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ORIGINAL ARTICLE

Bactericidal effect of 445-nm blue diode laser in the root canal dentin on *Enterococcus faecalis* of human teeth

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Abstract

Objectives The objective of this in vitro study was to determine the bactericidal effect of a blue diode laser with a wavelength of 445 nm in dentin slices of human teeth.

Materials and methods A total of 81 dentin slices (longitudinal section) with different thicknesses of (300, 500, and 1000 μm) were used in this study. The dental slices were divided into three laser test groups and four control groups (positive and negative). All (except the negative control groups) were inoculated with a suspension of *Enterococcus faecalis* (2.0×10^7 CFU/ml). The tested groups were subjected to different irradiation parameters: group I (1.2 W peak power, 0.6 W average power), group II (0.6 W, CW), and group III (0.4 W, CW). The irradiation was performed using a bare fiber with a diameter of 200 μm at an angulation of approximately 5° to the slice under constant sinus-shaped movement of the slice. Identical irradiation conditions were applied using a computer-controlled table. This appliance allowed to move the slices in a sinusoidal motion in a constant velocity during irradiation.

Results The results of this in vitro study showed the irradiation with a chopped mode of 1.2 W eliminated an average of 96%, 97.6%, and 84.9% of *E. faecalis* at dentin depths of 300 μm, 500 μm, and 1000 μm, respectively. The irradiation with continuous mode 0.6 W eliminated 82.41% (300 μm), 83.75% (500 μm), and 77.42 (1000 μm). Whereas, lower power 0.4 W in continuous mode eliminated 6.90%, 32.50%, and − 9.68% at dentin depths of 300 μm, 500 μm, and 1000 μm, respectively.

Conclusion No statistical but conspicuous difference was found between group I and group II in all dentin slice thicknesses, with $p = 0.067$ ($p > 0.05$), whereas the bacterial counts in groups I and II compared to group III were significantly different with p values as $p = 0.002$ and $p = 0.01$ ($p < 0.05$) respectively. Reducing the power in continuous mode had decreased the antibacterial effect of this laser.

Keywords Diode laser · Bactericidal effect · *Enterococcus faecalis* · Endodontics · 445 nm

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Introduction

Since bacteria are the most important cause of endodontic infections, the eradication of persisting bacteria in the distant area of the tubular root canal system is a major challenge in the treatment techniques [1]. The role of microorganisms in the development and perpetuation of pulp and periapical diseases has clearly been demonstrated in animal models and human studies [2]. Therefore, many studies investigated the effect of different antibacterial strategies on the success of root canal treatment.

A study by Zou et al. showed that penetration of sodium hypochlorite into dentin was detected as a bleached zone from the root canal toward the periphery. The shortest penetration (77 μm) was measured after incubation with 1% NaOCl for

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2 min at room temperature. The highest penetration (300 μm) was obtained with 6% NaOCl for 20 min at 45 °C. After the initial penetration during the first 2 min, the depth of penetration doubled during the next 18 min of exposure. Temperature had a modest effect within each group on the depth of penetration and in most cases was not statistically significant ($P > .05$) Depth of penetration increased with increasing hypochlorite concentration, but the differences were small. Within each time group, depth of penetration with 1% NaOCl was about 50–80% of the values with the 6% solution, leading to the conclusion that temperature, time, and concentration all contribute to the penetration of sodium hypochlorite into dentinal tubules [3]. Given that many previous studies have also used this bacterium, the results can be easily compared [4–8].

In order to increase the effectiveness of endodontic treatments, different laser wavelengths were investigated in the past years and the elimination of bacteria at different depths by several therapeutic procedures have been investigated in number of in vitro [9–12] and clinical [13–15] studies, inclusively with double wavelengths [16].

