ORIGINAL RESEARCH

Efficacy of Erbium, Chromium-doped Yttrium, Scandium, Gallium and Garnet Laser-activated Irrigation Compared with Passive Ultrasonic Irrigation, Conventional Irrigation, and Photodynamic Therapy against *Enterococcus faecalis*

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Abstract

Aim: To compare the antimicrobial effects of two different irrigation solutions activated with erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG) laser or an ultrasonic system and a photodynamic therapy (PDT) on *Enterococcus faecalis* (*E. faecalis*).

Materials and methods: The root canals of 72 single-rooted human permanent incisors were prepared with ProTaper Universal rotary instruments and incubated with *E. faecalis* (ATCC 29212) for 4 weeks. Then the teeth were randomly divided into seven experimental groups with 10 specimens for canal disinfection procedures. Group I, standard needle irrigation (SNI) with 2.5% sodium hypochlorite (NaOCl); group II, SNI with 2% chlorhexidine gluconate (CHX); group III, laser-activated irrigation (LAI) by Er,Cr:YSGG of NaOCl; group IV, LAI of CHX; and group V, passive ultrasonic irrigation (PUI) of NaOCl; group VI, PUI of CHX; group VII, PDT. The remaining two teeth were used as the control group. After the disinfection procedures were completed, the root canals were filled with phosphate-buffered saline and bacterial samples were taken with sterile paper cones. The cultivation was performed on Mueller-Hinton agar (MHA) plates. The live bacteria were calculated by counting the colonies on these plaques. The statistical analysis was performed using Kruskal-Wallis *H* test and Miller's multiple comparison technique.

Results: Both LAI and PUI of NaOCl and PUI of CHX were more successful than the PDT on root canal disinfection ($p < 0.05$).

Conclusion: Within the limitation of the present study, the activation of NaOCl solution by Er,Cr:YSGG laser or an ultrasonic system can be useful in the elimination of the *E. faecalis* from the canal. The PUI of CHX also has similar results. Photodynamic therapy showed a lower performance compared to these methods.

Clinical significance: The activation of the sodium hypochlorite with Er,Cr:YSGG laser or PUI may be useful for removal of the *E. faecalis* biofilm layer in the root canal.

Keywords: Er,Cr:YSGG laser, Laser-activated irrigation, Passive ultrasonic irrigation, Photoactivated disinfection, Root canal disinfection.

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Introduction

Posttreatment apical periodontitis results from the low standard of endodontic procedures for eliminating primary root canal infection.[1,2] The most important etiological factor in the most nonhealing cases is the continuation of microbial infection within the root canal system. These microorganisms are usually in direct contact with the nutrient source in the periradicular tissues and in areas where the instruments and irrigants are difficult to reach within the root canal system.[3–5] Sometimes the infection cannot be eliminated due to the complexity of the root canal system, though endodontic procedures are rigorously applied, and apical periodontitis may persist as an asymptomatic radiolucency.[6,7]

Enterococcus faecalis is a microorganism that is frequently involved in the etiology of the posttreatment endodontic disease. The potential of biofilm formation in root canals of *E. faecalis* chains and its adaptation to changing environmental conditions are well known.[8] This microorganism is resistant to many antimicrobial agents and is difficult to remove completely from the root canals.[9]

Nowadays, one of the recommended methods for increasing the disinfection of root canals is the use of lasers. Because laser energy has the ability to penetrate the dentin tissue, dental lasers can access areas that are not accessible in the root canal system.[10] Many studies in the literature discuss about the antimicrobial activity of the lasers in the canals.[11–14] Laser activation of irrigant creates cavitation and large elliptic gas bubbles, which expand by increasing the pressure and raising the fluid in the canal. When there is quick constriction, the pressure decreases and the liquid returns

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to the canal to form a secondary cavitation effect. Therefore, the laser works like a kind of liquid pump.[15]

