



Comparison of Antibacterial Effects of Photodynamic Therapy and an Irrigation Activation System on Root Canals Infected With *Enterococcus faecalis*: An In Vitro Study

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Abstract

Introduction: *Enterococcus faecalis* is a resistant bacterium which is the most abundant species in infected root canals. Photodynamic therapy (PDT) is a method for killing the bacteria with active Oxygen radicals generated in a photosensitizer when exposed to centralized light. Furthermore, as a new method of canal disinfection, a variety of irrigation activation systems have been introduced, one of which is GentleFile (GF) with rotary movements and spiral effects for antibacterial action. This study aimed to compare the effectiveness of the two mentioned methods when used with and without Sodium Hypochlorite in eliminating *E. faecalis* from infected root canals.

Methods: Fifty-eight uniradicular teeth were randomly divided into 4 experimental groups of 14. Two specimens were selected for later scanning electron microscopy in order to screen the procedure steps. In each experimental group, 10 samples were selected to be treated with GF or PDT; 3 of them were selected as positive controls and the other one sample was chosen as a negative control. Experimental groups were as follows: (1) Irrigation activation system, (2) Irrigation activation system + sodium hypochlorite, (3) PDT, and (4) PDT+ sodium hypochlorite. The specimens were then cultured for a bacterial colony count.

Results The decrease in the bacterial count after the treatment with the irrigation activation system was 99.8% ($P=0.011$) and when the system was used with sodium hypochlorite, it was 100% ($P=0.001$). The antibacterial effect of PDT was 90.08% ($P=0.011$) and it was 99.7% when PDT was combined with sodium hypochlorite ($P=0.011$).

Conclusion: All four methods can be administered as complementary methods in root canal disinfection. According to the results of disinfection in the experimental groups of current study it is concluded that integration of new technologies such as activation irrigation system or PDT in Combination with NaOCl ameliorates disinfection of root canal and can provide several advantages in the endodontic outcome.

Keywords: *Enterococcus faecalis*; Photodynamic therapy; GentleFile; Irrigation activation system; Sodium hypochlorite.



Introduction

Insufficient disinfection of the root canal system results in treatment failure and thus prevents the healing of apical periodontitis.¹ According to previous studies, the deep penetration of microorganisms into complex anatomical regions such as lateral canals, apical deltas, dentinal tubule constrictions and into the smear layer decreases the efficacy of the common irrigation.²⁻⁴ In addition, several studies indicated that even after a thorough endodontic treatment, there is a chance of failure due to resistant

bacteria remaining in root canal systems.^{3,5-7}

Enterococcus faecalis is a gram-positive anaerobic coccus which is the most abundant species in root canals, causing primary or secondary infections.^{8,9} This bacterium shows resistance to dressings such as calcium hydroxide and to irrigators through biofilm formation, deep penetration into dentinal tubules, and hydrogen-active components in its plasma membrane.¹⁰⁻¹²

Sodium hypochlorite (NaOCl) is a commonly used irrigant, dissolving debris and necrotic tissues.¹³ Its

dentinal penetration depth is 60-150 μm while *E. faecalis* has been detected 1200 μm inside dentinal tubules.¹⁰

A new method introduced for canal disinfection and eliminating pathogenic bacteria is photodynamic therapy (PDT). In this method, light absorption in a photosensitizer causes the generation of active oxygen radicals and the destruction of microorganisms.¹⁴⁻¹⁶ There are 3 main components in PDT: a photosensitizer, centralized light or laser, and oxygen; methylene blue photosensitizer is a hydrophilic phenothiazine derivative with light absorption at 660 nm. This maximum lies well within the emission range of common diode lasers used for PDT.¹⁷ Methylene blue is able to pass the protein channels in gram-negative bacteria's plasma membrane. It has shown the capability of destroying 83% of *E. faecalis* bacteria inside a canal if used alone and 97% of *E. faecalis* in biofilms if used with red light exposure.^{18,19}

A variety of techniques and irrigant delivery devices have been introduced for more effective canal disinfection following cleaning and shaping.²⁰ A newly introduced irrigation activation system known as GentleFile (Medic NRG, Kibbutz Afikim, Israel) is a rotary system with improved mechanical properties. The single-use stainless steel files that abrade/scrape the dentinal walls are operated by a fully automated handpiece at a maximum speed of 6500 RPM.

The pecking motion of the files causes a spiral effect on the irrigant, making the antibacterial effect stronger.²¹

The present study aimed to compare the effectiveness of an irrigation activation system (Gentlefile) and PDT in eliminating *E. faecalis* from infected canals.

