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Bactericidal Efficacy of Different Diode Laser Operation Modes Against *Enterococcus faecalis* Compared with Sodium Hypochlorite Irrigation

Skuteczność bakteriobójcza promieniowania lasera diodowego względem *Enterococcus faecalis* w porównaniu z irygacją podchlorynem sodu

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Microorganisms surviving in the root canal system despite the chemomechanical preparation are the main reason for reinfection and endodontic treatment failure. The introduction of laser-assisted endodontics resulted in higher success rates and less recurrent infections.

Objectives. To compare the antibacterial action of diode laser irradiation (CV – 1500 W, IMP – 3000 W), photo-activated disinfection (PAD), and 5.25% sodium hypochlorite (NaOCl) on *Enterococcus faecalis* in an infected tooth model.

Material and Methods. Fifty freshly extracted bovine teeth with single canals were prepared to apical size 45° ISO, autoclaved and contaminated with *Enterococcus faecalis*. After a 48-hour incubation the specimens were randomly divided into four treatment groups and one control group (n = 10): Group 1 was irradiated with 1500 mW (continuous wave diode laser irradiation); Group 2 was irradiated with 3000 mW (impulse diode laser irradiation, 300 ms – pulse, 300 ms – pause); Group 3 was subjected to photo-activated disinfection and Group 4 was irrigated with a 5.25% NaOCl solution for 15 min. Group 5 was rinsed with saline solution (control). After treatment, the dentin samples were collected and the number of surviving bacteria was determined by classical quantitative microbiologic evaluation.

Results. The differences in the mean number of the colony forming units (CFU/mg) between all the groups analyzed were statistically significant (p < 0.001). The highest number of microorganisms was observed in the control group (52.7 x 10³ CFU/mg). Both diode laser applications resulted in a significant reduction in the number of *E. faecalis* cells: G1 – 1.4 x 10³ CFU/mg, G2 – 4.2 x 10³ CFU/mg, whereas in the group subjected to photo-activated disinfection the extent of reduction was significantly less (G3 – 42.8 x 10³ CFU/mg).

Conclusions. The present study demonstrates that CW and pulse diode laser applications are both suitable for root canal disinfection, but do not eradicate all bacteria (**Dent. Med. Probl.** 2016, 53, 2, 216–221).

Key words: photodynamic therapy, disinfection, root canal treatment.

Słowa kluczowe: terapia fotodynamiczna, dezynfekcja, leczenie kanałowe.

Microorganisms surviving in the root canal system despite the chemomechanical preparation are the main reason for reinfection and endodontic treatment failure [1]. Among them, *Enterococ-*

cus faecalis is the most commonly isolated bacterium from teeth with periapical periodontitis with or without periodontal lesions [2–4]. Because of its ability to form intra- and extraradicular biofilms,

the proton-pump mechanism and many different virulence factors (*i.e.* collagen binding protein – Ace, lipoteichoic acid – LTA, enterococcus aggregation substance – EAS), *E. faecalis* penetrates the tubular network deeper than other microorganisms and is more resistant to calcium hydroxide as an intracanal medication between appointments [5–8]. Once in a biofilm, pathogens are more resistant to antibiotics or antiseptic agents, and are able to withstand host-defense measures, such as antibody-mediated phagocytic killing, unlike those in planktonic forms [9, 10]. There are numerous studies proving that different combinations of antiseptic agents in various solutions (chlorhexidine, calcium hydroxide, sodium hypochlorite) are not able to eradicate *Enterococcus faecalis* completely. Microorganisms colonize dentinal tubules as deeply as 1100 µm from the canal lumen, while the irrigation solutions used in the standard chemomechanical treatment penetrate only up to 130 – 300 µm deep in the dentinal tubules [11, 12]. Additional considerations in this regard are anatomical features like anastomoses, lateral canals, isthmuses and apical deltas, which are difficult to reach with endodontic instruments. Thus, some regions of the root canal system may remain untouched after conventional chemomechanical preparation [13].

The introduction of laser-assisted endodontics resulted in higher success rates and less recurrent infections. Laser light penetrates deeply into dentinal tissues (> 1100 µm), and can also be redirected in multiple directions by dentinal tubules acting as fiber-optic channels. Both factors improve the disinfecting capabilities of laser light [12].