The results in a study by Gouw-Soares et al. revealed a high significant bacterial elimination with a minimum of 83.65% in 500-μm slices irradiated with Ho:YAG laser, when compared with the untreated control group or even with the group treated with NaOCl plus H_2O_2 [17]. In a study performed by Gutknecht et al. [18], a bacterial reduction on an average of 99.91% of the *Enterococcus faecalis* injected into the root canals prepared in vitro (97.12% minimum, 99.9997% maximum) was able to be achieved by means of direct irradiation with Nd:YAG laser and an output of 1.5 W at 15 Hz. This in vitro work was performed on 40 roots of extracted human teeth. The setup suggests that the bactericidal effect of laser therapy in the framework presented here can also be achieved in vivo in clinical cases. In another study by Moritz et al. with a diode laser in comparison with a conventionally treated control group, the microbiological examination revealed streptococci at relevant concentrations in 20 cases and staphylococci in five cases. Extensive bacterial reduction was achieved in all cases by repeating high-power diode laser treatment only once, following the first irradiation. Minimal streptococcal growth was observed in seven root canals and minimal staphylococcal growth in two root canals. The maximum log kill was 4.22 for streptococci and 3.33 for staphylococci. In the control group, a maximum reduction by only one log step could be achieved in 6 out of 10 patients (40 patients with infected root canals underwent diode laser treatment. To verify the findings, microbiological tests were performed, and the results compared to those obtained with conventional antibacterial treatment) [19].

Another research done by Gutknecht et al. showed that a 980-nm diode laser can eliminate bacteria that have immigrated deep into the dentin, thus being able to increase the success rate in endodontic therapy, with an average (a maximum) bacterial reduction reached up to 13% (57%) at 1.75 W, 9% (66%) at 2.3 W, and 32% (86%) at 2.8 W with 500-μm slice thickness [7]. A diode laser with wavelength of 908 nm was used in conjunction with conventional chemo-mechanical techniques demonstrated a significant elimination of *E. faecalis* in the apical third of root dentin, also a result of study by Preethee [1]. Another study by Franzen et al. was done to evaluate the depth of effectiveness of erbium, chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser irradiation on microorganism reduction. Scanning electron microscopy (SEM) micrographs of the contaminated and irradiated surfaces showed the absence of a smear layer and opened dentinal tubule besides the significant bacterial reduction up to a dentin thickness of 500 μ ($P < 0.05$) using a low pulse energy of 3.13 mJ [20].

Materials and methods

The present study included 81 dental slices prepared from human teeth, previously extracted and collected for medical reasons. The slices consisted of three different thicknesses (300, 500, 1000 μm). First, the cleaned roots were separated from the crown at the enamel-dentin junction using a cutting/gridding system (Exakt, Hamburg, Germany). Afterwards, an endodontic treatment was performed manually from ISO 10 to 40. Then, the dental slices were sectioned parallel to the root canal lumen using a sawing microtome (Leitz 1600, Wetzlar, Germany) and stored in 0.08% thymol at a temperature of 4 °C until further use. The samples were separated into three test groups I, II, and III and four control groups, two positive control groups C_I and $C_{II + III}$, and two negative control groups N_I and $N_{II + III}$. The experiment was divided into two parts; each was performed on individual day.

The samples were treated with EDTA 17% for 30 s, then thoroughly washed in saline solution for 10 s. Directly before the bacteria inoculation, the dentin slices were sterilized in an autoclave (Sauter, Solda, Germany) for 20 min at 121 °C to ensure the sterility of the samples throughout the two experiments. One side of the dentin slices was inoculated via microliter injection with 1 μl of a defined concentration of a standard (2.0×10^7 CFU/ml) *Enterococcus faecalis* (strain ATC 29212) suspension. The side of the slice opposing to the inoculated surface was irradiated. After each five slices, the laser fiber was substituted with a new one (to ensure an optimal beam profile and clean fiber end).

Day one: group I (1.2 W, 10 Hz), day 2: groups II (0.6 W, CW) and III (0.4 W, CW). Two different control groups for both positive and negative settings were indispensable. C_I: 15 slices (5 of each thickness) represent the positive control group performed simultaneously with group I. N_I: three slices of each thickness were for the negative control group. $C_{II + III}$: 15 (5 of each thickness) samples indicate the positive control group. It has been conducted in the same time with groups II

and III. $N_{II + III}$: three slices of each thickness were used for the negative control group.

