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Investigating the Antibacterial Effect of Passive Ultrasonic Irrigation, Photodynamic Therapy and Their Combination on Root Canal Disinfection

Zohreh Ahangari[1], Mohammad Asnaashari[2], Nazila Akbarian Rad[1], Mehdi Shokri[3], Saranaz Azari-Marhabi[2*], Negin Asnaashari[2]

[1]Department of Endodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Dental Biomaterials, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

***Correspondence to**
Saranaz Azari-Marhabi, Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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Abstract

Introduction: *Enterococcus faecalis* is a gram-positive, facultative anaerobic bacterium associated with persistent endodontic infections. Conventional disinfection methods may not completely eradicate the bacteria within the root canal system. Therefore, novel modalities have been suggested to optimize root canal disinfection. The aim of this study was to evaluate and compare the antibacterial effect of photodynamic therapy (PDT), passive ultrasonic irrigation (PUI) and their combination in addition to conventional endodontic irrigation against *E. faecalis* biofilms in root canals.

Methods: Root canals of 50 single-rooted extracted human teeth were prepared and incubated with *E. faecalis* for 21 days. They were then divided into 4 treatment groups and a control group as follows: (1) NaOCl—Syringe irrigation with 2.5% NaOCl, (2) PUI—Passive ultrasonic irrigation with NaOCl, (3) NaOCl+PDT—Photodynamic therapy following syringe irrigation with NaOCl, (4) PUI+PDT, (5) Control—Syringe irrigation with saline. Colony-forming units were counted and bacterial reduction was calculated for each treatment group.

Results: All treatments led to significant reductions in the bacterial load compared to the control group. PUI and PUI+PDT led to the complete elimination of the bacteria from the root canals. NaOCl and NaOCl+PDT treatments reduced the bacteria by 99.9% and 99.5% respectively. NaOCl+PDT was significantly less effective in reducing the bacteria compared to other treatment groups. There were no significant differences between the NaOCl, PUI, and PUI+PDT groups.

Conclusion: Passive ultrasonic irrigation with or without the combination of Photodynamic therapy completely eradicated the bacteria. The use of PDT as an adjunction to NaOCl syringe irrigation and PUI did not enhance their antibacterial effect.

Keywords: Antimicrobial photodynamic therapy; Passive ultrasonic irrigation; Sodium hypochlorite; *Enterococcus faecalis*

Introduction

Persistent intraradicular infection is a major cause of endodontic treatment failure.[1] Therefore, efficient disinfection of the root canal system is of great importance.[2] Due to the complex anatomy of the root canals[3,4] and resistance of bacterial biofilms to disinfectants,[5] conventional methods of chemomechanical preparation are not able to completely eradicate the bacteria within the root canal system.[4,6] Following instrumentation and NaOCl irrigation, approximately 40%-60% of the canals remain infected with cultivable bacteria.[7] According to a research study, a negative culture before the root canal obturation resulted in a success rate of 94%, while a positive culture reduced the success rate to 68%.[8]

Enterococcus faecalis is the most commonly detected species in the previously root canal-filled teeth with persistent periradicular lesions.[1,9] Different factors including its ability to form biofilms, competition with other microorganisms, invading dentinal tubules, and survival in nutritional starvation contribute to its resistance and high prevalence.[9]

Novel modalities such as irrigant activation methods and photodynamic therapy (PDT) have been investigated to optimize root canal disinfection techniques.[10-14]

The effectiveness of intracanal irrigants relies on their direct contact with the root canal walls. The fluid exchange caused by conventional endodontic irrigation with a syringe and needle does not exceed 1 mm from the needle tip.[14,15] Moreover, the apical vapor lock created during syringe irrigation hinders irrigant displacement at

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the apical third of the root canal system.[15,16] To overcome these limitations, the activation/agitation of the irrigants using different techniques including manual dynamic activation, passive ultrasonic irrigation, sonic irrigation and laser-activated irrigation has been proposed.[14,17]

Passive ultrasonic irrigation (PUI) is one of the most widely used irrigant activation systems[16] in which the acoustic energy is transmitted from an oscillating non-cutting file or smooth wire to the irrigating solution in the prepared root canal through ultrasonic waves, leading to acoustic streaming and cavitation of the irrigant.[13] This procedure may enhance the penetration of the disinfecting irrigants, increasing their antimicrobial efficacy.[18]

PDT is a treatment characterized by the inactivation of cells, microorganisms, or molecules by means of a light of a specific wavelength.[19] The exposure of a non-toxic dye (photosensitizer) to light in the presence of oxygen leads to the generation of highly reactive chemical species, such as singlet oxygen and free radicals which induce cell death.[20]

Several studies have been carried out to evaluate the antimicrobial efficacy of PDT (or PAD: photo-activated disinfection) in root canal treatment.[6,21-24] Although conflicting results exist regarding its superiority over other decontamination strategies, preclinical data recommend PDT as a promising adjunctive method to the conventional chemomechanical preparation for further bacterial reduction.[11,12]

PDT can be performed by lasers, LED and halogen lamps.[25] The use of LED as a safer light source for PDT leads to less heat generation[26] and consequently less tissue injury.