The bactericidal effects of laser light are based on dose-dependent generation of heat. To avoid the risk of charring the dentin or possibly causing thermal injury to the periodontal tissues, the pulse operation module of the diode laser or the photo-activated disinfection procedure is suggested in some studies [14]. Low power laser activates a photosensitizer (a dye such as toluidine blue ortho), which has a bactericidal effect on various species, even within oral biofilms [15].

Taking into consideration these notions, the present *in vitro* study was performed to compare the efficacy of three different diode laser operation modes, (CW 1500 mW, pulse 3000 mW, photo-activated disinfection (PAD) with a standard irrigation solution (5.25% NaOCl) in root canals contaminated with *E. faecalis*. A comparison of the effectiveness of these laser-based procedures in eliminating such a resistant pathogen has not been conducted as a research project and has not been published either. The following study exceeds the existing publications in the laser-assisted endodontics domain, because it provides an analysis of different laser-based

bacterial eradication methods compared with standard antiseptic methods (sodium hypochlorite irrigation). Single-rooted bovine teeth were chosen as a substitute to human teeth to allow the simultaneous laser/NaOCl applications in all the groups, as bovine dentin is a proved satisfactory replacement for human dentin in microbiological studies [16].

Material and Methods

Preparation of Teeth

Fifty freshly extracted single-rooted bovine teeth were decoronated with a diamond flame-shaped bur. Calculus and periodontal soft tissues were removed from the external root surface with curettes, and the root canals were prepared with Largo[®] Peeso Reamers 2-3-4 (Dentsply, Maillefer, Ballaigues, Switzerland) to an apical size equal to #45 ISO to standardize the specimens. The smear layer was removed by sequential irrigations of 17% EDTA and 5.25% NaOCl, for 5 min each. The canals were then rinsed with saline solution and dried with paper points. All the samples were sterilized by autoclaving (121°C, 30 min) to remove all preexisting bacteria.

Bacteria and Culture Conditions

A pure bacterial culture of *Enterococcus faecalis* (ATCC 11420) was obtained from the Culture Collection (Polish Academy of Sciences, Wrocław, Poland) and transferred onto a microbank system (MVIM VIA Bank, UK). Before inoculation, the bacterial sample was incubated for 24 h in a broth culture medium (Brain Heart Infusion, supplemented with 7% sheep blood; Oxoid Ltd., Basingtoke, UK) at 37°C under aerobic conditions. After the incubation period, the turbidity of the broth was measured. The concentration of the inoculum was adjusted to a degree of turbidity of 0.5 according to the McFarland scale, using a CrystalSpec[™] nephelometer (Becton Dickinson, Warsaw, Poland), which corresponds to a bacterial concentration of 1.5×10^8 CFU/ml. All the samples were then immersed in the prepared broth and incubated at 37°C for 24 h under aerobic conditions to allow the propagation of the bacteria into the dentinal tubules. After incubation, the teeth were allocated randomly to the experimental or control groups.

Laser Irradiation

Group 1 (n = 10) was treated with a high-power diode laser (DiodeLX Mini, Lasotronix, Warsaw, Poland) emitting at a wavelength of 980 nm and set at a power of 1500 mW, operated in CW mode.

Group 2 (n = 10) was treated with the same laser device set at a power of 3000 mW, operated in pulsed mode, using a pulse duration of 300 ms and a pulse interval of 300 ms. Irradiation followed the oscillatory technique developed by Gutknecht et al. [17]. A fiber tip with a diameter of 200 μm was inserted into each root canal at a distance of 1 mm from the apical foramen and was withdrawn coronally with helicoidal movements at a speed of approximately 2 mm/sec. The laser was adjusted for an effective average output power of 1500 mW for Group 1 and 3000 mW for Group 2, measured directly on the fiber tip using a laser power meter (Gentec UNO UP19K-15S-H5 – 15 J, Gentec Electro-Optics, Quebec City, Canada) before each irradiation cycle. This procedure ensures standardized irradiation schemes for each specimen.