Irradiation

The applied laser wavelength was 445 nm (SIROLaser Blue, Sirona Dental Systems GmbH, Germany). The delivery system was via a 200-μm-diameter EasyTip (REF 6535905), endodontic fiber (Sirona Dental Systems GmbH, Germany). A stable and constant output power, emitted from the distal end of laser fiber, was confirmed by a power meter (FieldMaxII-Top, Coherent Inc., CAWA). The display settings for groups I and II were 0.6 W, and for group III was 0.4 W.

The irradiation was performed under an angulation of the fiber of 5° on the side opposite to the bacterial inoculation surface. This protocol was chosen in accordance with well-established literature to make results comparable [7, 21, 22]. Furthermore, the indirect irradiation was preformed to investigate the influence of the dentin thickness on the bactericidal efficiency of the laser.

During the irradiation, the fiber was fixed and the slice was hold mechanically on a small computer-controlled table (Fig. 1). It was designed specially to achieve identical automatic irradiation in a *sinusoidal* motion with a lateral movement component of approximately 2 mm/s and amplitude of 1 mm [23] (Fig. 2).

Group I: 15 slices (5 of each thickness) were irradiated with 1.2 W (P_{Avg} = 600 mW) in chopped mode, 10 Hz, 50-ms pulse duration (50% DC). Group II: 15 samples (5 of each thickness) irradiated with 0.6 W, CW. Group III: 15 slices (5 of each thickness) were exposed to laser irradiation with power of 0.4 W, CW. In the previously mentioned groups, the irradiation was performed four times per slice, each time for 10 s,

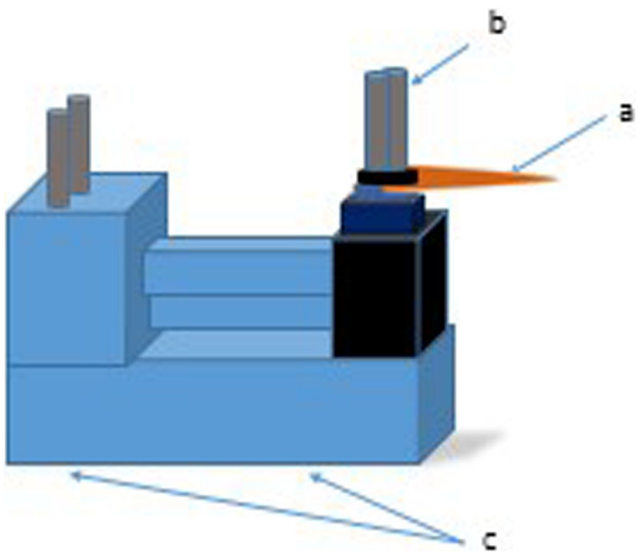


Fig. 1 The computer-controlled table. **a**) Dental slice, **b**) Springs to move the black piece and place the dental slice, **c**) Magnets circles, to fix it on the lasing table

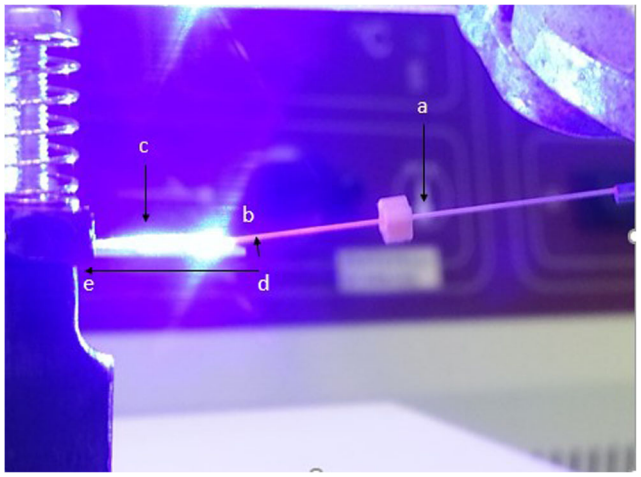


Fig. 2 **a**) EasyTip Fiber, **b**) 5° angle, **c**) The dental slice, **d**-**e**) The direction of the slice movement during irradiation

resulting in a total irradiation time of 40 s. Between each two irradiations, 10 s was left for cooling.