The present study aimed to evaluate and compare the efficacy of PUI, LED-mediated PDT in adjunction with the routine NaOCl irrigation, and their combination in root canal disinfection.

Materials and Methods

Specimen Preparation

Fifty-five single-rooted extracted human teeth (incisors and single-rooted premolars) with intact, fully developed roots were collected. The presence of a single canal was radiographically confirmed. The teeth were stored and disinfected in 5.25% sodium hypochlorite (NaOCl) solution for 24 hours. They were then stored in sterile 0.9% saline solution at room temperature before the experiment.

The teeth were decoronated and the roots were shortened to a length of approximately 12 mm using a water-cooled diamond disk. The root canals were prepared using ProTaper Gold rotary files (Dentsply Maillefer, Tulsa, OK, USA) to a master apical file size F3. During all preparation steps, irrigation was performed with 10 mL of sterile saline solution.

After mechanical instrumentation, root canals were irrigated with 17% Ethylenediaminetetraacetic acid (EDTA) solution for 2 minutes followed by irrigation with 5.25% NaOCl for 2 minutes to remove the smear layer. They were finally rinsed with sterile saline solution to eliminate the remaining irrigants. The apical ends of the roots were sealed with composite resin.

The roots were individually immersed in test tubes containing 1 μL of Brain heart infusion (BHI) broth (Merck, Darmstadt, Germany), and they were then sterilized in an autoclave at a temperature of 121°C and a pressure of 15 Psi for 15 minutes. To verify the absence of bacterial contamination, 5 specimens were randomly selected. Samples were taken from the root canals and cultured on agar plates. No bacterial growth was detected after 24 hours.

Root Canal Contamination and Biofilm Formation

Root canals were contaminated with *Enterococcus faecalis* (ATCC9854) taken from a frozen stock as follows: 0.5 mL of a suspension containing *E. faecalis* bacteria equivalent to 0.5 McFarland was inoculated to the tubes containing 0.5 ml of BHI broth medium (Merck, Darmstadt, Germany) and sterile dental specimens. After the vortex, for bacterial biofilm formation and further penetration of the bacteria into the dentinal tubules, all the tubes were incubated at 37°C for 21 days. During this period the BHI media were refreshed on alternate days, and each time the tubes were swirled individually using a vortex mixer so that the medium and the bacteria could completely penetrate into the dentinal tubules.

Treatment Groups

The roots were placed in a 96-well plate and randomly assigned into 4 treatment groups (n=10) and a control group (n=10) using a random number table.

Group 1 (NaOCl): Root canal irrigation with NaOCl using a syringe and a 30-gauge needle placed 1 mm short of the apices

Group 2 (PUI): Passive ultrasonic irrigation

Group 3 (NaOCl+PDT): Photodynamic therapy following NaOCl irrigation

Group 4 (PUI+PDT): Passive ultrasonic irrigation followed by photodynamic therapy

Control group: Root canal irrigation with 10 ml of normal saline using a syringe and a 30-gauge needle placed 1 mm short of the apices

In all treatment groups following NaOCl irrigation, the solution was rinsed off the root canals using 10 ml of normal saline. In groups 1 and 3, the root canals were irrigated with 10 ml of 2.5% NaOCl for 2 minutes without activation.

Passive Ultrasonic Irrigation

Following 90-second syringe irrigation with 10 ml of 2.5% NaOCl, the solution was activated by a #25 Ufile (NSK Dental, Japan) driven by an NSK ultrasonic device (Varios 970 lux, NSK Dental, Japan) for 30 seconds.

Photodynamic Therapy

The root canals were dried using sterile paper points and were then filled with toluidine blue solution (0.1 mg/mL). The solution remained in the canals for 1 minute. PDT was performed using an ENDO tip of 0.5 mm diameter and a light-emitting diode (LED) (FotoSan® 630, CMS dental, Denmark) with a power peak at 630nm and output intensity of 2000-4000 mw/cm^2 for 60 seconds (Energy density = 120-240 J).

