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ORIGINAL ARTICLE

Antibacterial efficacy of 810-nm diode laser on the biofilm formation by *Enterococcus faecalis* in root canals: an in vitro study

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Abstract

Aim This study aimed to evaluate the antibacterial efficacy of 810-nm diode laser on the root canals infected with *Enterococcus faecalis* biofilm-like structure by using comparable and safe parameters.

Methodology The root canals of 52 extracted human single-rooted teeth were prepared, 4 teeth allocated as negative control and 48 teeth inoculated with *E. faecalis* for 3 weeks. The teeth were then randomly divided into the following 4 experimental groups: Group NaOCl ($n = 12$), 17% EDTA + 5.25% NaOCl + saline; Group DL1 ($n = 12$), 17% EDTA + 1 W diode laser + saline; Group DL2 ($n = 12$), 17% EDTA + 1.5 W diode laser + saline; Group S ($n = 12$), Saline. Samples were obtained from dentin chips before and after the interventions. A reduction in colony count was assessed by counting the colony-forming units.

Results Compared to the control group, significant reductions were noted in *E. faecalis* colony counts in all groups ($p < .05$) except Group S ($p > .05$). The greatest reduction in colony count (98.9%) was noted in the Group NaOCl. The difference in this respect between the Group DL2 and Group S ($p < .05$) was significant; however, no significant difference was noted between Group DL1 and Group S ($p > .05$).

Conclusion Our results demonstrated two different parameters of 810-nm diode laser showing the significant antibacterial effect on *E. faecalis* biofilm but it was not as effective as NaOCl irrigation.

Keywords *Enterococcus faecalis* · Root canal · Diode laser

Introduction

A successful outcome of endodontic treatment depends on the maximum reduction of intra-canal bacteria [1]. Anatomical complexity of root canal system is the main challenge of microbial control during endodontic treatment; apical ramifications, lateral canals, and isthmuses connecting main root canals are shown to be a reservoir for bacterial cells, which are also generally organized in biofilm-like structures [2–4]. It has been mentioned that bacteria could be 100–1000 times more resistant to antibacterial agents than their planktonic counterparts [5–7]. *Enterococcus faecalis* is a Gram-positive facultative anaerobic coccus known for its ability to form intra-radicular and extra-radicular biofilms responsible for many cases of endodontic treatment failures [8, 9]. It can survive in inadequate nutritional conditions and penetrate deep into dentin tubules thus protecting itself from drugs in the root canal [10, 11].

Conventional chemo-mechanical approaches have been considered as the basic element of root canal treatment [12, 13]. However, it has limited success in persistent endodontic infections because of untouched areas after completion of the preparation and poor penetration of the irrigants and medications [14, 15]. Sodium hypochlorite (NaOCl) as the most commonly used irrigation solution can penetrate the dentinal tubules by 130 mm, whereas bacteria can penetrate by 1000 mm [16].

Among many other techniques developed to improve the disinfection of root canal, high-power diode lasers have been suggested with their ability to reach areas that are impossible to do so with traditional techniques [17]. The dentin absorption coefficient is low for the 810-nm wavelength, which is why the dispersion is superior to the absorption. This causes

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the photons to be absorbed farther away from the irradiation surface so its antibacterial effect can be seen in deep dentin layers [18, 19]. In previous studies, its antimicrobial effectiveness against various microorganisms has been shown but according to some, it was not more effective than NaOCl irrigation [20, 21].

There are many studies on antibacterial efficacy of 810-nm laser; however, the parameters are not exactly comparable and its antibacterial activity on biofilm is not mentioned [22]. In this study, we aimed to evaluate the antibacterial activity of 810-nm diode laser on *E. faecalis* biofilm by using comparable and safe parameters and to compare it with conventional NaOCl irrigation.

Materials and method

Teeth selection and preparation

Single rooted fifty-two teeth with uniform dimensions and completely formed apices were selected. The teeth were radiographically confirmed to have a single canal. The approval for this study was obtained by the Ethics Committee of the Dentistry Faculty of Bezmialem Vakıf University (No: 2016/89). Tooth crowns were removed and all canals reached the standard size of 14 mm. The working length (WL) was established by inserting a K-file #15 (Dentsply, Maillefer, Tulsa OK, U.S.A.) in the canal until its tip was just seen at the apical foramen. The working length was considered 1-mm short of the apical foramen (13 mm). Roots were instrumented with step-back technique and hand stainless steel instruments used to size #40 (K-type file; Mani Inc., Nakaakutsu, Japan). During preparation, the canals were irrigated with 1 ml of 5.25% NaOCl between each instrument using a disposable 2-ml syringe and a 30-gauge needle (BD Microlance, Becton Dickinson, Madrid, Spain). After preparation, the canals were rinsed with 1 ml 17% EDTA for 3 min using a 30-gauge needle to remove the smear layer. Finally, all canals were rinsed with 5 ml saline solution. The apical foramen was then sealed with self-cure glass ionomer (GC Co, Tokyo, Japan) and the root surfaces were covered with 2 layers of nail varnish. The teeth were then transferred into 2-ml microtubes and autoclaved at 121 °C for 15 min.