After irradiation

All the dentin slices were put individually into a sterile plastic PCR tube (Eppendorf Vertrieb Deutschland GmbH, Wesseling-Berzdorf, Germany) containing 1 ml of a physiological salt solution (0.9% NaCl). The PCR tubes were stored on ice during the experiment to prevent unwanted bacterial growth or death. In general, the duration between inoculation with bacteria and resuspension to grow survivors was < 2 h. In order to detach and disperse the bacterial cells, the tubes were vortexed. Afterward, the calculation of bacterial suspension was serially diluted (dilution 1×10^6). Only the last three suspensions could be detected and were used for calculation of surviving bacteria. Of all suspensions, two times 20 μl were plated onto Columbia-Agar plates. The dentin slices of the untreated control groups (both positive and negative) were treated equally. The plates were then incubated overnight at 37 °C for 14 h in an atmosphere of 10% CO_2. After this incubation period, *E. faecalis* colony-forming units (CFU) were counted and analyzed. The results are measured in "log kill" according to Rooney [21].

$$\log \text{kill} = \log\left(\frac{a}{b}\right)$$

a CFU of control group,
b CFU of test group

Statistical analysis was performed by SPSS Statistics 20 (IBM Corp., Armonk, NY, USA). P values < 0.05 were considered as statistically significant. A distribution test was carried on showing that the data were not normally distributed. Thus, a comparison of CFU values among groups was analyzed by the nonparametric post hoc Dunnett's test.

Results

The results of the bactericidal activity of the diode laser radiation using chopped mode (group I) with 1.2-W peak power and 10 Hz as well as the standard deviation are first given in Table 1, which shows the decrease in the bacterial numbers in each thickness and the standard deviation of both test (group I) and the positive control group C_I at the three selected sample thicknesses. The resuspension solution (0.9% NaCl) of survivors of CFU after the chopped mode irradiation in all thicknesses was 10^5 CFU/ml.

However, after using a 445-nm diode laser in continuous mode with 0.6 W (group II), the CFU on slice thickness of 1000 μm were in resuspension solution of 10^5 of survivors which is in the same range as in the chopped mode (group I), as shown in Table 2. On the other hand, the bacteria recovered after irradiation of continuous wave with power of 0.4 W (group III) were almost comparable with the positive control group, with resuspension solution of survivors of colony-forming units 10^6 CFU/ml in all thicknesses, indicating no significant bacterial reduction in this group.

In Fig. 3, one can compare the log kill and the median bacterial reduction between the three test groups I, II, and III. A high bacterial reduction with median log kill as 0.82 and 0.65 could be observed in groups I and II on the samples with 1000-μm dentin thickness. At a slice thickness 500 μm, the highest log kill value of 1.62 with 0.8 log step difference in group I was recorded. In the same slice thickness but with different protocol in group II, a higher log kill of 0.79 was registered. The log kill values at a slice thickness of 300 μm were recorded as 1.5 and 0.75 in group I and II, respectively.

Thus, when the same slice thicknesses were irradiated in group III, the average log kill was less in 1.47 log steps in the same thickness compared group I. On the other hand, with a slice thickness of 1000 μm of the same group III, the log kill − 0.04 value indicates bacterial growth, whereas irradiating the slice thickness of 500 μm showed higher results than both other thicknesses with average log kill of 0.17.

One can have an overview of the average percentage of bacterial reduction (Fig. 4). With the chosen distal output of 1.2 W, an average of 82.8% of the inoculated *Enterococcus faecalis* bacteria could be eliminated through a 1000-μm-thick dentin slice. With a slice thickness of 500 μm, it was still an average of 93.8%. The average bacterial reduction in the same group on a slice thickness of 300 μm is 92.9%. The bacterial reduction was less using the continuous mode than the chopped mode. Bacterial elimination using 0.6 W was up to 77% in a 1000-μm slice thickness.