Root Canal Sampling

After the treatments, F4 ProTaper Gold rotary files (Dentsply Maillefer, Tulsa, OK, USA) driven by an NSK rotary motor (Endo-Mate DT, NSK Dental, Japan) were used in the root canals for 30 seconds at a speed of 250 rpm and a torque of 1.5 N.cm. The files were then detached from the device and transferred into microtubes containing a liquid BHI medium using a sterile plier in order to culture the attached dentinal debris. During all sampling steps rotary files were not in contact with hands**.**

Colony Counting

The microtubes were vortexed and bacterial suspensions of each sample were diluted using 10 ten-fold serial dilutions. 100 μL of the dilute solutions was plated on 8×8 cm^2 BHI agar plates using the spread plate technique. The plates were incubated at 37°C for 24 hours. *E. faecalis* CFU/mL was calculated using a colony counter (Teif Azma Teb, Iran).

Sample Preparation for SEM Imaging

In order to confirm the biofilm formation in the canals, one specimen was prepared for scanning electron microscopy imaging. For this reason, after preparation of the root canal 2 grooves were made on buccal and lingual surfaces of the root by a fissure diamond bur. The specimen was inoculated with *E. faecalis* and incubated at 37°C for 21 days. Then it was split into halves using a sterile chisel. The tooth sections were fixed in 2.5% glutaraldehyde solution at 4°C for 24 hours (Figure 1).

Statistical Analysis

The Kruskal-Wallis test was applied to compare the final CFU counts among the 5 treatment groups, and pairwise comparisons of the groups were performed using the Mann-Whitney U test. *P* value< 0.05 was considered to be statistically significant. The data were analyzed using the GraphPad Prism 7 software (Figure 2).

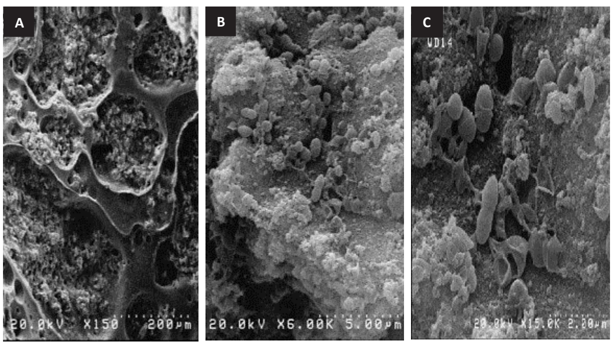

Figure 1. SEM Images of the Root Canal Wall After 3-Week Incubation With *Enterococcus faecalis*: A (150×), B (6000×), C (15000×).

Results

The mean colony counts (CFU/mL) of *E. faecalis* bacteria remaining in the root canals after treatments are presented in Table 1. The mean CFU in the control group served as the baseline for comparison. In all treatment groups, CFUs of *E. faecalis* significantly decreased compared to the control group, with the [PUI] and [PUI+PDT] treatments achieving a 100% reduction (Table 1). Bacterial reduction in groups [PUI], [PUI+PDT], and [NaOCl] was significantly higher than that of the [NaOCl+PDT] group. There were no significant differences between the [NaOCl], [PUI], and [PUI+PDT] groups (Table 2).

Discussion

The present study evaluated the antibacterial effect of PDT, PUI and their combination in adjunction to the routine application of NaOCl on root canals infected with *E. faecalis* biofilms.

Enterococcus faecalis was selected considering its ability to colonize root canals in biofilms and its resistance to antimicrobial agents.[2] In order to simulate

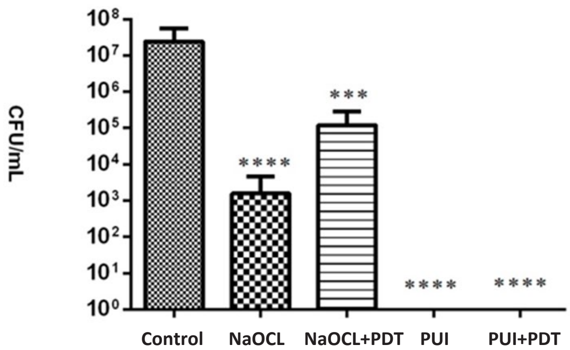

Figure 2. *Enterococcus faecalis* CFU/mL After Disinfection Protocols. The error bars indicate the mean CFU± SD. The asterisks represent statistically significant reductions relative to the control group (**P*<0.05, ** *P*<0.01, ****P*<0.001, *****P*<0.0001).

Table 1. CFU/mL Counts of *Enterococcus faecalis* and Percentage of Bacterial Reduction After Antibacterial Treatments

Group	Bacterial Reduction Relative to the Control Group (%)	Mean CFU/mL After Treatment	*P* Value
Control	-	2.4x10^7	-
NaOCl	99.93	1.5x10^4	<0.0001
NaOCl+PDT	99.5	1.21x10^5	0.0002
PDT+PUI	100	0.0	<0.0001
PUI	100	0.0	<0.0001

CFUs, colony-forming units; PDT, photodynamic therapy; PUI, passive ultrasonic irrigation.

