Photoacoustic Streaming (9), PUI: Passive ultrasonic irrigation (14)

er extrusion levels than the PUI group ($p<0.05$). At the same time, no significant difference was found between the PIPS and diode laser groups ($p>0.05$).

DISCUSSION

This study evaluated the effectiveness of PIPS, diode laser, and PUI in eliminating bacteria from root canals and assessing bacterial extrusion. All three activation protocols significantly reduced CFU counts compared to the negative control group ($p<0.05$); however, none completely eradicated intracanal bacteria or entirely prevented bacterial extrusion. Among the techniques, the diode laser demonstrated the highest efficacy in bacterial elimination and the lowest levels of bacterial extrusion, followed by PIPS and PUI. Conversely, PUI exhibited the highest levels of bacterial extrusion. These findings reject the null hypothesis that all activation modalities are equally effective. The results suggest that the acoustic streams generated by these activation techniques, while effective in reducing suspended bacteria, are insufficient for complete bacterial elimination. Consequently, these methods may be inadequate for targeting complex biofilm structures that adhere more firmly to canal walls.

One strength of this study is its robust experimental design, which included strict inclusion criteria for sample selection and standardisation of protocols. The use of a bacterial suspension of *Enterococcus faecalis*, a pathogen commonly associated with persistent endodontic infections (13, 19), further enhances the clinical relevance of the findings. Normal saline was used as the irrigant instead of an active antimicrobial solution to investigate the mechanical effects of the activation protocols. This deliberate

choice eliminates potential confounding effects from chemical disinfection but requires a cautious interpretation of the findings.

Previous studies have demonstrated favourable outcomes when activation systems are combined with antimicrobial irrigants, such as PIPS with NaOCl, which exhibited enhanced bactericidal efficacy (20, 21). By isolating the mechanical impact of laser activation, this study explores whether the activation energy alone can effectively eliminate intracanal bacteria. However, using extracted teeth may not fully replicate the complexities of *in vivo* conditions, including biofilm structure and host immune responses, which presents another limitation.

The findings of this study align with prior research on the efficacy of irrigation activation techniques but also reveal notable discrepancies with earlier studies. The diode laser and PUI demonstrated significant bacterial reduction, consistent with previous reports highlighting the effectiveness of these modalities in root canal disinfection. However, unlike studies that described PIPS as a highly effective method for biofilm disruption (16), the present study found PIPS less effective than diode laser and PUI. These discrepancies may result from differences in activation parameters, tip placement, root canal morphology, and study design variations, such as the use of antimicrobial irrigants like NaOCl in previous research.

Apical extrusion of bacteria was observed across all activation groups in this study, consistent with prior findings that no irrigation activation systems can entirely prevent extrusion (22, 23). The PUI showed the highest levels of bacterial extrusion among the tested techniques. This finding differs from previous studies, suggesting that the parameters of PUI, including activation time, frequency, and tip size, significantly influence its outcomes regarding bacterial reduction and extrusion (24, 25). Furthermore, discrepancies in the impact of root canal preparation size on extrusion outcomes were noted when compared with earlier findings (26, 27), indicating that further research is needed to standardise and optimise these variables. These findings emphasise the need for standardization in experimental design and highlight the influence of activation technique parameters on clinical outcomes. The variability in reported effectiveness across studies underscores the importance of further investigations to establish optimal protocols for irrigation activation.

This study used an F4 (40/.06) rotary file, which may have influenced apical extrusion levels. The findings align with Mitchell et al. (24), who reported increased extrusion with larger tapers, but differ from those of Yost et al. (25), who found no significant effect with files of smaller tapers. Exploring the interaction of file size, taper, and activation parameters across different irrigation systems is crucial for optimizing outcomes.

The study findings suggest that the eradication of intracanal *E. faecalis* by the diode laser was comparable to that achieved in the PUI group and superior to that of the PIPS group. The diode laser's superior performance in bacterial elimination and reduced extrusion can be attributed to its deeper tip placement and higher energy output than PIPS. When activated, the laser tip generates vapour bubbles that implode and explode, enhancing fluid dynamics (28). PIPS exhibits a higher absorption rate in water compared to the diode laser, which could theoretically offer greater effectiveness for root canal irrigants (16, 29). Nonetheless, this study suggests that while PIPS effectively delivers irrigant to the apical third, the fluid dynamics produced by the intra-canal tips of the diode laser and PUI are more effective. The diode laser's higher power activation (1 W for diode laser versus 0.3 W for PIPS) and deeper tip placement may counterbalance differences in water absorption coefficients between the two lasers.

The direction of acoustic streaming generated by the diode laser likely facilitated the coronal movement of debris, minimising apical extrusion. In contrast, the coronal tip placement in PIPS may have directed fluid dynamics toward the apex, increasing extrusion. These differences in debris movement underscore the importance of activation tip placement and energy settings in achieving safe and effective irrigation.

The activation mechanism of PUI is similar to that of LAI in that both generate vapour bubbles, though via different processes. In PUI, the bubbles are produced by pressure changes caused by the vibration of the ultrasonic instrument (30). Conversely, in LAI techniques, these effects are induced by the power of light. Understanding these mechanisms helps clinicians choose the appropriate method based on case-specific requirements.

Clinically, these findings suggest that diode lasers offer a promising alternative for enhancing root canal disinfection while minimising extrusion risks. However, practitioners should consider the technique's learning curve, cost of equipment, and specific clinical scenarios when selecting an irrigation activation technique. The study underscores the need to balance efficacy and safety when selecting irrigation activation techniques.

Further research is needed to evaluate the efficacy of activation techniques in complex clinical scenarios, particularly those involving biofilms and anatomically challenging canals. Studies investigating the combined effects of activation modalities with antimicrobial irrigants, such as sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA), would provide more comprehensive insights into their effectiveness. Additionally, optimising activation parameters, including power settings and tip placement, is crucial to enhance efficacy while minimising potential adverse effects.

Understanding the role of root canal preparation size and taper in bacterial extrusion is another critical area for future exploration. Long-term clinical trials are also required to assess the impact of these techniques on treatment outcomes, such as postoperative pain and periapical healing. Such studies are essential to validate laboratory findings and translate them into practical guidelines, bridging the gap between experimental research and clinical practice to achieve more predictable and successful endodontic outcomes.

CONCLUSION

Within the limitations of this study, although the tested activation techniques caused bacterial extrusion and were unable to completely eliminate the intracanal bacteria, the diode laser exhibited the best outcome in terms of bacterial elimination from the root canal and its extrusion.

Disclosures

Ethics Committee Approval: The study was approved by the University of Sharjah Research Ethics Committee (no: REC-21-10-12-S, date: 02/11/2021).

Authorship Contributions: Concept – S.A.S., M.M.A., S.S.Y.; Design – S.A.S., S.S.Y.; Supervision – S.A.S., O.M., S.S.Y.; Funding – S.A.S., O.M., S.S.Y.; Materials – S.A.S., M.M.A., S.S.Y.; Data collection and/or processing – M.M.A., A.S., S.S.Y.; Data analysis and/or interpretation – S.A.S., A.J., S.S.Y.; Literature search – M.M.A., A.J., S.A.S.; Writing – A.J., S.A.S.; Critical review – S.A.S., O.M., A.J.

Conflict of Interest: All authors declared no conflict of interest.

Use of AI for Writing Assistance: No artificial intelligence technologies were utilised in the creation or submission of this work.

Financial Disclosure: This work was funded by the College of Graduate Studies at the University of Sharjah, Sharjah. United Arab Emirates.

Peer-review: Externally peer-reviewed.

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