Materials and Methods

Specimen Preparation

Fifty-eight freshly extracted, intact, adult uniradicular human teeth were collected. They were placed in NaOCl 5.25% and then stored in sterile saline 0.9% at room temperature. Tooth crowns were cut with a disk bur so that all canals reached the standard working length (WL) of 14 mm. The WL was determined by introducing a K-file #15 (Dentsply/Maillefer /Tulsa/OK) in the canal until its tip was visualized at the apical foramen. Then, the canals were sequentially prepared within the 0.5 mm apical

end of the canal via the crown-down instrumentation technique up to master apical file size 40 with a rotary file (Protaper, sx,s1,s2,f1,f2) (Dentsply/Maillefer/Tulsa/OK) under irrigation with 2 cc of NaOCl 2.25 %.

Two specimens were selected for later scanning electron microscopy in order to screen the procedure steps. They were then cut in half using a disk bur.

Since the smear layer inhibits the contamination of tubules by *E. faecalis*, the teeth were placed in EDTA (ethylenediaminetetraacetic acid) 17% in a vortex instrument for 15 minutes to remove the smear layer.

Then, they were irrigated with normal saline and placed in NaOCl 2.25% in a vortex instrument for 10 minutes. Afterward, the canals were dried out using absorbent paper points and the apical foramina were sealed by a composite resin (Denfil, South Korea) under a biologic hood.

Each tooth was transferred into a lab tube containing sterile Phosphate-buffered solution (Germany, Merk). Then, the teeth were sterilized in an autoclave for 15 minutes at 121°C.

The teeth were randomly divided into 4 experimental groups of 14. In each group, 13 teeth were later infected with *E. faecalis*, 3 of them were selected as positive controls and 10 samples were selected to be treated with GF or PDT; the other one sample was chosen as a negative control not to be infected or to receive any kind of treatment (Figure 1).

Bacterial Culturing

A frozen pool of *E. faecalis* was administered for this purpose. The *E. faecalis* frozen bacterium (Accession number: ATCC9854) was transferred to Broth Hewitt Todd agar plate and was incubated for 24 hours at a temperature of 37°C. Single colonies were inoculated to a 10 ml BHA medium and were incubated for 24 hours at a temperature of 37°C. Then, a 1.5×10^8 CFU/mL suspension which equals 0.5 McFarland was prepared. In order to have a 0.5 McFarland sample, light absorption between 0.08–0.1 is needed in spectrophotometry with a wavelength of 600 nm.

Each tooth was placed in a sterile test tube, with 1 mL of the prepared *E. faecalis* bacterial suspension and BHA

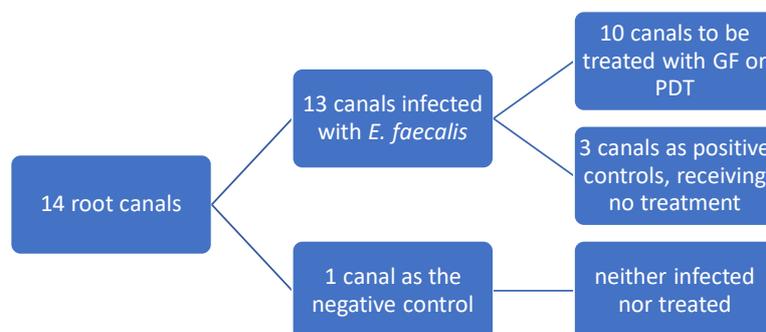


Figure 1. Divisions Inside Each of the 4 Groups With 14 Canals.

medium (Merck, Germany).

For deeper bacterial penetration into the dentinal tubules and for biofilm formation, the samples were incubated in anaerobic conditions at a temperature of 37°C for 21 days. The medium was replaced every two days with a fresh medium. During this period, some of the samples were randomly selected and cultured on a BHA medium for 24 hours in an incubator in order to make sure about the bacterial growth of the samples or their not being infected. This was carried out every 7 days.

Specimen Preparation for Scanning Electron Microscopy

The scanning electron microscopy (SEM) specimens were treated in the same way as the other samples were. A specimen underwent *E. faecalis* culturing for 21 days as the positive control and the other, as the negative control, was sterilized in an autoclave. Both the specimens were then placed in glutaraldehyde 2.5% in 4°C for 24 hours for fixation. They were washed with distilled water for 30 seconds and finally, sent for electron microscopy. The positive control was sent for electron microscopy to make sure of biofilm formation and the negative control underwent this procedure to make sure of the accuracy of the sterilization procedure.

Cleaning

After the 21-day period of incubation, the instrumentation was accomplished under a biologic hood under sterile conditions.

Each sample was taken out of its test tube and washed with 5 mL of sterile saline. In the first group, the pulp canals were irrigated with 100 µL of sterile saline and then instrumented using the irrigation activation system, Gentlefile. The system was used for 1 minute and was in contact with the canal walls. A #25 file was introduced in each canal within 1 mm of the WL with an up and down movement. The canal was then irrigated using 5ml of sterile saline.

