

# Effectiveness of Different Disinfection Methods in the Elimination of Enterococcus Faecalis Biofilm from the Infected Root Canals

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**Abstract: Background and Objectives:** This *ex vivo* study aimed to assess the antibacterial efficacy of diode laser, non-activated sodium hypochlorite (NaOCl), NaOCl activated by 980 nm diode laser or Passive Ultrasonic Irrigation (PUI) technique for elimination of *Enterococcus faecalis* (*E. faecalis*) from the infected root canals.

**Materials and Methods:** Root canals of 90 single-rooted human premolars were instrumented with ProTaper rotary system. The teeth were sterilized and their root canals were inoculated with *E. faecalis* except for five samples, which served as negative controls. The teeth were incubated at 37°C for three weeks and were then randomly divided into four groups (n=20). Group A root canals were rinsed with 5.25% NaOCl for 60 seconds; group B root canals underwent diode laser (980 nm) irradiation (1.5 W, CW, 4\*10 s) while filled with 5.25% NaOCl; group C root canals were subjected to passive ultrasonic irrigation (PUI) with 5.25% NaOCl for 60 seconds; group D root canals underwent diode laser irradiation (1.5 W, CW, 4\*10 s). Infected root canals in the positive control group (n=5) did not undergo any intervention. Samples were taken from the intracanal bacteria and cultured to determine the number of colony forming units (CFUs). Data were analyzed using the Kruskal-Wallis test.

**Results:** among the treatment groups, the lowest bacterial count was noted in sodium hypochlorite group agitated with ultrasonic while the highest bacterial count was noted in diode laser group and this difference was statistically significant (P=0.004). No other significant differences were noted (P>0.05).

**Conclusion:** All the tested methods are suitable for elimination of *E. faecalis* from the root canal system. However, it appears that NaOCl, with/without agitation, is more effective than diode laser for decreasing intracanal infection.

**Keywords:** Biofilm, Diode Laser, Sodium Hypochlorite, Passive Ultrasonic Irrigation.

## 1. INTRODUCTION

Elimination of bacteria from the root canal system is among the main goals of endodontic treatment because residual microorganisms in the root canal system, particularly *Enterococcus faecalis* (*E. faecalis*), are the most important cause of treatment failure and development of apical periodontitis [1].

Due to the complex anatomy of the root canal system and presence of isthmuses, fins and anastomoses, mechanical preparation alone by manual and rotary instruments cannot completely clean and disinfect the root canal system. Moreover, irrigating solutions may not be able to access all areas in the complex structure of root canals [2, 3]. To improve the efficacy of irrigating solutions and enhance their access to different areas of the root canal system, these solutions may be activated by different techniques. These techniques include agitation by gutta-percha, manual files, plastic instruments and sonic and ultrasonic devices. By doing so, destruction of bacterial biofilm is enhanced by the activated irrigating solution, leading to improved permeability of bacterial membrane to sodium hypochlorite (NaOCl) [4, 5].

Lasers are capable of effective cleaning and disinfection of the root canals and elimination of resistant bacterial species such as *E. faecalis* [6]. Also, laser is currently used for agitation of irrigating solutions. Laser agitation has introduced for not only the disinfectant effect of laser, but also activation of irrigant facilitating its access to complex areas of the canals [7].

Studies on efficient elimination of *E. faecalis* by this method are limited, and no consensus has been reached on the efficacy of this technique for root canal disinfection [8-11].

Since the solid state laser systems such as Er:YAG are big and expensive, a tendency exists among researchers and clinicians to use diode lasers. These systems are small, portable, non-expensive. Thus, they are appropriate for clinical use [12].

This study aimed to assess the efficacy of diode laser, non-activated NaOCl, NaOCl activated by 980 nm diode laser or by PUI technique in elimination of *E. faecalis* biofilm from the infected root canals to find the most efficient technique for root canal disinfection.

## 2. MATERIALS AND METHODS

This ex vivo study was carried out on 90 single-rooted, single-canal sound human mandibular premolars with closed apices. The teeth were stored in 0.5% chloramine T solution until the experiment. The crowns were cut with high speed hand piece such that the root length was standardized to have 13 mm length. All root canals were instrumented with ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland) up to F5 using the single length technique according to the manufacturer's instructions to have 12 mm of working length. Also, 2.5% NaOCl was used for root canal irrigation between instrumentations. After completion of cleaning and shaping, 1 mL of 17% EDTA (Premier Dental Products, Norristown, PA, USA) and 1 mL of 5.25% NaOCl (Golrang, Pakshoo Co. Tehran, Iran) were used each for 3 minutes for smear layer removal followed by a final rinse with 10 mL of saline. To prevent fluid penetration, apical foramen was sealed with self-cured glass ionomer cement (Fuji 2 Universal Restorative Material, GC Inc., Japan), and other surfaces were covered with two layers of nail varnish.

