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ORIGINAL ARTICLE

Bactericidal efficacy of three parameters of Nd:YAP laser irradiation against *Enterococcus faecalis* compared with NaOCl irrigation

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Abstract

The success of endodontic treatment depends on the thorough removal of microorganisms from the root canal system. The search for new ways to eliminate the microorganisms is therefore justified. Nd:YAP is a laser that uses yttrium aluminum perovskite, doped with neodymium crystal, as active laser medium. We used the Nd:YAP laser in an in vitro experiment to evaluate the bactericidal effect of three parameters of Nd:YAP laser-activated irrigation on biofilms of *Enterococcus faecalis* in root canals. The canals of 45 extracted human single-root teeth were prepared on a #35 Mtwo instrument and contaminated with *E. faecalis* for 14 days. Forty infected single-root teeth were then randomly divided into four groups according to the irrigation agitation protocols as follows: 5.25% sodium hypochlorite (NaOCl), Nd:YAP laser (180 mJ) + NaOCl, Nd:YAP laser (280 mJ) + NaOCl, and Nd:YAP laser (360 mJ) + NaOCl. The remaining bacteria were counted immediately using the cell count method. Teeth were firstly spilt and one half examined by scanning electron microscopy (SEM). The other half involved examination of bacterial colonization in dentinal tubules using confocal laser scanning microscopy (CLSM). Nd:YAP laser (280 mJ) + NaOCl and Nd:YAP laser (360 mJ) + NaOCl completely removed the *E. faecalis* biofilms from the root canal walls and made it the cleanest among the treatment groups. Bacterial reductions in the treatment groups for dentinal tubules are presented in a descending order as follows: Nd:YAP laser (360 mJ) (53.7%), Nd:YAP laser (280 mJ) (51.5%) > Nd:YAP laser (180 mJ) (45.3%) > 5.25% NaOCl (31.9%) > control (19.3%) ($p < 0.05$). Nd:YAP laser of 280 mJ and 360 mJ showed effective bactericidal effect in removing *E. faecalis* biofilm from the root canal walls and dentinal tubules.

Keywords Ed:YAP laser · *Enterococcus faecalis* · Parameters · Antibacterial effect

Introduction

Bacterial invasion in the root canal will result in a multi-bacterial infection process in which the strictly anaerobic microorganisms play an important role [1]. Insufficient disinfection of root canals would lead to treatment failure and persistent periapical pathology [2]. Therefore, endodontic treatment should promote proper cleaning and disinfection of the root canal system [3, 4]. One of the bacterial species most commonly found in root canal infections is *Enterococcus faecalis* [5–7], a facultative anaerobic gram-positive bacterium that can resist the defense systems of the host and produce pathological changes [8]. One of the most important characteristics of this bacterium is its ability to penetrate into dentinal tubules and survive extreme challenges which enable it to escape the action of endodontic treatments and irrigants used during chemomechanichal preparation [3, 5, 9, 10].

Sodium hypochlorite (NaOCl) solution is the most frequently used irrigants during the chemomechanical debridement of root canals because of its broad antimicrobial spectrum and capacity to dissolve organic tissue remnants [11–13]. However, NaOCl is highly irritating when in contact with periapical tissues, causing alterations in the dentin collagen and reducing the resistance of teeth to fracture. It also interferes negatively with bond strength of adhesive restorations to dentin [14–17]. Because of the mentioned side effects of this irrigant, researchers have developed alternative endodontic irrigants. Research has recently been conducted through application of multiple lasers in endodontology for intracanal application and their effects on infected root canals [18–21]. When the effect of Nd:YAG laser application was compared to root

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canal conventional irrigation, it was found that the laser was inferior to the commonly used 5.25% NaOCl [22]. Its bactericidal effect is based on the temperature rise inside the root canal but with a minimal thermal effect on surrounding tissues [19].

A new Nd:YAP laser has been studied recently. This laser is considered to be superior to Nd:YAG for both temperature elevation and antibacterial ability due to its 1.34-μm wavelength which is in the infrared range [35]. This wavelength is absorbed better in water than that of Nd:YAG. However, there are few studies published so far on comparison between bactericidal effect of Nd:YAP laser and NaOCl irrigation technique. The aim of our current study was to evaluate the bactericidal effect of three parameters of Nd:YAP laser on biofilms of *E. faecalis* in root canal system and compare them with NaOCl.