Bacterial inoculation of root canals and biofilm generation

The pure culture is prepared by *E. faecalis* (American Type Culture Collection ATCC 29212) passaged to 5% sheep blood agar (Salubris Inc., Istanbul, Turkey) from − 80 °C stock and the turbidity is adjusted to 0.5 McFarland (1.5 × 108 CFU/ml) spectrophotometrically in tryptic soy broth (TSB; Oxoid, Hampshire, England) for inoculation of the root canals. Teeth were placed in 1.5-ml sterile microtubes individually and filled with bacterial suspension. After the initial inoculation, the root canals were reinoculated every 48 h with the same amount of the bacterial suspension following the aspiration of the previous bacterial suspension. The identity and purity of the *E. faecalis* culture were checked both by VITEK MS (bioMerieux, Marcy-l'Étoile, France) Gram stain and observation of colony morphology on agar media before every inoculation. The samples were incubated at 35 °C at 5% CO_2 conditions for 3 weeks. The teeth were washed three times with phosphate-buffered saline when the incubation period was completed.

Four teeth were dispersed as a negative control and incubated in sterile TSB. During the 3 weeks of the incubation period, the media of the sterility control teeth was also replaced every other day to replicate the experimental procedure in order to check the possible contamination that could arise from both the procedure and the long incubation period.

Following the incubation period, two randomly selected teeth from negative and saline groups (Group S) were stored in 10% buffered formalin and prepared for the scanning electron microscopy (SEM) to visualize the non-infected dentin tubules and the pattern of colonization respectively (Figs. 1a, b and 2a, b).

Experimental procedures

The specimens were randomly divided into 4 experimental groups ($n = 12$) as follows:

Group NaOCl ($n = 12$) The root canals were rinsed conventionally with 5 ml 5.25% NaOCl, for 1 min. Next, 5 ml 5% saline was injected into the root canals by a 30-G syringe and remained in the root canals for 30 s to neutralize the NaOCl.

Group DL 1 (810 nm 1 W) ($n = 12$) Intra-canal irradiation was performed with an 810-nm DL (ARC Laser, Nurnberg, Germany) (1 W, CW). A DL with a 200-mm diameter fiber tip was used 1 mm short of the apex and moved from the apex toward the coronal part in a rotary motion for 7 s. This circle was repeated 4 times with a relaxation time of 20-s and 3 intervals.

Group DL 2 (810 nm 1.5 W) ($n = 12$) The procedure was the same as *Group DL 1*, except the laser was applied with 1.5 W power.

Group S ($n = 12$) The root canals were rinsed with 5 ml saline solution by 30-G syringe.

Sampling procedures

Bacterial samples were obtained from each specimen before and after each intervention protocol.