All teeth were then individually packed and autoclave-sterilized at 121°C and 15 psi pressure for 30 minutes. Samples were then taken randomly from the teeth to ensure the accuracy of sterilization process. Of all teeth, five were randomly chosen as the negative control group and incubated for three weeks. Under sterile conditions, the remaining teeth were placed in Cryo-tubes (Cryo. S, PP Greiner Bio-One GmbH., Frickenhausen, Germany) containing sterile brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI, USA); 50 µL of *E. faecalis* suspension (ATCC29212) was inoculated into 5 mL of BHI and incubated

at 37°C for 24 hours. The suspension concentration was adjusted at 0.5 McFarland standard turbidity ( $1.5 \times 10^8$  CFUs/mL). Under sterile conditions, the teeth were removed from the BHI tube and the canals were dried with sterile paper points. Next, 1 mL of the bacterial suspension was injected into the root canals by a 30-gauge irrigation needle. Each tooth was placed in a new tube containing fresh BHI and incubated at 37°C for 21 days under aerobic conditions. During this period, the suspension in the canals was replaced with fresh suspension every 48 hours. After three weeks of incubation, five teeth were randomly chosen and did not undergo any intervention to serve as positive control group. For the remaining teeth, the medium was aseptically aspirated from the tubes and the canals were dried with paper points. A total of 80 teeth were randomly divided into four groups (n=20).

In group A, canals were rinsed with 2 mL of NaOCl injected into the canals with a 30-gauge needle (Helal Iran Medical Device co., Iran) for 60 seconds.

In group B, NaOCl was injected into the canals and agitated with diode laser (GaAIAs) irradiation at 980 nm wavelength (Wiser, Doctor Smile Co., Italy) with 1.5 W output power in continuous mode. Diode laser was irradiated into the canals with an optical fiber with 200  $\mu$ m diameter to a length 1 mm shorter than the working length. Laser was irradiated in circular motion with 2 mm/s speed, starting from the apical third towards the coronal third of the root canal (step-back technique). The laser irradiation protocol was repeated with four cycles of 10-second irradiations and 20-second intervals. To ensure the presence of irrigating solution in the root canal during the process of laser irradiation, the irrigating solution was added whenever the pulp chamber seemed to be empty.

In group C, 2 mm of NaOCl was injected into the canal and agitated using a #20 stainless steel U-file connected to an ultrasonic device (Piezo engine, 230 v, Various 970, NSK co., Japan) set on E4 power such that the tip of the hand piece was 1 mm short of the root canal length (at 12 mm).

In group D, diode laser was used for dry canals as explained for group B.

After completion of the disinfection process and in order to neutralize the irrigating solution, the root canals were rinsed with 5 mL of 5% sodium thiosulfate for one minute. For sampling from the canals, after drying the canals with sterile paper points, sterile saline was used as the transfer medium in the root canals. Using a #40 Hedstrom file (Mani Inc., Tochigi, Japan), root canal walls were filed to take a sample from the root dentin. Using a #40 sterile paper point, the transfer medium and dentin chips in the canals were collected. Files and paper points were placed in separate tubes specific for each tooth that contained 10 mL of sterile saline and vortexed for 30 seconds using a vortex mixer in order for the bacteria to release into the solution. Eventually, 50  $\mu$ L of saline was vortexed and inoculated into plates containing blood agar. After incubation of the samples at 37°C for 48 hours, number of colony forming units (CFUs) was counted using a colony counter (Colony Star, Funk Gerber Product, Gebr Liebsch, Germany).

The Kruskal Wallis test was used to compare the groups in terms of the mean CFUs.  $P < 0.05$  was considered statistically significant.

### 3. RESULTS

The results showed that the positive control group had the highest bacterial colony count while no bacterial colony was noted in the negative control group.

Table 1 shows the number of *E. faecalis* CFUs in the groups. As shown, among the treatment groups, the lowest bacterial count was noted in sodium hypochlorite group agitated with ultrasonic while the highest bacterial count was noted in diode laser group and this difference was statistically significant ( $P = 0.004$ ). No other significant differences were noted ( $P > 0.05$ ).

Table 1: Mean (standard deviation) colony-forming units per milliliter (CFUs/mL) in the four experimental groups

Groups	Mean	Std. Deviation
NaOCl	$1.06 \times 10^4$	$3.06 \times 10^4$
NaOCl+ Laser	$1.11 \times 10^4$	$3.05 \times 10^4$
NaOCl+ Ultrasound	$7.0 \times 10^1$	$2.22 \times 10^2$
Laser	$6.63 \times 10^4$	$2.22 \times 10^5$

#### 4. DISCUSSION

This study compared the efficacy of diode laser and sodium hypochlorite activated with diode laser and ultrasonic for elimination of *E. faecalis*.

Microorganisms remaining in the root canal system following chemomechanical preparation are often responsible for root canal treatment failure. *E. faecalis* is commonly used in endodontic studies with in vitro design since it forms bacterial biofilm in the root canal system, colonizes and invades the dentinal tubules. Due to resistance to antimicrobial agents, it can cause mono-infection in the root canal system [13-15].

The time required for biofilm formation varies from 15 minutes to 60 days; however, some studies have shown that one or two days of incubation is sufficient for the formation of *E. faecalis* biofilm, longer incubation can lead to formation of more mature biofilm [16, 17]. Thus, in this study, similar to that of Preethee et al, [18] three weeks of incubation was allowed for the formation of mature biofilm of *E. faecalis*.