Materials and methods

Tooth preparation

This research protocol was approved by the Ethics Committee of the School of Dental Medicine, University of Nanjing, China. Fifty freshly extracted, adult, human, intact, and matured teeth with single canals were collected and restored in 0.9% sterile saline at 4 °C temperature. The study included teeth with complete root formation without previous endodontic treatment and root caries. Age of patients ranged between 18 and 60 years. The teeth crowns were cut with a water-cooled diamond fissure bur by standardizing the root length to 12 mm. Teeth with apical foramen that could be easily passed with a #20 K-file (Dentsply, Maillefer, Ballaigues, Switzerland) or could not be initially passed with a #08 K-file were not included in the study. The working length (WL) was established by introducing a #15 K-file in the canal until its tip was visualized at the apical foramen. The canals were then sequentially prepared within 0.5-mm apical end of the canal via crown-down instrumentation technique up to master apical file size #35 with Mtwo rotary system (VDW, Munich, Germany) accompanied with 17% EDTA gel (Beyotime, Shanghai, China) to remove the smear layer. Then the canals were irrigated using the ultrasound instrument (P5 Newtron XS, France), followed by activation with 5.25% NaOCl (Tianshi Biological Technology Co. Ltd., Henan, China) for 1 min. After that, the teeth were washed with normal saline and placed in NaOCl 5.25%, followed by vortexing for 4 min. The apical foramen was sealed with a restorative glass ionomer (Fugi, GC2, Tokyo, Japan). Each tooth was transformed to a lab tube which contained sterile BHI (brain heart infusion) broth medium (Oxoid, Basingstoke, England). The teeth were sterilized in an autoclave for 15 min at 121 °C. Five teeth were randomly selected as negative control group and incubated in the BHI broth for 24 h. Lack of bacterial growth indicated samples not being contaminated by bacteria.

Bacterial culture

E. faecalis frozen bacteria (ATCC9854) were transformed to BHI agar plate and incubated for 24 h at 37 °C temperature. Single colonies were inoculated with 10-mL BHI broth medium for 24 h at 37 °C temperature. A 1.5×10^8 CFU/mL which equaled 0.5 McFarland was then prepared. *E. faecalis* biofilm was grown in root canals using a standardized biofilm growth protocol. Briefly, 100 μL of the suspension was injected into each canal using syringe without overflowing. The suspension was carried to the entire root canal length using a #15 K-file. The roots were incubated at 37 °C for 2 weeks to allow propagation of the bacteria into the dentinal tubules. The medium was replaced every 2 days with fresh medium. After incubation, five teeth were randomly selected as positive control group. The five teeth were then split longitudinally with an acuminate chisel and hammer, with one root-half randomly selected from each root and stained with a fluorescent LIVE/DEAD BacLight bacterial viability stain (Molecular Probes, Eugene, OR, USA) according to the manufacturer's instructions. Another half was observed using a scanning electron microscopy (SEM) to visualize the pattern of *E. faecalis* biofilm.

Final irrigation with different agitation protocols

Five canals were left untreated (control) and the remaining 40 samples were randomly divided into four experimental groups ($n = 10$/each). The groups were treated as follows:

In group 1: the roots were treated with syringe irrigation using 5.25% NaOCl (5 mL, 60 s).

In group 2: the 5.25% NaOCl was activated by the Nd:YAP laser (Lokki dt, Vienne, France) at 180 mJ, 0.9 W.

In group 3: the 5.25% NaOCl was activated by the Nd:YAP laser at 280 mJ, 1.4 W.

In group 4: the 5.25% NaOCl was activated by the Nd:YAP laser at 360 mJ, 1.8 W.

For group 1, the needle was moved back and forth between the site of 1 mm short to the WL and 1 mm below the orifice of the canals. For groups 2–4, the 200-μm flexible endodontic fiber was placed 3 mm away from the apex for 5 s with a 10-s interval in an apical-to-coronal motion along the long axes of the canals and repeated four times in a row.

Inhibition of *E. faecalis*

Evaluation of bacterial reductions

The five untreated and ten treated canals in each treatment group were used to measure the number of viable bacteria before and after treatments, respectively. Each sample of bacteria was collected by sequential placement of #30 sterile paper points (ProTaper NEXT paper points, Dentsply, Switzerland): before treated with NaOCl and Nd:YAP laser, sterile saline moisturizing paper point was remained in the canal for 1 min to obtain the sample of bacteria, then the canals were treated with NaOCl and Nd:YAP laser, followed by collecting the sample of bacteria again as mentioned above. The bacteria from five untreated canals as control were also collected by sterile paper points twice with 1-min interval. To avoid contamination, all the procedures were performed in a laminar flow hood. The paper points were then transferred into a vial containing 1 mL of sterile distilled water, followed by vortexing for 1 min. Bacterial suspensions were diluted in tenfold steps and 100 μL of each dilution was spread onto BHI agar plates that were incubated at 37 °C for 48 h, followed then by recording of their CFUs. The numbers of CFUs in each group before treated multiplied by the corresponding dilution ratios represented a close estimate number of viable bacteria before treatments. The number of viable bacteria before and after treatments was recorded.

Confocal laser scanning microscopy analysis

Grooves were first made along the buccal and lingual surfaces using a diamond disc. The roots were then split into two halves longitudinally in the buccolingual direction with a hammer and acuminate chisel to expose the dentine surface of the canal wall. A LIVE/DEAD BacLight bacterial viability stain (Molecular Probes, Eugene, OR, USA) containing SYTO 9 and propidium iodide (PI) was used according to the manufacturer's instructions. The specimens were washed with sterile saline, then stained with 20 μL of Live/Dead reagent in the dark for 20 min. After that the stained specimens were washed with sterile distilled water for 1 min. The stained root-halves were examined with confocal laser scanning microscopy (CLSM) (Nikon A1 Si; Nikon Corporation, Tokyo, Japan). The excitation/emission wavelengths were 500/530 nm for SYTO 9 and 552/617 nm for PI, respectively. Simultaneous dual-channel imaging was used to display green fluorescence (live cells) and red fluorescence (dead cells). For CLSM analyses, the percentage of combined red to red-green was calculated for each group: killing rates = intensity of red/(intensity of red + intensity of green) × 100. The measured red/green fluorescence intensities were used to calculate the percentage of dead bacteria over both dead and live bacteria.