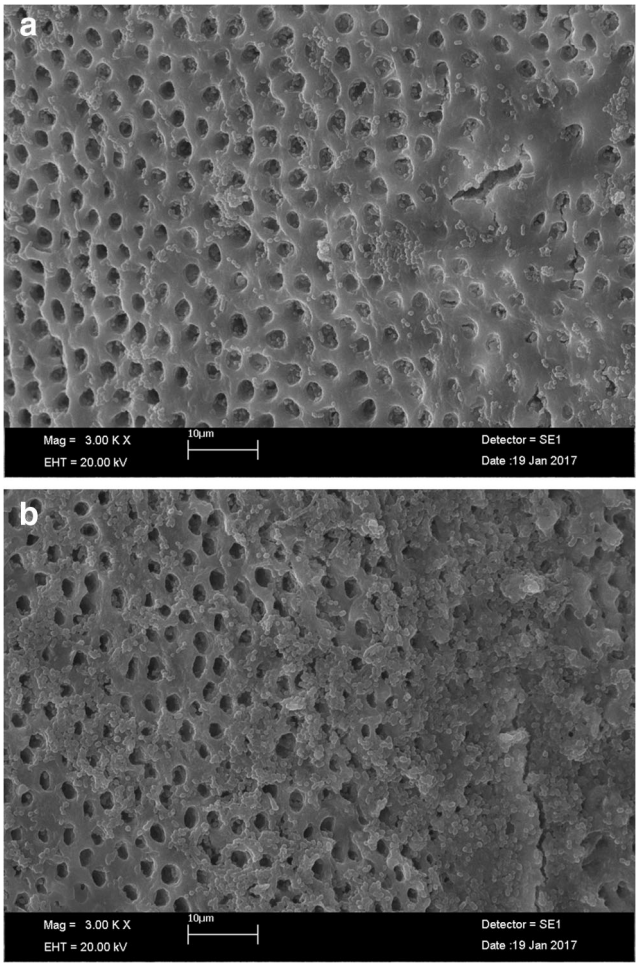

Fig. 1 **a** Dentinal tubules infected with *Enterococcus faecalis* biofilm. **b** Dentinal tubules infected with *Enterococcus faecalis* biofilm

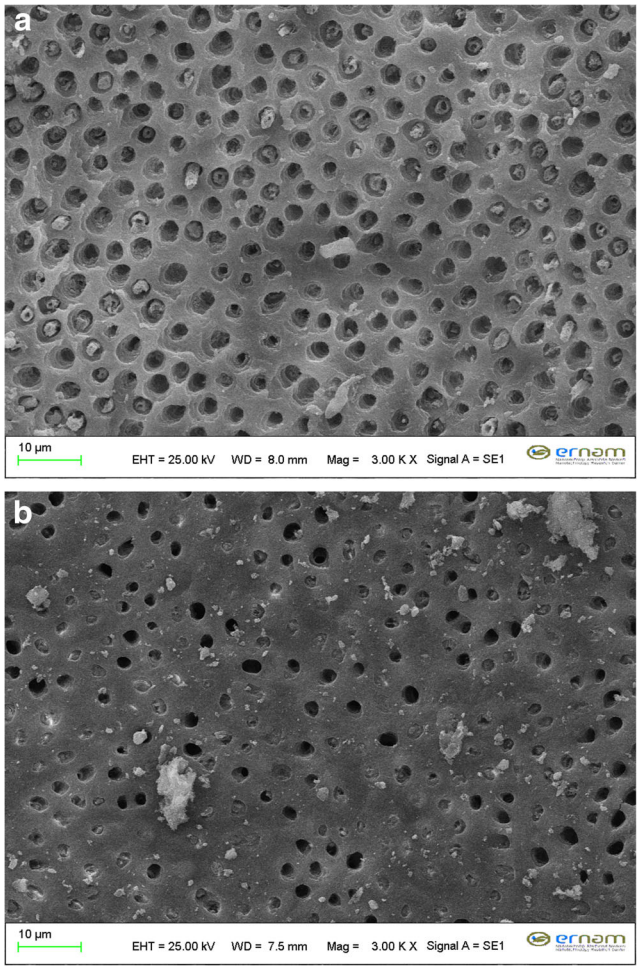

Fig. 2 **a** SEM image of the tooth from the negative control group. **b** SEM image of the tooth from the negative control group

Before intervention

The root canals were rinsed with a sterile saline solution using a 30-G syringe to eliminate planktonic bacteria and then to scrape biofilm samples from inside the root canals, a #40 Hedstrom file (Mani Inc., Tochigi, Japan) was used for 10 s with circumferential filing movement. A #40 sterile paper point (Gapadent Co, Hamburg, Germany) was placed inside the canals for 30 s. Both the H files and paper points were then transferred into sterile microtubes containing 1 ml saline solution.

After intervention

To standardize all groups and eliminate the smear layer caused by primary sampling procedure root canals were irrigated with 5 ml 17% EDTA canals for 30 s. For laser groups, the laser was calibrated to confirm and check real output powers before each usage then treatments were applied. A #45 Hedstrom file (Mani Inc., Tochigi, Japan) was used and the same sampling procedure was run.

Statistical analysis

Data were analyzed using SPSS version 22 (IBM SPSS, Türkiye). The normal distribution of variables was evaluated by the Kolmogorov-Smirnov test. The Kruskal-Wallis test was used to compare the percentage of reduction in colony count (RCC%) among the understudy group and the Mann-Whitney *U* test was used to determine the group causing the difference. Wilcoxon sign test was used for intra-group comparison. Significance was assessed at $p < 0.05$.