Group 3 (n = 10) was subjected to photo-activated disinfection. Toluidine Blue Ortho (TBO) (Lasotronix, Warsaw, Poland) as a photosensitizing agent, was introduced into each canal for 60 s. After this pre-irradiation time, irradiation was performed with a diode laser (Diode LX Mini, Lasotronix, Warsaw, Poland) emitting at a wavelength of 635 nm and set at a power of 100 mW. Laser light was applied through a single-use endodontic diffuser, which was gently moved up and down the canal during the irradiation time of 30 s.

Group 4 (n = 10) was irrigated with 5.25% NaOCl solution for 15 min with a side-vented endodontic needle, size 30 ga (CanalPro™, Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA).

Group 5 (n = 10) was rinsed with saline solution using side-vented endodontic needles, size 30 ga, and served as a negative control.

Root Canal Sampling

The dentin chips were collected with Largo® Peeso Reamers 5-6 (Dentsply, Maillefer, Ballaigues, Switzerland) and placed onto sterile plates, weighed before and after dentin collection on an electronic balance (AS, Radwag, Poland). The portions were then transferred to Eppendorf test tubes

containing 2 ml of sterile physiological saline solution and sonificated for 30 s (Ultrasonic Disintegrator, MSE, UK). For each sample, three serial 10-fold dilutions were prepared: 10^{-1} , 10^{-2} , 10^{-3} , providing 150 portions. One hundred microliters of each dilution were applied to a selective culture medium (Enterococcosel Agar, Graso, Poland) and incubated at 37°C for 24 h under aerobic conditions. All procedures were conducted in a laminar flow chamber, using sterile instruments to avoid contamination.

Plate Count/Bacteriological Evaluation

After the incubation period, the number of CFU/mg of dentin was counted for each plate.

Statistical Analysis

The mean value and the standard deviation of CFU values were calculated. As the data did not have a normal distribution, a nonparametric test was warranted. For significant differences between the CFU, values were subjected to the Kruskal-Wallis test. For group comparisons the Mann-Whitney U-test was used.

Results

The results are given in Figure 1. The highest number of CFU/mg was observed in the negative control group (Group 5, rinsed with saline solution only). The complete eradication of *E. faecalis* was achieved in the 5.25% NaOCl group (Group 4, positive control), as no bacterial cells were detected on the Enterococcosel Agar plates in any of the samples. All the groups' CFU/mg results were compared with Group 4 and Group 5 results and the groups subjected to laser-based eradication methods results (CFU/mg) were compared separately (in pairs). A statistically important reduction of CFU/mg was observed in Group 1 (1500 mW, CW) and Group 4 (5.25% NaOCl) – positive control,

Table 1. Efficacy of the diode laser, photo-activated disinfection (PAD) and 5.25% NaOCl irrigation in eliminating *Enterococcus faecalis* ($p < 0.001$)

Experimental groups	No. of Samples (n)	CFU/mg	SD
Group 1 (1500 mW, CW)	10	1.4×10^3	2.2×10^3
Group 2 (3000 mW, pulse)	10	4.2×10^3	5.4×10^3
Group 3 (PAD)	10	42.8×10^3	50×10^3
Group 4 (5.25% NaOCl)	10	0	–
Group 5 (control)	10	52.7×10^3	52.1×10^3

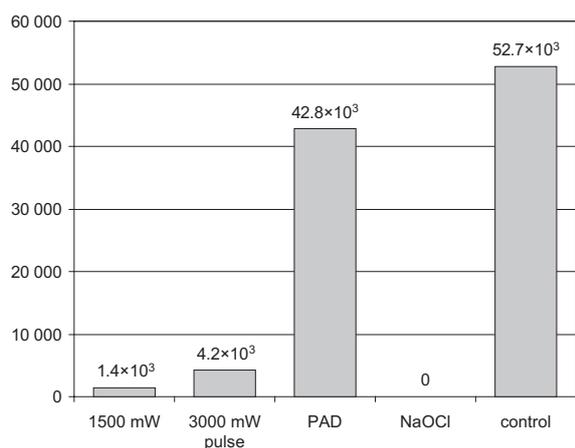


Fig. 1. Effect of CW and pulse mode diode laser irradiation, photo-activated disinfection and sodium hypochlorite irrigation on the survival of *Enterococcus faecalis* in the infected tooth model. Vertical bars represent the mean number of recovered cells

in comparison with Group 5 (negative control), $p < 0.001$. Among the groups of samples treated with laser-based bacterial eradication methods (Group 1, 2 and 3), only the 1500 mW applied in the continuous wave mode was effective (Group 1), statistically important reduction of *E. faecalis*.