Scanning electron microscopic evaluation

The specimens from each group were prepared and examined by SEM to identify the morphology and distribution of bacteria on wall of root canals. The split specimens were stored in 2.5% glutaraldehyde for 24 h, followed by dehydration with ethyl alcohol (30%, 50%, 60%, 90%, and absolute alcohol twice for 20 min each) sequentially, drying in alyophilized (ES-2030, Hitachi, Japan), sputter-coating with platinum (Ion Sputter E-1045, Hitachi, Japan), and observation by SEM (Tescan Vega TS5136LS, Brno, Czech Republic) to visualize the pattern of colonization. When viewing the root specimens, each sample was aligned so that the long axis of the root canal was vertical on the visual display unit (VDU) of the scanning electron microscope. And the same area of each specimen could be observed in both coronal and apical area.

Statistical analysis

The number of viable bacteria was expressed as the mean ± standard deviation and analyzed using a one-way analysis of variance followed by the Tukey test using the SPSS statistics package for Windows (version13.0; SPSS, Inc., Chicago, IL, USA). The statistical significance level was set at $\alpha = 0.05$. The percentage of live cells (green) was assessed by using the Kruskal-Wallis test and Dunn test for multiple comparisons ($\alpha = 0.05$).

Results

Microbiological analysis

Antibacterial test against *E. faecalis* revealed that Nd:YAP of 280 mJ, 1.4 W, and 360 mJ, 1.8 W, almost totally inhibited the growth of *E. faecalis*. The 5.25% NaOCl showed limited inhibitory effect on *E. faecalis* in Fig. 1. The mean and standard deviation for contamination levels between different treatment protocols before treatment and after treatment are expressed in $\log^{10}$ CFU/mL in Fig. 1f. We found in our experiment that the amounts of *E. faecalis* decreased from 9.665 $\log^{10}$ CFU/mL to 6.634 $\log^{10}$ CFU/mL after treatment with 5.25% NaOCl ($p < 0.05$), as shown in Fig. 1f. Groups 3 and 4 showed the lowest mean contamination (0 $\log^{10}$ CFU/mL) and when compared with 5.25% NaOCl, the Nd:YAP laser presented stronger antibacterial activity ($p < 0.05$).

Scanning electron microscopy

Figure 2 shows the SEM images of split-fractured root-halves showing that numerous *E. faecalis* cells were colonized on the root canal wall of the untreated samples, aggregating into grapelike colonies to develop a biofilm,

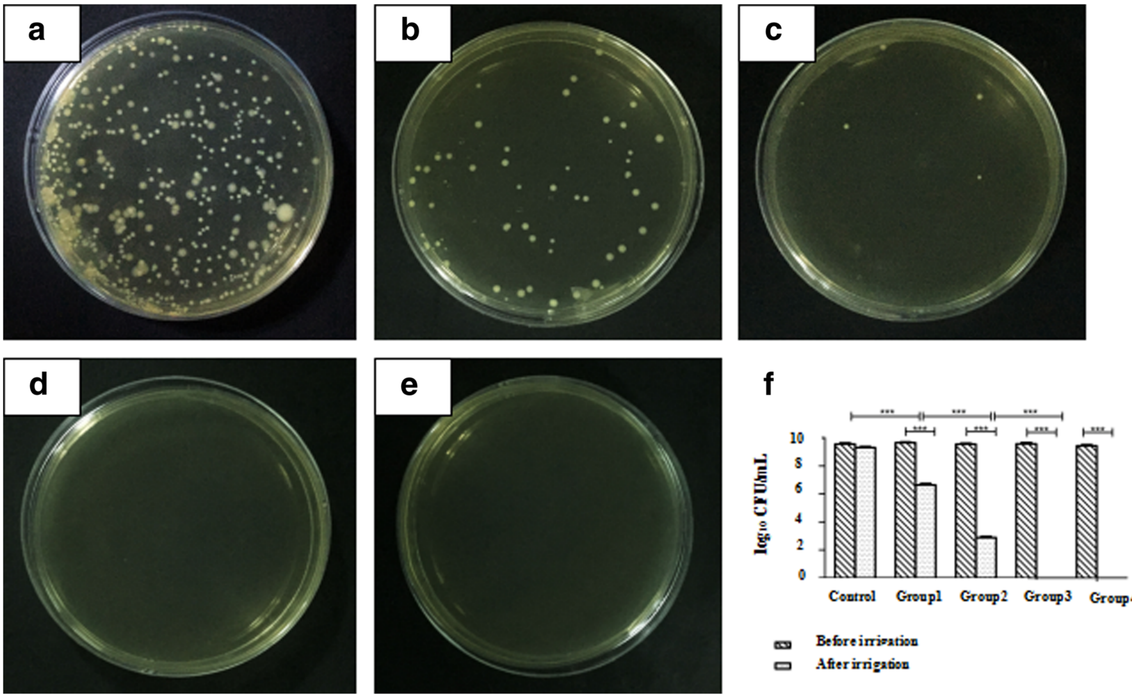


Fig. 1 Antibacterial effects of different irrigation protocols against *E. faecalis*. **a** A representative image of CFUs before irrigation. **b** A representative image of CFUs of 5.25% NaClO. **c** A representative image of CFUs for Nd:YAP laser (180 mJ) activated irrigation using 5.25% NaClO. **d** A representative image of CFUs for Nd:YAP laser (280 mJ) activated irrigation using 5.25% NaClO. **e** A representative image of CFUs for Nd:YAP laser (360 mJ) activated irrigation using 5.25% NaClO. **f** CFU/ml counts of *E. faecalis* bacteria before and after irrigation. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{*}p < 0.05$