In the second group, 100 µL of NaOCl 2.25% was injected in each canal and remained for 1 minute. The canal was then irrigated using 5ml of sterile saline and instrumented using the irrigation activation system

In the third group, the teeth were treated with PDT with methylene blue. 100 µL of methylene blue 25 µg/mL was injected in each canal followed by diode laser emission inside the canal. The Konftec Laser device (Taiwan) with a wavelength of 660 nm, 150 mW, 60 second irradiation and 9-joule energy was used according to the manufacturer's manual.

The system was coupled to an optical fiber with a diameter of 200 µm. The optical fiber was initially placed 1 mm shorter than WL, and spiral movements, from apical to cervical, were performed to allow adequate distribution of the light throughout the root canal (step-back technique). Each exposure took 60 seconds (20 seconds of exposure followed by a 10-second pause),

repeated three times. The canals were then irrigated with sterile saline.

In the fourth group, 100 µL of NaOCl 2.25% was injected in each canal and remained for 1 minute initially. The canals were then irrigated with 5 mL of sterile saline and treated with PDT.

Sampling

Immediately after the cleaning stage, a sterile Protaper F3 file was introduced for 30 seconds in each canal for sampling. The Protaper file was then vortexed in a lab tube containing 10 mL of normal saline.

Sample Culturing

The vortexed saline inside the tube was a 10-fold serial dilution in multiple tubes. 100 µL of the diluted solutions were cultured in 8*8 cm plates containing BHA with spread technique. Then, the cultures were placed in a 37°C incubator for 24 hours.

All the procedures above were carried out under a biologic hood to achieve sterile conditions.

Counting the colony-forming units (CFU) of *E. faecalis* (CFU/mL) was carried out using a colony counter system (Tayf Azma, Iran).

Statistics

The gathered data on bacterial growth (CFU) was analyzed with the Mann-Whitney U test using SPSS version 21 software.

Results

Counting the CFUs of *E. faecalis* (CFU/mL), using a colony counter system, revealed the data presented in Table 1.

In the second group, treated with NaOCl and the irrigation activation system, 0 CFUs were observed after treatment, and hence there was a reduction of 100% in the CFUs of *E. faecalis* ($P=0.001$). The results in the other groups were not significantly different from one another. The reduction in CFUs was the minimum in the fourth group, treated with PDT only, with a 90.08% reduction in the CFUs ($P=0.011$).

The SEM images revealed sterile negative control specimens (Figure 2A) and a suitably formed *E. faecalis* biofilm in positive control specimens (Figure 2A).

Discussion

Insufficient cleaning of the root canal system leads to failures in the root canal treatment and hinders the healing process of apical periodontitis.¹

Since the irrigants' efficacy depends on their close contact with the canal walls, the microorganisms penetrating the deeper layers of dentin will usually remain untouched.²² The aim of this study was to compare the antibacterial effect of PDT with an irrigation activator system on canals infected with *E. faecalis* in laboratory

Table 1. The Number of CFUs of *E. faecalis* Before and After Each Treatment and the Reduction Percentage in CFUs

Group Number	Treatment	CFUs Before Treatment	CFUs After Treatment	Percentage of Reduction in CFUs	P Value
1	Irrigation activation system	$4 \times 10^7 \pm 5.5 \times 10^6$	$8.7 \times 10^4 \pm 4.8 \times 10^4$	99.8%	0.011
2	Irrigation activation system + NaOCl	$2 \times 10^5 \pm 1.4 \times 10^5$	0	100%	0.001
3	PDT	$4 \times 10^7 \pm 5.5 \times 10^6$	$3.7 \times 10^6 \pm 2.7 \times 10^6$	90.08%	0.011
4	PDT + NaOCl	$6 \times 10^7 \pm 5.8 \times 10^7$	$1.8 \times 10^5 \pm 2.4 \times 10^6$	99.7%	0.011

conditions.

The irrigation activator system used in this study is a product of MIB Company, which has not been compared to PDT in any other studies. The results indicated that this system could be effective in eliminating the *E. faecalis* biofilm when used with NaOCl. In addition, PDT was significantly effective in *E. faecalis* biofilm reduction when used with or without NaOCl. The irrigation activation system showed the strongest antibacterial effect when used with NaOCl. The second most effective method was PDT used in combination with NaOCl, which was almost as effective as the irrigation system used without NaOCl (PDT was performed after disinfection with sodium hypochlorite for further reduction of the *E. faecalis* bacteria), both stronger than PDT when applied without NaOCl.