The RCC(%) calculation made with the following equation;

$$\frac{\text{CFUs (before treatment)} - \text{CFUs (after treatment)}}{\text{CFUs (before treatment)}} \times 100 = \text{RCC}(\%)$$

Results

The variables evaluated with the Kolmogorov-Smirnov test did not show normal distribution. The significant reductions in the *E. faecalis* count were seen in all groups except Group S

($p < 0.05$) (Table 1). The highest RCC(%) was noted in the Group NaOCl and was significantly different from other groups. The lowest RCC(%) was found in Group S (Fig. 3). Laser groups (Group DL 1, Group DL 2) were not significantly different in terms of RCC(%) ($p > .05$). The difference in this respect between Group DL2 ($p < .05$) and Group S ($p < .05$) was significant, but no significant difference was noted between Group DL1 and Group S ($P > .05$) (Table 2).

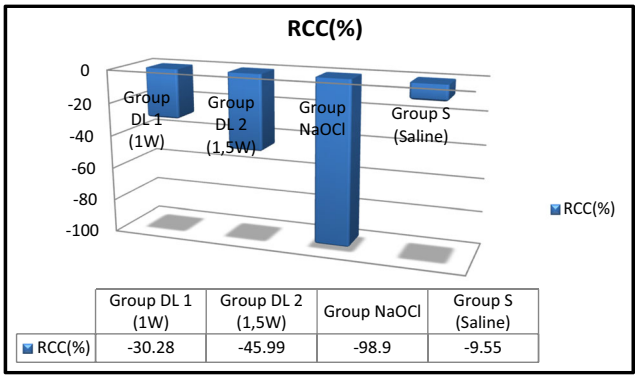

Fig. 3 RCC(%) after the intervention

Discussion

Traditional chemo-mechanic preparation is the most common way of attaining successful root canal treatment [16]. However, according to a previous study, chemo-mechanical preparation is only effective at the entrances of lateral canals and dentinal walls in 75% of the teeth investigated [2]. On account of that, near-infrared may be considered as an alternative because of their ability to reach deep dentin layers [19]. In this study, we aimed to assess the antibacterial activity of the 810-nm diode laser on *E. faecalis* biofilm alone and compare it with the most common NaOCl irrigation.

Endodontic infections are often related to multiple species; however, in the current study, the monospecies infection model was used to reproduce the same biofilm-like structure in each root canal of specimens [23]. *E. faecalis* was chosen as a microbiological marker because it has been identified frequently in cases with refractory endodontic infections [24, 25]. Besides that, it can colonize deep tubules and form biofilm [9]. Biofilm growth is a continuous process and for in vitro studies, there is no consensus of a specific biofilm model [26]. The significance of biofilm age has been demonstrated and also it has been mentioned that after 3 weeks of incubation, the bacterial biofilm becomes resistant to antibacterial agents [14, 27]. Because of that, in the present study, the incubation time was determined as 3 weeks.

NaOCl is the only irrigant in endodontics that can dissolve organic tissue, commonly used in concentrations between 0.5% and 6% NaOCl. Although the toxicity of NaOCl increase with concentration, previous studies demonstrated that 6% NaOCl solution is the only agent able to remove *E. faecalis* biofilm [28, 29]. In the present study, 5.25% NaOCl was chosen because of its outstanding antibacterial effect.

Nelakantan et al. [30] reported that the NaOCl-EDTA combination results in better dentinal tubule disinfection than the other combinations. Furthermore, using chelating agents before diode laser irradiation also has a positive effect on antibacterial activity [31, 32]. This can be explained with the deeper penetration of the laser beam into dentinal tubules because of additional smear layer removal [33]. In this study, to enhance the antibacterial activity in all experimental groups, each specimen irrigated with 17% EDTA after the primary sampling procedure.

Beer et al. [34] evaluated the bactericidal effect of two diode lasers and for 810-nm diode laser, 98.8% reduction has been reported. That result did not corroborate our study. The inconsistency might result from the differences in the incubation periods between studies. In the study of Beer et al., a 2-h incubation was performed, whereas in the present study, the samples were incubated for 3 weeks. Moreover, in a recent study, Ghorbanzadeh et al. showed that the biofilm maturation times had a significant effect on the antibacterial properties of evaluated disinfection methods [35].