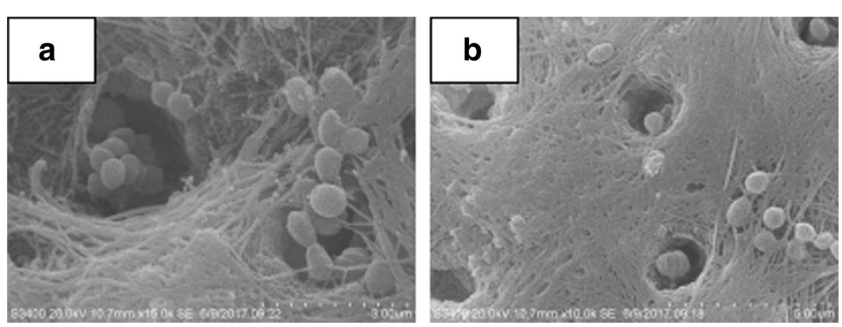
and there was large number of mycelial connections between *E. faecalis* cells (Fig. 2a, b). *E. faecalis* biofilm was still observed on the root canal walls treated with 5.25% NaOCl, but there were no mycelial connections between *E. faecalis* cells (Fig. 3(a1–a4)).

Only a few *E. faecalis* were observed in the coronal of canals treated with Nd:YAP of 180 mJ, 0.9 W (Fig. 3(b1–b2)), and no *E. faecalis* bacteria were observed in the canals treated with Nd:YAP of 280 mJ, 1.4 W, and 360 mJ, 1.8 W (Fig. 3(c, d)). Melting areas were present on the specimens' surfaces of coronal with craters treated with Nd:YAP of 280 mJ, 1.4 W (Fig. 3(c1–c2)). Very few or no open tubules were observed after treatment with Nd:YAP of 360 mJ, 1.8 W, and the dentinal tubules were sealed or partially sealed, with diameters ranging from 0 to 2 μm. Melting areas were also present on the specimens' surfaces craters. Moreover, double-layered structures of tubules were observed in the apical area of the roots after treatment with Nd:YAP of 280 mJ, 1.4 W, and 360 mJ, 1.8 W.

Efficiency of killing Bacteria in dentinal tubules

We applied CLSM to detect live versus dead bacteria in the dentinal tubules after staining. As shown in Fig. 4, representative images depict live/dead bacteria in the dentinal tubules in the roots treated by four different disinfection procedures. The results from analysis of those images are presented in Fig. 4(f). Bacterial reductions in the treatment groups for dentinal tubules are presented in a descending order as follows: group 4 (53.7%), group 3 (51.5%) > group 2 (45.3%) > group 1 (31.9%) > control (19.3%) ($p < 0.05$). There was no statistically significant difference between group 3 and group 4 ($p > 0.05$). Overall, the

Fig. 2 2-week-old *E. faecalis* biofilm colonization on the root canal wall. **a** Represents coronal of the root canal. **b** Represents apical of the root canal