In the studies of Elumalai et al and Ragul et al on novel canal irrigation systems compared with conventional irrigation, it was observed that they facilitate conveyance of irrigants inside the canals in higher volumes and lead to higher effectiveness in canal debridement.^{23,24} The irrigation system used in our study removes the biofilm by functioning similar to rotary files and can improve the disinfection effectiveness of the irrigant and thorough removal of microbes.²⁴ Also, interestingly significant reduction of biofilm was observed in the irrigation activation system group without NaOCl, which could be attributed to removing the infected dentin by abrading/scraping of the dentinal walls by Gentlefile.

Siddiqui et al conducted a systematic review of 17 studies on the antibacterial effectiveness of PDT against *E. faecalis* in infected root canals and they reported that in 12 studies, PDT was successful in eliminating

E. faecalis from infected root canals, in four studies, it was less efficient than conventional irrigation and instrumentation, and in one study, PDT was as effective as conventional endodontic irrigation and instrumentation. It was also reported that the intensity of PDT depends on the wavelength of the laser, its power, the exposure time, and the photosensitizer. In addition, in articles in which PDT was shown to be effective, the wavelengths used were between 600-805 nm and the photosensitizers were toluidine blue or methylene blue, which is in accordance with the results of our study.²⁵

Fimple et al. observed that PDT with the same parameters of light and photosensitizer as those in our study resulted in an 80% decrease of the bacteria inside the canal.²⁶ The bacterial reduction was 90.08% in our study.

In an experiment evaluating the effect of different energy levels, bacterial loads and different exposure cycles on the results of PDT, Soares et al administered the diode laser with a wavelength of 660 nm and a power of 40 mW along with methylene blue 50 µg/mL. They found PDT very effective against bacteria.²⁷

Similar to the results of our study, the results of a study by Hoedke et al revealed that the application of NaOCl with PDT improved its antibacterial function.²⁸

Souzo et al observed that NaOCl had a significantly better antibacterial function than PDT. A possible explanation may be the low concentration of Oxygen available for generating cytotoxic oxygen derivatives in the canal and particularly in the irregularities and dentinal tubules. The photosensitizer materials, in addition, may not penetrate these irregularities, ultimately resulting in the bacteria's remaining in these areas. They concluded that the antibacterial effect of PDT is restricted to areas accessed by NaOCl and depends on the penetration depth of the photosensitizer. They also observed that methylene blue and toluidine blue were not significantly different in PDT against *E. faecalis*.¹⁷

According to the results of the present study, complete eradication of the *E. faecalis* biofilm could be the result of this activation irrigation system in delivering and conveying NaOCl irrigant to the dental canal. The results of a systematic review by Nagendrababu et al regarding the effectiveness of ultrasonically activated irrigation in root canal disinfection revealed that the use of ultrasonically activation irrigation systems could result in superior microbial reduction within the root canal

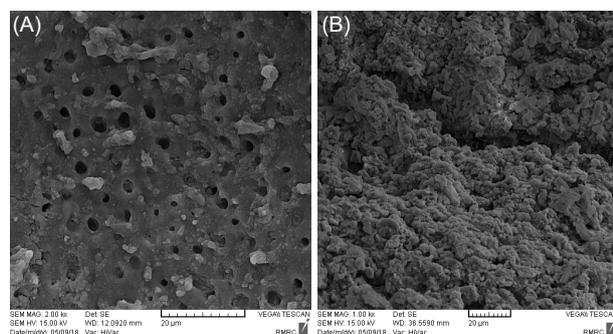


Figure 2. (A) SEM image of the negative control specimen. (B) SEM image of the positive specimen.

system compared to other types of irrigant activation and conventional syringe irrigation.²⁹ However, according to the results of a clinical trial by Orozco et al, there was no significant difference between passive ultrasonic irrigation and conventional irrigation in decreasing bacterial counts.³⁰ Furthermore, the results of a systematic review by Susila and Minu showed that the utilization of mechanical active irrigation devices is advantageous in root canal treatment. They stated that mechanical active irrigation devices are clinically efficient in the conveyance of the irrigant in the root canal, which leads to more cleanliness in root canals.³¹

Finally, it should be stated that GF Brush²¹ which improves the debridement of canals prepared with Gentlefile has also been introduced, but due to the limitations of sanctions in our country, it could not be accessible.

Conclusion

Within the limitations of the present study, it was concluded that both PDT and the irrigation activation system were significantly effective in decreasing CFU/mL. According to the results of disinfection in the experimental groups of current study it can be stated that integration of new technologies such as activation irrigation system or PDT in combination with NaOCl ameliorates disinfection of root canal and can provide several advantages in the endodontic outcome. They can be administered as complementary methods for root canal debridement and disinfection.

Ethical Considerations

Ethical approval was obtained from the research ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1396.1118).

Conflict of Interests

The authors declare no conflict of interest.

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