No consensus has been reached regarding the time required for elimination of *E. faecalis* by sodium hypochlorite. The one-minute period allowed in this study was suggested by Sahar-Helft et al, [11] in their disinfection protocol to prevent the possible side effects of this irrigating solution on root dentin and interference with resin sealers used for root canal filling [11, 19, 20].

Several techniques have been suggested to improve the efficacy of irrigating solutions such as changing the concentration of irrigating solution or its temperature, addition of surfactant and agitation [21]. To prevent/treat postoperative infection, PUI has been suggested for agitation of the irrigating solution as an adjunct to enhance disinfection of the root canal system [22].

Acoustic streaming and cavitation have been described as the main mechanisms of bacterial destruction following the application of ultrasound because increase in temperature, pressure and free radicals generated after collapse of gas bubbles formed in cavitation can damage the membrane and cell wall of bacteria [23, 24].

Some studies have reported a significant increase in the efficacy of irrigating solutions following ultrasonic activation [25, 26]. In the current study, the greatest reduction in colony count was noted in the group subjected to agitation of sodium hypochlorite by ultrasound but no significant difference was noted between the conventional irrigation and PUI; this finding was in agreement with that of Gründling et al, [27] and Bhuva et al [28].

At present, use of laser for disinfection of the root canal system is increasing. Diode laser is more popular for this purpose due to low cost, small size and its easy handling [12].

The difference between the current study and similar ones is that this study also evaluated the efficacy of diode laser alone for elimination of *E. faecalis* in addition to other methods such as sodium hypochlorite agitation by diode laser and ultrasound and conventional irrigation. In this study, 980 nm diode laser was used to activate sodium hypochlorite because this wavelength has a stronger absorption into water among the near infrared wavelengths [29].

Humed et al. showed that 980 nm diode laser can induce cavitation by explosive formation of water vapor in aqueous-based media and consequently increase the kinetics of the irrigating solution [12]. Also, increase in temperature of the irrigating solution during laser irradiation can affect the disinfecting ability of the irrigating solution [6]. The current study failed to show any significant difference in agitation of the irrigating solution by diode laser and ultrasound and conventional irrigation. These results were similar to those of De Meyer et al, [8] in 2016 who reported no significant difference between the laser activation group by ER: YAG laser, PUI and conventional irrigation using sodium hypochlorite in terms of bacterial biofilm reduction. Also, Bago Jurič et al, [30] in 2014 demonstrated that laser activation by Er, Cr:YSGG laser and PUI had equal efficacy in terms of elimination of *E. faecalis*. However, Neelakantan et al, [10] in 2015 indicated that agitation of irrigating solutions with diode and Er:YAG lasers for disinfection of dentinal tubules was more effective than PUI and conventional irrigation of root canals. Sahar-Helft et al, [11] and Al-Aqeedi et al. [31] concluded that laser activation by Er:YAG and Er,Cr:YSGG lasers was more efficient than conventional irrigation for elimination of *E. faecalis*. Difference between their results and ours may be due to different methodology and difference in the type of irrigating solution, its concentration, duration of use, type of laser and laser irradiation parameters used for agitation of the irrigating solution.

According to Gutknecht et al, 980 nm wavelength of diode laser has the highest absorption in water compared to other wavelengths of diode and Nd:YAG lasers. Thus, when irradiating 980 nm diode laser, most of the irradiated energy is absorbed by the superficial parts of dentinal tubules filled with water; therefore, the greatest antibacterial effects are exerted at this area. As the result, deep parts of dentinal tubules would be affected the least. Therefore, bacteria that have penetrated deep into dentinal tubules remain safe from the irradiation, and the disinfection efficacy of 980 nm diode laser decreases as such. This can explain the lower disinfecting efficacy of laser in some studies compared to sodium hypochlorite and its activated forms[29, 32]. According to Mithra et al, [33] this finding can be related to the fact that when smear layer is removed before microbial inoculation, *E. faecalis* deeply penetrates into dentin. To eliminate the deeply lodged bacteria, laser needs to be irradiated directly and penetrate deeply into the tubules, which is not always possible in root canals.

Aside from its antimicrobial effects, application of laser in endodontics has some other benefits as well. Parirokh et al. [34] indicated that irradiation of diode laser after elimination of smear layer can successfully obstruct the dentinal tubules especially in the apical third of the root canal and consequently decrease the risk of re-infection of the root canal system. However, temperature rise, which damages the tooth surrounding tissues and difficult access of laser light to root canal surfaces are among the limitations of laser irradiation [35].

Future studies are required to assess the efficacy of different types of lasers with varying powers and irradiation times. Also, this technique should be compared with laser-assisted endodontic treatment (chemical irrigation before laser irradiation).

## 5. CONCLUSION

According to the results of this study, all techniques assessed are suitable for elimination of *E. faecalis*. However, it seems that use of sodium hypochlorite with/without agitation is more effective than diode laser for decreasing intracanal infection.

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