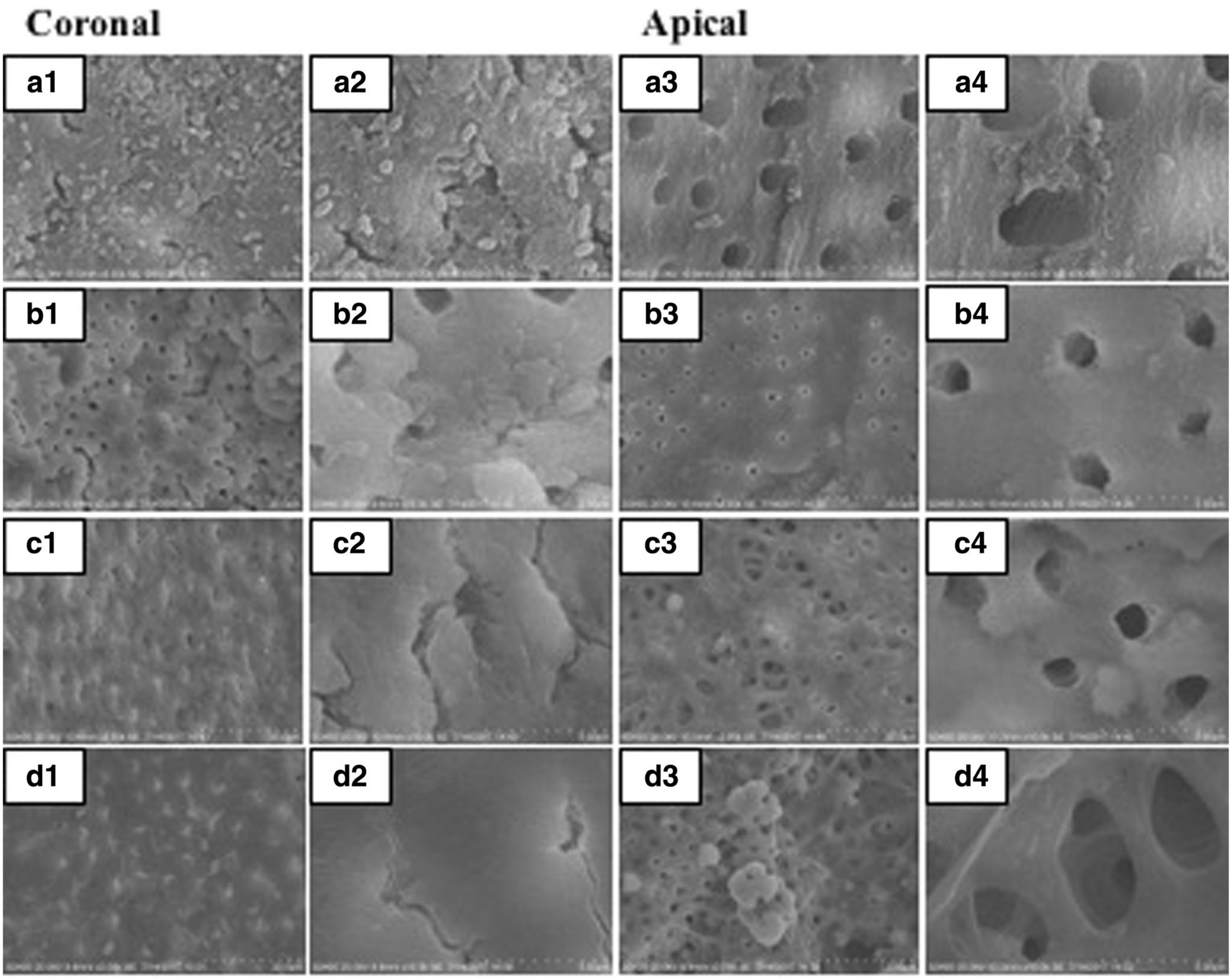


Fig. 3 Representative SEM images of root canal wall after different irrigation protocols. Coronal and apical representing the coronal and apical regions of the root canals. **a**–**d** Represent the root canals treated with NaOCl, Nd:YAP (180 mJ) + NaOCl, Nd:YAP (280 mJ) + NaOCl, and Nd:YAP (360 mJ) + NaOCl. Scale bar = 20 μm and 5 μm. SEM scanning electron microscopy

Nd:YAP laser of 280 mJ, 1.4 W, and 360 mJ, 1.8 W, had the highest level of dead bacteria in the dentinal tubules.

Discussion

E. faecalis is a gram-positive facultative anaerobic coccus bacteria commonly detected in asymptomatic, persistent endodontic infections that have been identified as a frequent cause of treatment failure in root canal therapy [21]. Its prevalence in such infections ranges from 24 to 77% [23]. *E. faecalis* can maintain a quiescent phase with a low metabolic activity in canals of root-filled teeth for a period of time and then cause a relapse of infection when there is a favorable nutritional condition [24]. Furthermore, the bacteria survive thermal variations because they grow at 10 °C and 45 °C and are resistant to several antimicrobial agents, including calcium hydroxide [1]. The resistance of *E. faecalis* correlates greatly to its biofilm formation ability [25, 26].

The antibacterial effects of biofilm can be up to 100–1000 times higher than planktonic bacteria [27, 28]. Elimination of bacteria in the root canal system, especially the bacterial biofilm, is therefore crucial to the success of root canal therapy [25]. The time of *E. faecalis* colonization or biofilm formation in the root canals was different in many studies [26]. Some studies incubated *E. faecalis* for a few hours, the others claimed 1 day incubation time was sufficient for the biofilm formation [14, 29]. A longer incubation time for the bacteria biofilm was more similar to the actual condition, which is why it is increasingly used in recent studies [30, 31]. Antibacterial protocols in this study were evaluated on 2-week *E. faecalis* biofilm and the pattern of colonization was confirmed by SEM. In our study, bacterial inoculation was performed at a concentration of 1.5×10^8 CFU/mL as done by other researchers such as Ivona Bago Juric et al. [32] and