Activation of irrigation solutions with erbium lasers is a recommended method for root canal disinfection.[16] The erbium, chromium-doped yttrium, scandium, gallium and garnet (Er,Cr:YSGG) is a type of laser with a wavelength of 2,780 nm, which is highly absorbable by water. It has been claimed that it increases the disinfection of the root canal system without causing thermal damage to the surrounding tissues, thanks to the hydrokinetic energy it uses.[11,17,18]

The objective of this study was to comparatively investigate the use of two different irrigation solutions with laser-activated irrigation (LAI), passive ultrasonic irrigation (PUI), and standard needle irrigation (SNI) methods with each other and with a photodynamic therapy (PDT) method on the removal of *E. faecalis* inside the canal. Our null hypothesis is that different irrigant activation methods and PDT will show a similar performance against *E. faecalis*.

Materials and Methods

This study was carried out in the research laboratories of the Faculty of Dentistry and Medical Microbiology, Department of Karadeniz Technical University, Turkey.

Preparation of Samples

Seventy-two human mature incisors, extracted for periodontal reasons, were used in this *in vitro* study. They had single-root, single-canal, and a closed apex with a maximum root curvature of 10°. The teeth purified from the tissue debris on the external root surfaces were stored in 0.1% thymol solution at room temperature for the time period until the experimental phase. The crowns of the teeth were removed with a diamond disc, so that the root length was 15 mm. An ISO 10 K-file was run in the canal until the tip of the instrument was visible through the main foramen and 1 mm was subtracted to determine the working length. The glide path was obtained by using manually, respectively, with ISO sizes 15–20 K-files. By using ProTaper Universal rotary nickel–titanium instruments (Dentsply Maillefer, Ballaigues, Switzerland), the coronal and middle thirds of the canal were shaped with S1, S2, and S*X* files and the apical finishing process was completed by using F1, F2, and F3 files, respectively. After the use of each instrument, the root canals were irrigated with a 1-mL volume of 2.5% sodium hypochlorite [(NaOCl) Wizard; Rehber Kimya, Turkey]. After using the last instrument, the canals were irrigated with 5 mL of 17% EDTA (Wizard) for 1 minute, followed by a final irrigation with 2 mL of distilled water, and followed by 5 mL of 2.5% NaOCl. The canals were irrigated with 5 mL of 5% sodium thiosulfate (Zag; Bereket Kimya, Turkey) and finally with 2 mL of distilled water to neutralize the NaOCl in the canal.

The working blocks were prepared by covering the root ends of the teeth with cyanoacrylate and immersing them into an acrylic resin (Fig. 1). Care was taken to ensure that the upper surface of the coronal region of the roots was at the same level as the surface of the acrylic resin material. After the acrylic resin was polymerized, the samples were placed in autoclaved plastic carrier containers and sterilized for 15 minutes in an autoclave at 121°C and 1 atm pressure.

Contamination of Root Canals with *E. faecalis*

Enterococcus faecalis strain (ATCC 29212, American Type Culture Collection) was obtained from the collection in the Faculty of Medicine, Department of Medical Microbiology, Karadeniz Technical University.