Table 2. The Results of Pairwise Comparison of the Groups Using Mann-Whitney Test

	NaOCL	NaOCl+PDT	PUI	PUI+PDT
NaOCL	-	*P* =0.00028	*P* =0.08	*P* =0.08
NaOCl+PDT	*P*=0.00028	-	*P* <0.0001	*P* <0.0001
PUI	*P* =0.08	*P* <0.0001	-	*P* >0.9999
PUI+PDT	*P* =0.08	*P* <0.0001	*P* >0.9999	-

PDT, photodynamic therapy; PUI, passive ultrasonic irrigation.

the in vivo situation, a biofilm model was used in this research. Bacteria within a biofilm are less susceptible to antimicrobials compared to their planktonic counterparts. This may be due to the biofilm-specific protection against oxidative stress, biofilm-specific expression of efflux pumps, and decreased penetration of antimicrobial agents through the biofilm matrix.[27,28] Shen et al reported that the bacteria in mature (3-week old) biofilms were more resistant to antimicrobial treatments than those in young biofilms.[29] Therefore, in the present study, the root canals were incubated with *E. faecalis* for 3 weeks.

Given that NaOCl is the "gold standard" irrigant for root canal disinfection,[30] in this study, irrigation with NaOCl was carried out in the samples of all treatment groups (PDT, PUI, and PUI+PDT) to evaluate the combination effect.

The results showed that all treatments caused a significant reduction in the CFUs of *E. faecalis* within the root canals. This reduction was significantly higher in the PUI, PUI+PDT, and 2.5% NaOCl treatments compared to NaOCl+PDT. PUI with or without PDT eradicated the bacteria within the root canal system.

PDT acts through the activation of a photosensitizer by exposure to a light at a compatible wavelength.[4,12] In this study, toluidine blue was employed in PDT, which is a common type of photosensitizer used in numerous studies.[2,3,26,31-34] FotoSan 630 was used as the light source, emitting light in the red spectrum with peak power at 630 nm.

Rios et al[34] evaluated the antimicrobial effect of PDT using an LED and toluidine blue. The Results demonstrated that PDT in adjunction to 6% NaOCl irrigation led to a greater reduction of the bacteria compared to the sole use of PDT or NaOCl irrigation. Likewise, de Oliveira et al[35] reported that the association of 5.25% NaOCl irrigation with PDT using a diode laser and methylene blue resulted in significant additional antimicrobial effect against *E. faecalis* compared to the NaOCl irrigation alone, whereas in the present study CFU reduction caused by 2.5% NaOCl irrigation was greater than that of adjunctive PDT. This difference may be attributed to the use of sodium thiosulfate for NaOCl inactivation in the aforementioned study. In the current study, the remaining NaOCl in the dentinal tubules may have restricted the penetration of the photosensitizer or negated its effect, and thus interfered with the function of PDT. Furthermore, the use of a different PDT protocol and a more mature biofilm model in the present study may have led to the reduced effect of PDT. It should be noted that in clinical practice, sodium thiosulfate is not commonly used after root canal irrigation with NaOCl.

In the present study, PUI yielded the best bactericidal effects (100% bacterial reduction) which were significantly greater than those of syringe irrigation and adjunctive PDT. Similarly, studies conducted by Mohammed et al. and Eneide et al demonstrated superior antibiofilm efficacy of PUI over syringe irrigation.[36,37] The acoustic microstreaming and cavitation created by PUI produce shear stress, disrupting the bacterial biofilm on the root canal walls.[16]

Xhevdet et al[38] compared the disinfection efficacy of PDT, NaOCl irrigation and PUI. The results showed that while PDT caused a significant decrease in microorganisms, ultrasonic irrigation was more effective in reducing the bacterial load, which is in accordance with the findings of the present study.

Wang et al[39] investigated the synergistic antibacterial effect of MB-mediated PDT and ultrasonic irrigation with NaOCl on *E. faecalis* bacteria within the root canals of bovine incisors. They found the combination treatment to be significantly more effective than stand-alone treatments. In the present study, although the combination treatment led to the complete elimination of the bacteria, no synergistic effect was found between PDT and PUI.

Conclusion

Within the limitations of this study ultrasonic activation of 2.5% NaOCl solution resulted in the complete elimination of the bacteria within the root canals. The adjunction of PDT to NaOCl irrigation did not enhance the antibacterial effect.

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