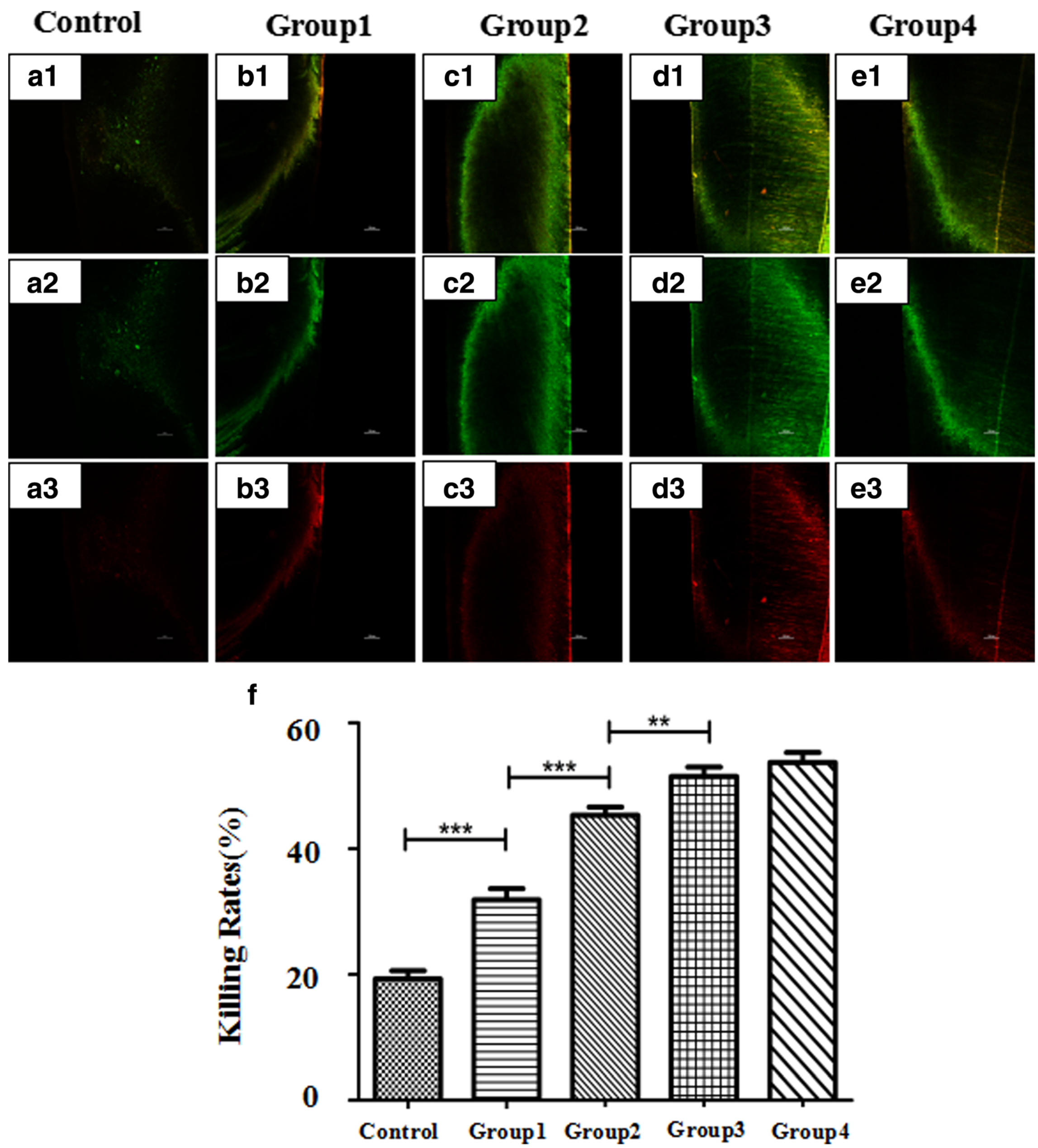


Fig. 4 CLSM analysis of live/dead bacteria in dentinal tubules. **a**2–**e**2 Represent the live bacteria in dentinal tubules. **a**3–**e**3 Represent dead bacteria in dentinal tubules. **a** Represents root canals before treatment. **b**–**e** Represent root canals treated with NaOCl, Nd:YAP (180 mJ) + NaOCl, Nd:YAP (280 mJ) + NaOCl, and Nd:YAP (360 mJ) + NaOCl. Scale bar = 100 μm. **f** Represents the cell viability in *E. faecalis* biofilms after treatment with different irrigants. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{*}p < 0.05$

Mohammad Asnaashari et al. In light of the results obtained from the positive control group by the SEM, this concentration was seen to be effective and achieved satisfactory experimental result in vitro root infection that could form *E. faecalis* biofilm [33]. In order to allow better penetration of the bacteria into the dentinal tubules, the smear layer was removed by irrigation with 17% EDTA.

NaOCl is currently the most widely used irrigation agent due to its wide antibacterial spectrum and its capacity to dissolve organic debris and necrotic tissue [34]. In this study, the application of 5.25% NaOCl reduced the *E. faecalis* count in root canals with statistically significant difference compared to the positive control group. However, *E. faecalis* bacteria were still attached to the root canal although the mycelium between the bacteria was destroyed. The Nd:YAP laser is a laser using yttrium aluminum perovskite doped with neodymium crystal as active laser medium [35]. The Nd:YAP laser beam shows clinically interesting properties, such as its good absorption by dark materials and metals. It is also 20 times more absorbed by water than the Nd:YAG laser. Moreover, its flexible fiber optic allows delivering energy in curved root canals where the effect of ultrasonic instrumentation is limited due to the constraining effect of the curvature of the canal [20].