Table 1 Results of day one: group I (1.2 W, 10 Hz, 50 ms) and the positive control group C_I

Slice thickness	Group	CFU/ml	Standard deviation
1000 μm	I	7.05×10^5	1.50×10^5
	C_I	4.67×10^6	1.34×10^6
500 μm	I	8.30×10^4	4.01×10^5
	C_I	3.45×10^6	2.96×10^5
300 μm	I	1.39×10^5	4.04×10^5
	C_I	4.38×10^6	1.58×10^6

Table 2 Results of day two: group II (0.6 W, CW), group III (0.4 W, CW), and the positive control group $C_{II + III}$

Slice thickness	Group	CFU/ml	Standard deviation
1000 μm	II	8.75×10^5	6.51×10^5
	III	4.25×10^6	1.63×10^6
	$C_{II + III}$	3.85×10^6	1.78×10^6
500 μm	II	1.08×10^5	7.42×10^5
	III	4.50×10^6	2.18×10^6
	$C_{II + III}$	6.67×10^6	2.09×10^6
300 μm	II	8.50×10^5	1.09×10^5
	III	4.50×10^6	2.18×10^6
	$C_{II + III}$	4.83×10^6	3.48×10^6

Comparative statistics

There were statistical differences between group I and group II compared with the positive control groups over all dentin thicknesses, with $p < 0.001$. However, there were no statistical differences in group III bacterial counts over the three selected dentin thicknesses ($p = 0.127$) compared to the positive control group.

One can see that, with an increasing power, the average bacterial reduction also rose (Fig. 4). The ultimate bactericidal effect of the laser radiation at the different operation modes with different powers (1.2 W, 10 Hz), 0.6 W and 0.4 W, has been determined using the log kill values of the groups, placed in relation, and then given as a percentage. In this way, the average bacterial reduction in the performed settings could be assessed in relation to the dentin slice thickness.

Comparative analysis

The results showed that no statistical but conspicuous difference was found between group I and group II in all dentin slice thicknesses, with $p = 0.067$ ($p > 0.05$), whereas the bacterial counts in groups I and II compared to group III were significantly different with p values as $p = 0.002$ and $p = 0.01$ ($p < 0.05$), respectively. Though, comparing the colony-forming units between the three different slices in each group individually has shown no significant difference ($p > 0.05$). Thus, a reasonable high bacterial reduction could be observed

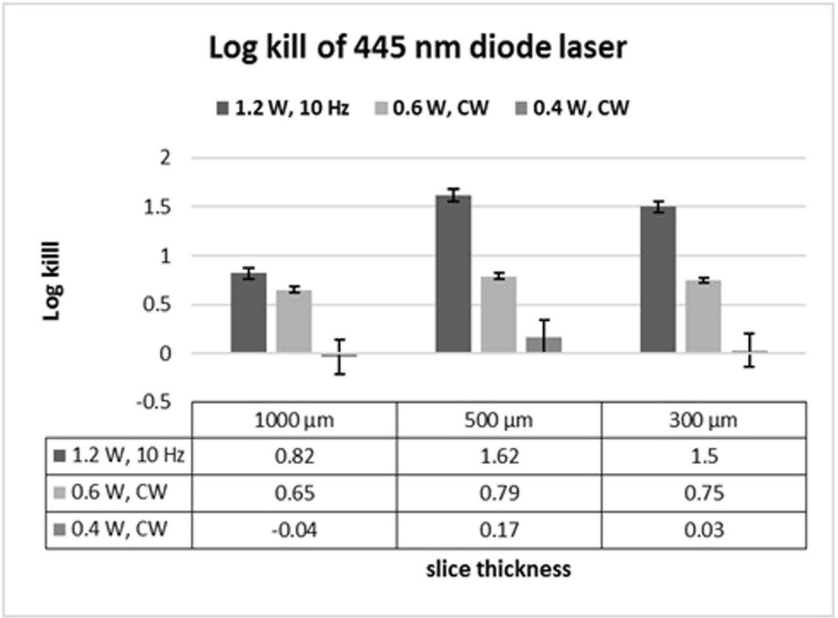


Fig. 3 Log kill of the 445-nm diode laser

in all three dentin thicknesses (including 1000 µm) in the first and second groups.