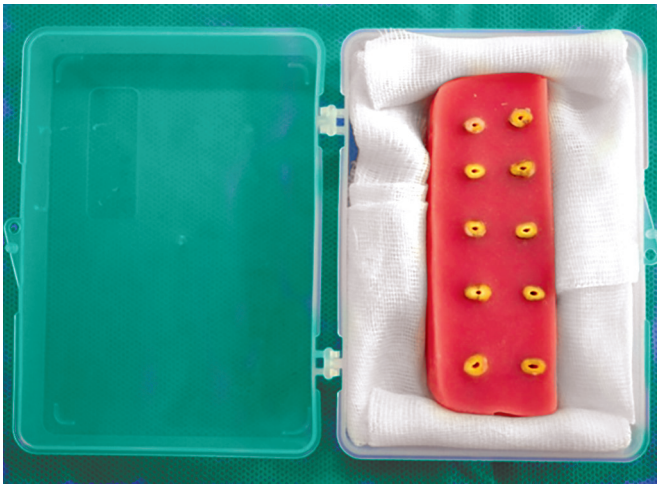


Fig. 1: Working blocks

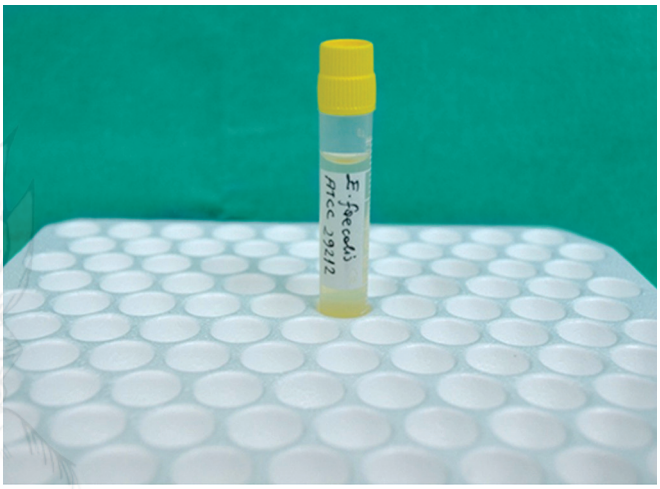


Fig. 2: Root canals were filled using 10 µL of the bacterial suspension with McFarland 0.5 turbidity of *Enterococcus faecalis* ATCC 29212 strain

The root canals were filled with 10 µL of the bacterial suspension of *E. faecalis* in order to infect (Fig. 2). Using a sterile 30-gauge irrigation needle (Kerr, USA), the bacteria were enabled to reach the apical part of the canal. In order to prevent the teeth from drying out, moistened cotton was placed in the teeth containers and the containers were incubated at 37°C for 48 hours in the incubator. This procedure was continued for every other day for 4 weeks.

After 4 weeks of putative *E. faecalis* biofilm formation, the samples were randomly divided into seven groups of 10 teeth (the remaining 2 teeth were used as the control group). On the experimental groups, the first microbial sampling (S1) were performed from the root canals before disinfection procedures. The root canals were soaked with sterile phosphate-buffered saline (PBS) and sterile ISO 25/0.02 paper cone (DiaDent, Canada) was placed inside and waited for 60 seconds. The removed paper cone was placed in a tube with 1 mL of sterile PBS inside. This procedure was repeated 3 times for each root canal. The bacteria were transferred to the liquid medium by vortexing the tubes

with paper cones. Suspensions of 100 µL were pipetted into sterile 1.5 mL centrifuge tubes containing 0.9 mL of PBS (10^{-1} dilution tube). Dilution tubes of 10^{-2} were made by diluting 10^{-1} dilution tube at a rate of 1/10 using PBS. From each stock and dilution tubes, 10 µL was pipetted onto the Mueller–Hinton agar (MHA) media and spread over. After incubating the petri plates at 37°C for 48 hours, the colonies were counted in the most countable dilution plate, and the number obtained was multiplied by the dilution rate (100 for plate, 1,000 for 10^{-1} dilution plate, 10,000 for 10^{-2} dilution plate) to determine the number of bacteria collected from each root canal (CFU/mL). After the S1 samples were taken from all the groups, the canal disinfection process was started.

Disinfection of the Root Canals

Group I: SNI with NaOCl (n = 10)

Standard needle irrigation was performed using a 30-gauge irrigating needle with side-vented close ended (KerrHawe SA, Bioggio, Switzerland) and a syringe (Ayset A.Ş., Istanbul, Turkey) with 5 mL of 2.5% NaOCl (Wizard) for 60 seconds. The irrigation needle was moved within the root canal to a distance of 1 mm shorter than the working length. The needle was moved up and down slightly in the canal without contacting the root canal walls during the irrigation process.