The Nd:YAP laser with energies of 180 mJ, 280 mJ, and 360 mJ was thus chosen for bacterial reduction in this study. It has been used in oral surgeries for lingual frenulectomy and frenectomy and for initial treatment of periodontitis in adult [36]. The Nd:YAP laser has been used to enhance canal cleanliness in endodontic and restorative dentistry; however, there are few publications so far on its antimicrobial efficacy [37]. Substantial CFUs reductions of bacteria were obtained immediately after irradiation with Nd:YAP laser in our current study (28.8% in group 3, 0% in group 4, and group 5). Our results confirmed the possibility of using the Nd:YAP laser in the elimination of bacterial biofilm instead of conventional irrigation, which had never been reported in previous studies. Many researchers have studied other types of lasers for eliminating the bacterial biofilm. Sahar-Helfit et al. studied Er, Cr:YSGG laser which reduced the viable microbial population from the root canals with small and large apical foramina but did not eradicate all bacteria [38]. Neelakantan P et al. found that diode and Er:YAG laser were more effective in reducing the *E. fecalis* biofilms than ultrasonic activation and conventional syringe irrigation. Bahrololoomi Z et al. found that 1.5 W diode laser was effective in reduction of *E. faecalis* bacterial count without damaging periodontal structures [16]. Golob BS et al. found that Er:YAG laser activated irrigation with 5% NaOCl was effective in eradication of the *E. faecalis* biofilm and removal of the smear layer [39].

The Nd:YAP of 280 mJ and 360 mJ in our study resulted in the largest number of sterile samples. This feature was confirmed by CLSM examination in the present study. Red fluorescence, which is indicator of presence of dead bacteria, was significantly stronger in the Nd:YAP laser groups than 5.25% NaOCl, and some other morphological changes of root dentin walls were also seen following the Nd:YAP laser irradiation. The laser caused ablation of the dentin surface, vaporizing the intertubular and peritubular dentin in the apical region of the canals, thus exposing the dentinal tubules and showing double-layered structures of tubules. The melting areas were also present on the specimens' surfaces of coronal with craters. All these morphological aspects were probably due to the laser ablation process and thermal denaturing of the organic portion of the dentine, even when water irrigation was used [40]. These changes could thus be desirable for endodontic treatment, to allow the irrigant solutions as well endodontic sealer to penetrate further into the tubules. Yet it is not known whether the morphological changes have any side effects, such as reduction of resistance of teeth to fracture, hence further research is needed to confirm or rule out this.

Conclusion

The novel Nd:YAP laser of 280 mJ and 360 mJ showed effective bactericidal effect in removing *E. faecalis* biofilms from the root canal walls and dentinal tubules under presented conditions in this study.

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Compliance with ethical standards

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

References

1. Dos Santos Antonio MP, Moura-Netto C, Camargo SE, Davidowicz H, Marques MM, Maranhao de Moura AA (2012) Bactericidal effects of two parameters of Er:YAG laser intracanal irradiation: ex-vivo study. Lasers Med Sci 27(6):1165–1168
2. Sjogren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 30(5):297–306
3. Chivatxaranukul P, Dashper SG, Messer HH (2008) Dentinal tubule invasion and adherence by *Enterococcus faecalis*. Int Endod J 41(10):873–882
4. Kayaoglu G, Orstavik D (2004) Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. Critical reviews in oral biology and medicine: an official publication of the American Association of Oral Biologists 15(5):308–320
5. Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ (2008) Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. J Endod 34(5):537–540
6. Haapasalo M, Orstavik D (1987) In vitro infection and disinfection of dentinal tubules. J Dent Res 66(8):1375–1379
7. Sangalli J, Junior EGJ, Bueno CRE, Jacinto RC, Sivieri-Araujo G, Filho JEG, et al. Antimicrobial activity of *Psidium cattleianum* associated with calcium hydroxide against *Enterococcus faecalis* and *Candida albicans*: an in vitro study. Clin Oral Investig 2018
8. Siqueira JF Jr, Batista MM, Fraga RC, de Uzeda M (1998) Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. J Endod 24(6):414–416
9. Niazi SA, Clark D, Do T, Gilbert SC, Foschi F, Mannocci F et al (2014) The effectiveness of enzymic irrigation in removing a nutrient-stressed endodontic multispecies biofilm. Int Endod J 47(8):756–768
10. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M (2014) Effect of long-term exposure to endodontic disinfecting solutions on young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod 40(4):509–514
11. Tanomaru Filho M, Leonardo MR, Silva LA, Anibal FF, Faccioli LH (2002) Inflammatory response to different endodontic irrigating solutions. Int Endod J 35(9):735–739