Table 1 *Enterococcus faecalis* colony-forming units before and after treatment

	Before treatment Mean ± SD	After treatment Mean ± SD	[2]*p*
Group DL1 (1w)	$36.5 \times 10^4 \pm 21.8 \times 10^4$	$14.6 \times 10^4 \pm 15.3 \times 10^4$	0.012*
Group DL2 (1.5w)	$13.7 \times 10^4 \pm 7.7 \times 10^4$	$6.3 \times 10^4 \pm 4.7 \times 10^4$	0.012*
Group NaOCl	$44.9 \times 10^4 \pm 79.3 \times 10^4$	$0 \pm 0.3 \times 10^4$	0.002*
Group S	$10.9 \times 10^4 \pm 7 \times 10^4$	$10.6 \times 10^4 \pm 9.9 \times 10^4$	0.386
[1]*p*	0.017*	0.000*	

[1] Kruskal-Wallis test

[2] Wilcoxon sign test

* $p < 0.05$

Table 2 Post hoc evaluation

	RCC(%) *p*
Group 1 (1 W)–Group 2 (1,5 W)	0.564
Group 1 (1 W)–Group 3 (NaOCl)	0.000*
Group 1 (1 W)–Group 4 (SF)	0.086
Group2 (1,5 W)–Group 3 (NaOCl)	0.000*
Group 2 (1,5 W)–Group 4 (SF)	0.012*
Group 3 (NaOCl)–Group 4 (SF)	0.000*

Mann-Whitney *U* test

* $p < 0.05$

Moritz et al. introduced the diode laser system to root canal treatment and a follow-up in vivo study has shown its bactericidal effects under clinical conditions [36, 37]. After those studies, comparable results were obtained by other researchers [19, 38]. According to a recent study, 810-nm diode laser has eliminated 97% of the bacteria, which is as effective and even better than 2.5% NaOCl irrigation [20]. However, Ghorbanzadeh et al. demonstrated that diode laser alone was ineffective in the elimination of *E. faecalis* biofilm [35]. This result is consistent with the present study. The discrepancies between studies presumably arise from differences in methodology, for example, the concentration of NaOCl, duration of irrigation, and applied laser parameters.

In our study, 810-nm diode laser has shown a significant amount of antibacterial activity. Similar results were noticed by others where 810-nm diode laser was significantly more effective than the saline group [39, 40]. Even though NaOCl treatment seems most effective in reducing colony-forming units of bacteria, the total volume of irrigation was different and more in NaOCl group (5 ml NaOCl and 5 ml saline, 10 ml) compared to the other groups (5 ml saline) and this might lead to better antibacterial effect for the NaOCl group. Also according to previous studies, 810-nm laser showed better penetration into the dentin tubules than the NaOCl irrigation but in this study, the sampling procedure could interfere with better results for NaOCl group because #45 Hendstrom was not enough to get deep dentin samples [19, 32].

In the present study, the limitations of the sampling procedure, such as collecting the bacteria mostly from main root canal walls, impeded the acquisition of information about the penetration and disinfection effect of the 810-nm diode laser and the NaOCl irrigation on deep dentinal tubules. Recently, quantification of the antibacterial effect of irrigants and medicaments extending into dentinal tubules could be achieved by confocal laser scanning microscope (CLSM) and viability bacterial stains which permitted the evaluation of the antibacterial effect extending into dentinal tubules [41]. In addition to that, the antibacterial efficacy in this study was evaluated with a culture-based reduction colony count (RCC%) method which is an old method and could not provide data on bacterial survival rate. On the other hand, direct observation techniques using CLSM could give information about microbial viability [42].

At the end of the experimental procedure, the data was analyzed with a non-parametric test (Mann-Whitney *U* test). As a limitation of the study, high S deviation was detected and it might have been due to the limited sample groups, sampling procedure, and individual differences.

Conclusion

Within the limitation of the present study, the results have shown that the application of the 1.5 W 810-nm diode laser alone compared to the saline group was significantly more effective in reducing colony-forming units of *Enterococcus faecalis*, the most notable culprit of treatment-resistant endodontic infections. On the other hand, no difference was noticed between the 1 W 810-nm diode laser and the saline group in terms of antibacterial activity. These results indicate the importance of the power of the laser.

Consequently, the drawback associated with NaOCl treatment keeps 810-nm diode laser usage as an alternative. Additional studies are warranted to investigate the details harnessing benefit from its antibacterial activity and define evidence-based "gold standard" for the treatment outline, the diameter of fiber, the settings of the laser parameters (power, pulse frequency), the duration of irradiation, and the irrigation protocol before laser application.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The approval for this study was obtained by the Ethics Committee of the Dentistry Faculty of Bezmialem Vakıf University (No: 2016/89).

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