Group II: SNI with chlorhexidine gluconate (CHX; n = 10)

Standard needle irrigation was performed by the same method used in group I with 5 mL of 2% CHX (Consepsis; Ultradent, South Jordan, UT, USA) for 60 seconds.

Group III: LAI of NaOCl using Er,Cr:YSGG laser (n = 10)

The root canals were irrigated for 30 seconds using 5 mL of 2.5% NaOCl and the Er,Cr:YSGG laser (Waterlase; Biolase, Irvine CA, USA) was applied to the canal filled with the solution. The 4-mm section of the RFT2 fiber tip (Waterlase) was inserted into the canal and activated for 30 seconds. The laser parameters used were 0.25 W, 20 Hz, 10% air, and waterless mode.[19]

Group IV: LAI of CHX using Er,Cr:YSGG laser (n = 10)

Laser-activated irrigation was performed by the same method used in group III with 2% CHX.

Group V: PUI with NaOCl (n = 10)

The root canals were irrigated for 30 seconds using 5 mL of 2.5% NaOCl, and the stainless steel file numbered 15 (Varios U file; Nakanishi Inc., Tochigi, Japan) was mounted on a piezo-electric ultrasonic unit (EMS, Nyon, Switzerland), and it was placed 1 mm shorter than the working length into the canal filled with the solution and activated by short vertical movements for 30 seconds ultrasonically.

Group VI: PUI with CHX (n = 10)

Passive ultrasonic irrigation was performed by the same method used in group V with 2% CHX.

Group VII: Photodynamic Therapy

A light-emitting diode lamp (Fotosan; CMS Dental, Copenhagen, Denmark) emitting light with a power of 628 nm has been used for root canal disinfection. The root canals were filled with a low-viscosity photosensitizing agent (toluidine blue) using the 30-gauge needle and an injector. In accordance with the recommendations of the manufacturer, the photosensory activation was carried out for 30 seconds after the Fotosan endodontic tip was felt in the canal until resistance was felt. After the procedure was completed, the canals were irrigated with 5 mL of distilled water ($n = 10$).

In groups I, III, and V, the canals were irrigated with 5 mL of 5% sodium thiosulfate solution to neutralize the NaOCl in the canal, and the solution was kept in the canal for 5 minutes. In groups II, IV, and VII, the canals were washed with Tween 80 solution for 1 minute, then with 2 mL of distilled water, and finally with 2 mL of 5% sodium thiosulfate, respectively. The last solution waited in the canal for 5 minutes in order to neutralize the CHX.

After the canal disinfection procedures were completed in all groups, the same method used for the bacteria count of S1 was used for the second sampling (S2).

Control Group

After 4 weeks of inoculation time, in order to control the formation of the *E. faecalis* biofilm layer in the canal, two roots were split longitudinally. Each section was dehydrated in the graded concentration of alcohol and gold sputtered to achieve a conductive coating. Then samples were examined under scanning electron microscope [(SEM) JSM-6400; JEOL, Tokyo, Japan].

Statistical Analysis

Kruskal–Wallis *H* test was applied to the groups using the average number of rows to compare the differences between the disinfection performances of the groups because the data were not normally distributed.[20] When the absence hypothesis was disapproved as a result of the Kruskal–Wallis *H* test, the multiple comparison techniques developed by Miller were used to determine the difference between the groups. Statistical analysis was performed at $p < 0.05$ significance level.