Discussion

A root canal system infection is a mixed infection of gram-negative and gram-positive bacteria [23]. These bacteria usually permeate the three-dimensional tubular network, forming a focus for the potential reinfection of endodontically treated teeth [24]. The results of this study showed that the diode laser with a wavelength of 445 nm has a greater bacterial reduction potential for all of the tested dentin slice thicknesses than the Er,Cr:YSGG laser, Er:YAG, and other diode lasers used separately. This reduction was significant in the 1000-µm dentin slices.

In this study, the 445-nm laser's parameters were set in a range where no thermal damage would be expected on root surface, and adjacent ligament during irradiation according to a previous study was performed on a total of 15 human single-rooted extracted teeth. Thee acceptable safe powers are suspected to deviate from those of near-infrared diode lasers due to the different absorption characteristics of hydroxyapatite in the visible part of the electromagnetic spectrum at 445 nm. The mean temperature rise inside the roots was below 10 °C. Whereas the maximum rise of temperature 9.83 °C

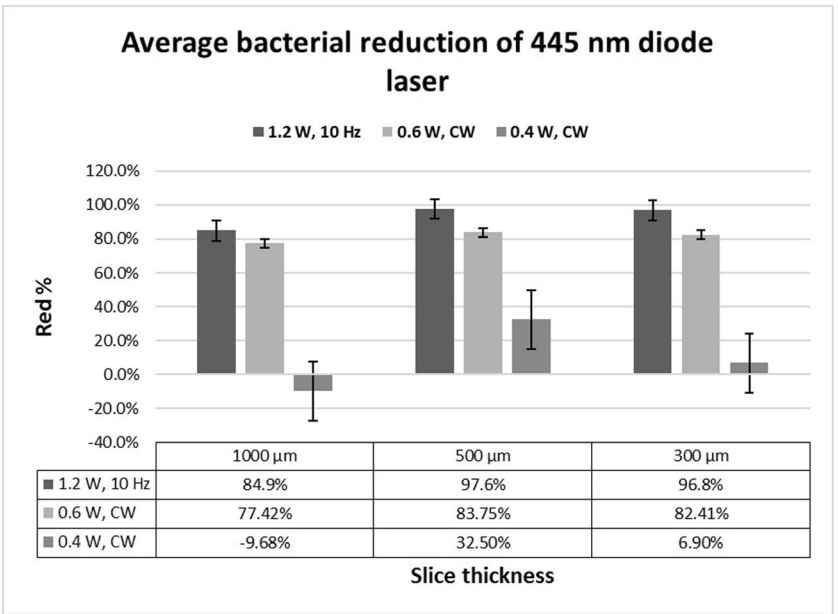


Fig. 4 Average bacterial reduction of the 445-nm diode laser

(0.6 W CW) and 9.81 °C (1.2 W gated mode, repetition rate 10 Hz, and pulse duration 50 ms) was registered in lower incisors, and 4.97 °C was detected at 0.4 W CW. On the other hand, the mean temperature rises in that study were 1.45 °C for 0.6 W and 1.78 °C W for 1.2 W gated (50% duty cycle). The thermal changes seen in the apical thirds of the root were statically significant ($p < 0.001$) and were greater than those at the coronal and middle thirds. The results of this study suggest that 445-nm diode lasers may work under a safe temperature during endodontic applications [25].

The major factor responsible for the long-term failure and therapy resistance in endodontics is the discrepancy in the penetration depth of microorganisms and chemical solutions [26]. Because of second factors, the endodontic solutions possess an approximately one order lower penetration ability than microorganisms [27]. In many previous studies, the bacterial suspension was inoculated directly inside the root canal, and the irradiation by the laser fiber tip was in direct contact with the bacteria over the dental slices. The presented study was designed to investigate the bactericidal effect of the diode laser with wavelength of 445 nm by subjecting dentin slices of 300-, 500-, and 1000-μm thicknesses to irradiation of the opposite area of bacterial inoculation, thus at a distance of 300, 500, and 1000 μm from the bacteria. This established method was applied in the same way in studies conducted by Klinke et al. [9], Gutknecht et al. [28], and Franzen et al. [20].