Discussion

Root canal treatment failure, resulting in periapical lesion formation may be caused by an infection with *Enterococcus faecalis* [1, 3, 18, 19]. The high resistance of the microorganism to commonly applied disinfectants or inter-appointment medications [8] contributes to its high prevalence in primary and secondary endodontic infections [9], and has prompted a search for effective elimination methods [20, 21].

Different laser devices are used currently in root canal treatment [4, 22, 23]. The high power diode laser, emitting at a wavelength of 980 nm, was first introduced for use in endodontics in 1997 [22]. Its light is better absorbed by water than dental tissues, which results in greater dentin penetration and less laser light-dentin interactions, especially compared with the Nd-YAG laser [4]. Enamel prisms and dentin tubules acting as optical fibers permit laser light propagation as far as 1100 μm , and possibly more, from the canal lumen [24]. Microorganisms are capable of invading the tubular 3D network up to a depth of 1100 μm . However, the conventionally used irrigation solutions penetrate periradicular dentin to no more than 130 μm deep [12]. Three different mechanisms of laser light bactericidal action have been

reported: A rise in the temperature of the bacterial environment above lethal values, a temperature rise inside the bacteria (due to laser light-sensitive chromophores), and light-induced modulation of enzymatic activity [23]. The high power diode laser (810 nm) was previously used to disinfect root canal systems in the pulsed operation mode with a repetition rate of 1.5 Hz and an output power of 4 W [22] or 3 W [4]. In the present study the bactericidal effect of the high power diode laser operated in the pulse mode (Group 2) was not as significant, possibly because only one irradiation cycle was performed instead of several cycles in the previous studies [4, 22]. The aim of our study was to compare the bactericidal effect of a single application of different procedures that are used in current practice. Single irradiation with a 980-nm diode laser operated at a power output of 1500 mW in the continuous wave (CW) mode (Group 1) resulted in a significant reduction of *E. faecalis* CFU/mg in comparison with the control group (Group 5). Complete *E. faecalis* eradication was demonstrated in the 5.25% NaOCl-irrigated group (Group 4), which is in accordance with other studies [23, 25]. This effect results from the antimicrobial action of hypochlorous acid and active chlorine [26]. The irrigation time in the present study corresponds to the chemomechanical preparation time of root canals of average difficulty [27]. The method of sampling applied in the present study did not entail continuing use of NaOCl on the agar plates, which is why no neutralizing agent was necessary [27]. Several methods of sample collection were applied in previous studies: Use of paper points, rinsing [28], and immersion in culture broth or in physiological saline solution [29, 30]. In the present study the dentin shaving method was applied to guarantee precise quantification of the recovered volumes. The literature largely presents satisfactory disinfection rates achieved by several irradiation cycles with the high power diode laser operated in pulse mode [22, 31]. In the present study a single irradiation cycle of each laser-treated group was performed to standardize the procedure. Repetitive laser pulse irradiation could probably improve the bactericidal effect. High *E. faecalis* resistance to photo-activated disinfection is commonly associated with its Gram-positive cell wall structure, and/or the natural resistance of starved bacterial cells to adverse conditions [32]. Despite dentin tubules acting as optical fibers, some bacteria may invade anatomical root canal system complexities deeper than others and form biofilms, thereby hindering complete eradication [13].

Further investigation of laser-assisted endotherapies should be conducted to establish ap-

propriate laser parameters and eliminate the risk of recurrent periapical infections. According to the authors, research that mimics *in vivo* conditions, in which different microorganisms interact among themselves, would be of invaluable clinical significance.

The present study demonstrates that the most effective laser-based method in *E. faecalis* elimination is CW laser light application (1500 mW). Both pulse diode laser application and photo-activated disinfection can be suitable for root canal disinfection, but do not eradicate all bacteria.

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