Results

The SEM images taken from the samples in the control group confirmed that a microbial biofilm layer was formed in the canals (Fig. 3). The microbial reduction rates of the groups after S1 and S2 analyses and the number of rows given according to these ratios are presented in Table 1. In the present study, the number of rows was given by the microbial reduction percentages. For example, when the microbial reduction is 100%, the number of rows of the sample would be 78, while the number of rows given to the samples

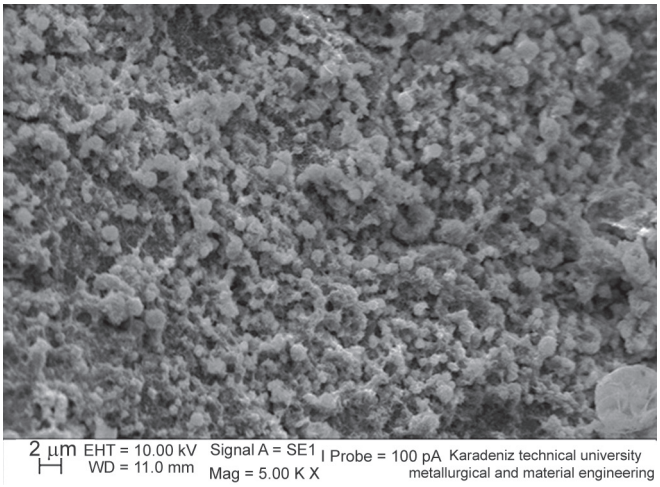


Fig. 3: Positive control sample (scanning electron microscope image with 5,000× magnification of *Enterococcus faecalis* biofilm)

with more microbial reduction would be lower. The PDT showed significantly lower performance than NaOCl activated by Er,Cr:YSGG laser or PUI and CHX activated by PUI ($p < 0.05$; Figs 4 and 5). No statistically significant difference was observed between the other groups ($p > 0.05$).

Discussion

Enterococcus Faecalis is the most frequently used microorganism in endodontic microbiology studies because of its high resistance to many antimicrobial agents.[13,17,18] In previous studies, different materials were used to create an *E. faecalis* biofilm layer. For example, nitrocellulose membrane filters,[21] hydroxyapatite discs,[22] dentinal sections[23] or extracted teeth[11,24] have been used for this purpose. In this study, the extracted human teeth used were experimentally infected with *E. faecalis* ATCC 29212.

In previous studies, both culture methods and molecular techniques have been used to analyze the number of living bacteria in root canals and dentine tubules.[25] The culture method has been reported to be sensitive in determining the amount of *E. Faecalis* in the root canals.[25,26] This study used a culture method that is easy to apply and more economical. On the other hand, this kind of *in vitro* tests should be interpreted with caution as the findings may not fully reflect clinical conditions.[26]

Sodium hypochlorite is an irrigation solution that is widely used in endodontic treatments because of its ability to dissolve vital and necrotic pulp tissue and also to have broad-spectrum antimicrobial activity. However, the using of solutions had lower toxicity, such as CHX, has been proposed due to some problems, such as high-toxicity NaOCl induces an inflammatory reaction when in contact with vital tissues and NaOCL is a highly corrosive material.[27,28] Onçağ et al.[27] evaluated the antibacterial properties of 5.25% NaOCl, 2% CHX, and 0.2% CHX plus 0.2% cetrimide (Cetrexidin) in root canals infected with *E. faecalis*. The combination of CHX and Setrexidine on *E. faecalis* was significantly more effective than NaOCl alone. Ercan et al.[28] reported that both CHX and NaOCl were significantly effective in reducing microorganisms in teeth with necrotic pulp, periapical pathology, or both and could be used successfully as an irrigant. However, the efficacy of both 1% NaOCl and 6% NaOCl solutions against *E. faecalis* biofilm has been reported to be superior to the Smear Clear, 2% CHX, REDTA, and BioPure MTAD solutions.[29] Giardino et al.[30] compared the activity of 5.25% NaOCl, TetraClean, and MTAD on *E. faecalis* biofilm and reported that only 5.25% NaOCl disintegrates and removes the biofilm. Only 2% of CHX-containing medications was able to eliminate *E. faecalis* biofilms in a study examining the efficacy of CHX or antibiotic (clindamycin with metronidazole)-based medications.[31] Williamson et al.[32] found CHX to be less effective in their studies comparing the efficacy of 6% NaOCl and 2% CHX against the biofilm of *E. faecalis*. Agrawal et al.[33] reported that the 2% CHX solution is less effective compared to other solutions of 5.25% NaOCl and BioPure MTAD on *E. faecalis*. According to our results, when SNI method is used, though the performance of 2.5% NaOCl solution on *E. faecalis* biofilm is superior to 2% CHX solution, no statistical significant difference was observed between the two solutions. In general, although studies comparing the antibacterial effects of CHX and NaOCl exhibited slightly conflicting results, both *in vitro* and *in vivo* antibacterial effects appear to be similar.[34]