12. Santos JN, Carrilho MR, De Goes MF, Zaia AA, Gomes BP, Souza-Filho FJ et al (2006) Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. J Endod 32(11):1088–1090
13. Farina AP, Cecchin D, Barbizam JV, Carlini-Junior B (2011) Influence of endodontic irrigants on bond strength of a self-etching adhesive. Australian endodontic journal: the journal of the Australian Society of Endodontology Inc 37(1):26–30
14. Cheng X, Guan S, Lu H, Zhao C, Chen X, Li N et al (2012) Evaluation of the bactericidal effect of Nd:YAG, Er:YAG, Er,Cr:YSGG laser radiation, and antimicrobial photodynamic therapy (aPDT) in experimentally infected root canals. Lasers Surg Med 44(10):824–831
15. Oter B, Topcuog LN, Tank MK, Cehreli SB. Evaluation of antibacterial efficiency of different root canal disinfection techniques in primary teeth. Photomed Laser Surg 2018
16. Bahrololoomi Z, Fekrazad R, Zamaninejad S (2017) Antibacterial effect of diode laser in pulpectomy of primary teeth. Journal of Lasers in Medical Sciences 8(4):197–200
17. Abuhaimed TS, Abou Neel EA (2017) Sodium hypochlorite irrigation and its effect on bond strength to dentin. Biomed Res Int 2017:1930360
18. Gurbuz T, Ozdemir Y, Kara N, Zehir C, Kurudirek M (2008) Evaluation of root canal dentin after Nd:YAG laser irradiation and treatment with five different irrigation solutions: a preliminary study. J Endod 34(3):318–321
19. Franzen R, Esteves-Oliveira M, Meister J, Wallerang A, Vanweersch L, Lampert F et al (2009) Decontamination of deep dentin by means of erbium, chromium: yttrium-scandium-gallium-garnet laser irradiation. Lasers Med Sci 24(1):75–80
20. Namour A, Geerts S, Zeinoun T, De Moor R, Nammour S (2016) Safety irradiation parameters of Nd:YAP laser beam for endodontic treatments: an in vitro study. Biomed Res Int 2016:4741516
21. Estrela C, Estrela CR, Decurcio DA, Hollanda AC, Silva JA (2007) Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. Int Endod J 40(2):85–93
22. Moshonov J, Peretz B, Brown T, Rotstein I (2003) Cleaning of the root canal using Nd:YAP laser and its effect on the mineral content of the dentin. J Clin Laser Med Surg 21(5):279–282
23. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB (2006) *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod 32(2):93–98
24. Love RM (2001) *Enterococcus faecalis*–a mechanism for its role in endodontic failure. Int Endod J 34(5):399–405
25. Swartz DB, Skidmore AE, Griffin JA Jr (1983) Twenty years of endodontic success and failure. J Endod 9(5):198–202
26. Pasqualini D, Cuffini AM, Scotti N, Mandras N, Scalas D, Pera F et al (2010) Comparative evaluation of the antimicrobial efficacy of a 5% sodium hypochlorite subsonic-activated solution. J Endod 36(8):1358–1360
27. Li Y, Sun K, Ye G, Liang Y, Pan H, Wang G et al (2015) Evaluation of cold plasma treatment and safety in disinfecting 3-week root canal enterococcus faecalis biofilm in vitro. J Endod 41(8):1325–1330
28. Hoyle BD, Costerton JW (1991) Bacterial resistance to antibiotics: the role of biofilms. Prog Drug Res 37:91–105
29. Siqueira JF Jr, Rocas IN (2005) Exploiting molecular methods to explore endodontic infections: part 1–current molecular technologies for microbiological diagnosis. J Endod 31(6):411–423
30. Pedulla E, Genovese C, Campagna E, Tempera G, Rapisarda E (2012) Decontamination efficacy of photon-initiated photoacoustic streaming (PIPS) of irrigants using low-energy laser settings: an ex vivo study. Int Endod J 45(9):865–870
31. Ordinola-Zapata R, Bramante CM, Aprecio RM, Handysides R, Jaramillo DE (2014) Biofilm removal by 6% sodium hypochlorite activated by different irrigation techniques. Int Endod J 47(7):659–666
32. Bago Juric I, Plecko V, Anic I (2014) Antimicrobial efficacy of Er,Cr:YSGG laser-activated irrigation compared with passive ultrasonic irrigation and RinsEndo((R)) against intracanal *Enterococcus faecalis*. Photomed Laser Surg 32(11):600–605
33. Asnaashari M, Mojahedi SM, Asadi Z, Azari-Marhabi S, Maleki A (2016) A comparison of the antibacterial activity of the two methods of photodynamic therapy (using diode laser 810 nm and LED lamp 630 nm) against *Enterococcus faecalis* in extracted human anterior teeth. Photodiagn Photodyn Ther 13:233–237
34. Baumgartner JC, Cuenin PR (1992) Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod 18(12):605–612
35. Gu Y, Perinpanayagam H, Kum DJ, Yoo YJ, Jeong JS, Lim SM et al (2017) Effect of different agitation techniques on the penetration of irrigant and sealer into dentinal tubules. Photomed Laser Surg 35(2):71–77
36. Ambrosini P, Miller N, Briancon S, Gallina S, Penaud J (2005) Clinical and microbiological evaluation of the effectiveness of the Nd:Yap laser for the initial treatment of adult periodontitis. A randomized controlled study. J Clin Periodontol 32(6):670–676
37. Moshonov J, Peretz B, Brown T, Rotstein I (2004) Cleaning of the root canal using Nd:YAP laser and its effect on the mineral content of the dentin. J Clin Laser Med Surg 22(2):87–89
38. Eldeniz AU, Ozer F, Hadimli HH, Erganis O (2007) Bactericidal efficacy of Er,Cr:YSGG laser irradiation against *Enterococcus faecalis* compared with NaOCl irrigation: an ex vivo pilot study. Int Endod J 40(2):112–119
39. Golob BS, Olivi G, Vrabec M, El Feghali R, Parker S, Benedicenti S (2017) Efficacy of photon-induced photoacoustic streaming in the reduction of *Enterococcus faecalis* within the root canal: different settings and different sodium hypochlorite concentrations. J Endod 43(10):1730–1735
40. Folwaczny M, Aggstaller H, Mehl A, Hickel R (2003) Removal of bacterial endotoxin from root surface with Er:YAG laser. Am J Dent 16(1):3–5