Gutknecht et al. reported an average bacterial reduction of 32% when dentin slices of 500-μm thickness were irradiated with 980 nm at an output power of 2.8 W in CW mode. The delivery was via a 200-μm quartz fiber, under 5° angulation. This wavelength shows higher absorption coefficient in water than 810 nm and 1064 nm, yielding a lower penetration depth [7]. Another study by Gutknecht et al. with 810 nm showed bacterial reduction up to 74% in a 500-μm slice thickness using a setting of 3 W in continuous mode (CW) delivered in 400-μm fiber [28]. A bacterial reduction of 98% was conducted using a 445-nm diode laser in a slice thickness of 500 μm after irradiating the slice with power of 1.2 W, 10 Hz, and pulse duration of 50 ms (group I), delivered with 200-μm fiber. However, using continuous wave with 0.6 W (group II) gave less average bacterial reduction of 84%. Different results were obtained by irradiating a 1-mm slices thickness with Nd:YAG laser using an output power of 1.5 W, 15 Hz, and 15 ms. Maximum bacterial reduction of 85% (average of 29.2%) was achieved in 1 mm [29], whereas using chopped mode of 445-nm blue diode laser in the same slice thickness with 1.2 W, 10 Hz, and 50 ms pulse duration (I) had an average bacterial reduction up to 85%. However, by using the same wavelength with the same power (0.6 W) but in different mode, continuous mode (II), less average bacterial reduction was obtained, 77% in the same slice thickness.

Klinke et al. [9] evaluated the decontamination effect of Nd:YAG laser with an output power of 1.5 and 20 Hz delivered through a 200-μm glass fiber on *E. faecalis* and pulses of 100 μs and repetition rate of 15 Hz. After 10–20-s irradiation time, a comparable result of 85% average bacterial reduction was achieved in a slice thickness of 1000 μm.

Additionally, a superior result was achieved using dual laser wavelength. The average bacterial reduction reached up to 99.96% using 2780 nm Er,Cr:YSGG laser alternating with 940-nm diode laser. Delivery system was via a 415-μm-diameter and 21.17-mm-length fiber (type RFT), respectively. The power settings of Er,Cr:YSGG laser were 1.5 W average power 50 Hz and 50-μs pulse duration, with 60% water and 30% air pressure, whereas for diode laser, the average power was 2 W in continuous mode [30].

In this experiment, the number of CFUs in the bacteria solution was remarkable (average concentration (2.0×10^7 bacteria/ml); the control groups and the test groups fluctuated by more than one order of magnitude. Even though the suspensions were placed on a shaker before every use, it was apparently not possible to achieve a constant number of CFUs in the suspension, which could be explained by the fact that *E. faecalis* not only occurs as solitary organisms but also in pairs and chains which could be insufficiently detached by vibration [31, 32].

There was no statically significant difference in the numbers of bacteria survivors between groups I (1.2 W, 10 Hz) and II (0.6 W, CW) when applying a diode laser with a wavelength of 445 nm on the same slice thicknesses (300, 500, and 1000 μm). But in continuous mode, one can see less efficiency of a 445-nm wavelength as the power decreased from 0.6 W (group II) to 0. 4 W (group III). This difference was the only considered statically significant. We suppose that there were no statistical differences between groups I and II due to the same energy delivered in all samples during 40 s (24 J), while in group III, only 16 J was applied. The fact that different dentin thicknesses did not interfere with results using such energy outputs may be attributed to the high energy transmission of radiation of 455-nm wavelength in hydroxyapatite.

Conclusion

The objective of this in vitro study was to simulate clinically laser-supported endodontic treatment to investigate the efficiency of the 445-nm blue diode laser using different modes in depths up to 1000 μm of human dentine. The difference was found not statically significant between groups I and group II in all dentin slice thicknesses, whereas the bacterial counts in groups I and II compared to group III were significantly different. To irradiate the root canal walls homogeneously in vivo conditions, it would be preferable that CW would yield a better energy distribution throughout the dentin, while chopped mode would likely provide more inconsistent results. However, comparing the tested groups with the

control groups shows that a statistical difference between group I, group II, and their control groups at all dentin depths was observed. Whereas no such difference was recorded with group III indicating to yielding less effective results in terms of the bactericidal effect. Clinical studies are necessary to confirm the results and to investigate the efficiency of this wavelength under in vivo conditions.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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