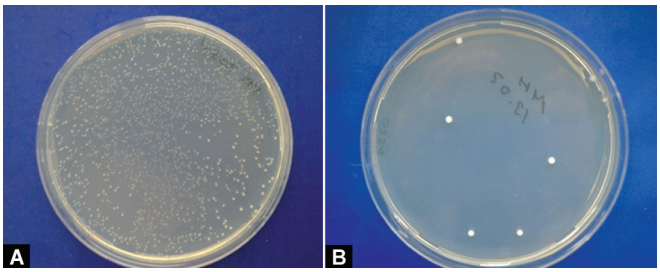
It has been reported that the use of PUI after manual or rotary instrumentation significantly reduced the number of bacteria in the canal.[35] These positive findings can be attributed to two main factors. The first is that the acoustic microstreaming generated by ultrasonic activation causes the dissolution of bacterial biofilm in the root canals. By dispersing bacterial biofilm, planktonic bacteria are exposed, which are more sensitive to the bactericidal effects of irrigation agents. The second ultrasonic factor that makes the cells more permeable to irrigants by weakening the cell membrane is cavitation.[35,36] However, in many studies, the application of NaOCl

Table 1: Microbial reduction rates (%) after disinfection protocols and number of rows

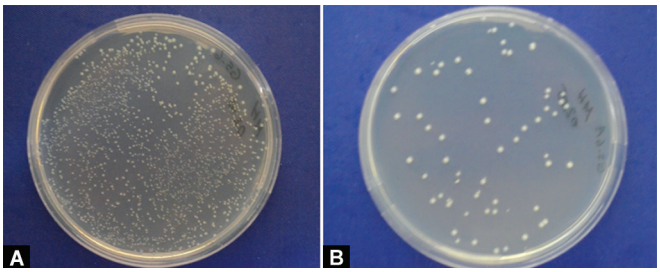
Groups	*Microbial reduction rate % (mean ± SD)*	*Number of rows*
Group I (SNI with 2.5% NaOCl)	99.7000 ± 0.39611	37.2[a,b]
Group II (SNI with 2% CHX)	99.6092 ± 0.51771	35.5[a,b]
Group III (LAI with 2.5% NaOCl)	99.9658 ± 0.03075	56.4[a]
Group IV (LAI with 2% CHX)	99.9551 ± 0.03852	52.2[a,b]
Group V (PUI with 2.5% NaOCl)	99.9616 ± 0.11401	70.3[a]
Group VI (PUI with 2% CHX)	99.9524 ± 0.08276	63.1[a]
Group VII (PDT)	97.8911 ± 1.88444	9.1[b]

[a,b]There are statistically significant differences between the groups marked with a and b letters ($p < 0.05$)

CHX, chlorhexidine gluconate; SNI, standard needle irrigation; NaOCl, sodium hypochlorite; PDT, photodynamic therapy; LAI, laser-activated irrigation; SD, standard deviation



Figs 4A and B: Laser-activated irrigation with sodium hypochlorite. Image of *Enterococcus faecalis* colonies formed on Mueller-Hinton agar medium of a randomly selected sample. (A) S1 sampling (before disinfection); (B) S2 sampling (after disinfection)



Figs 5A and B: Photodynamic therapy group. Image of *Enterococcus faecalis* colonies formed on Mueller-Hinton agar medium of a randomly selected sample. (A) S1 sampling (before disinfection); (B) S2 sampling (after disinfection)

solution with SNI or PUI has been reported to have a similar effect on *E. faecalis*.[37–39] Similarly, in our study, no significant difference was observed between SNI or PUI using NaOCl and CHX.

In order to increase the success of endodontic treatment, it has been proposed to activate irrigation solutions with lasers of different wavelengths to help conventional cleaning procedures.[17–19] It is proposed to use Er:YAG or Er,Cr:YSGG lasers for this purpose.[40,41] In the study conducted by Christo et al.[41] comparing the efficacy of different concentrations of NaOCl solution with SNI or Er,Cr:YSGG laser activations on the *E. faecalis* biofilm found that LAI performed with 4% NaOCl was more successful than SNI. Betancourt et al.[18] reported that the LAI with Er,Cr:YSGG increased the bactericidal efficiency of 0.5% NaOCl against *E. faecalis* biofilms. Pedulla et al.[40] reported that when using 5% NaOCl as an irrigation solution, SNI and LAI performed with Er:YAG laser for 30 seconds showed similar efficacy against *E. faecalis*. Sahar-Helft et al.[42] compared the activities of SNI performed with 2% CHX and LAI performed with Er:YAG on *E. faecalis*. They found that the number of bacteria after LAI performed with CHX decreased significantly. Peters et al.[43] reported that when using 6% NaOCl as an irrigation solution, LAI obtained more negative sample number than SNI and PUI, but no statistical difference was observed between the three groups. In the present study, the percentage of microbial reduction in the LAI group with NaOCl was 99.9658% and 99.9551% in the LAI group with CHX. Although there were higher success rates compared to PUI or SNI group using the same solutions in both groups practiced with LAI, no statistically significant difference was observed.

According to the results of the present study, the mean percentage of microbial reduction in PDT-applied root canals is 97.8911%. So PDT used in the removal of the biofilm of *E. faecalis* is less successful than the LAI of NaOCl or the PUI of NaOCl and CHX. Ng et al.[44] suggested that PDT application in addition to chemomechanical preparation contributed to the canal disinfection. In contrast, Souza et al.[45] reported that PDT applied with two different photosensitizing agents (methylene blue or toluidine blue) did not contribute significantly to the chemomechanical preparation in *E. faecalis* elimination. Tennert et al.[46] detected a success rate of 92.7% in the experimental group with PDT after primary infection, 99.9% in the SNI with NaOCl and 99.9% in the group with PDT and SNI combined. They found only PDT application to be significantly less successful. Wang and Huang[47] compared the PUI of 2.5% NaOCl in terms of their efficacy in PDT and combined the use of both systems on *E. faecalis*. All three groups were found to be successful in eliminating *E. faecalis*, but the combined treatment was the most effective method.

Meire et al.[48] compared two different PDT systems with Er:YAG laser and Nd:YAG laser and reported that both PTD systems were weak in the elimination of the *E. faecalis*. The use of different PDT systems, the methodological differences of different photosensitizing agents, and the use of different light parameters make it difficult to compare the studies evaluating the microbial activity of PDT systems. It has been claimed that bacterial species and reproductive modes in the root canal system may affect PDT susceptibility.[49] It has also been shown that the dentin, the dentin matrix, the pulp tissue, and the bacterial lipopolysaccharides can also affect the antimicrobial ability of PDT.[50] Care should be taken when interpreting the results of the present study because the *in vitro* studies may not always reflect clinical conditions.

Conclusion

In the light of the results of this study, activation of NaOCl and CHX irrigation solutions by Er,Cr:YSGG laser or an ultrasonic system can be useful in the elimination of the *E. faecalis* from the canal. Photodynamic therapy showed a lower performance compared to these methods.

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