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The photo-activated and photo-thermal effect of the 445/970 nm diode laser on the mixed biofilm inside root canals of human teeth *in vitro*: A pilot study

Ivan Katalinić[a,*], Ana Budimir[b], Zrinka Bošnjak[b], Suzana Jakovljević[c], Ivica Anić[a]

[a] Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb, Gundulićeva 5, 10000, Zagreb, Croatia
[b] Department of Clinical and Molecular Microbiology, University Hospital Centre Zagreb, Kišpatićeva 12, 10000, Zagreb, Croatia
[c] Department for Materials, Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb, Lučićeva 1, 10000, Zagreb, Croatia

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ABSTRACT

Aims: 1) Evaluation of the photo-thermal (PT) and photo-activated (PAD) antibacterial effect of the 445/970 nm diode laser on *E. faecalis*, *S. aureus* and *C. albicans* mixed biofilms grown together inside root canals of human teeth. 2) Defining a potentially efficient clinical protocol for safe and predictable usage in endodontic procedures.

Methodology: The root canals of 100 extracted human teeth with single straight canals were prepared with ProTaper NEXT files, sterilized, contaminated with a combination of three cultures (*E. faecalis*, *S. aureus*, *C. albicans*) and incubated for 15 days. The samples were randomly distributed into three groups (n = 20) and treated as follows: Group 1 (G1) – the 445 nm photo-thermal (PT) effect, Group 2 (G2) – a combination of the 445 nm and 970 nm PT effect, Group 3 (G3) – the 445 nm photo-activated (PAD) effect with 0.1% riboflavin, Group 4 (G4) – a combination of 3% sodium hypochlorite (NaOCl) and the 445 nm PAD effect. Four samples were used as positive control (non-treated) and four as a negative control. 12 aditional samples were used as a control for the G4 (3% NaOCl rinse without the laser). The number of viable microbes in each canal was determined by the colony forming unit (CFU) count.

Results: A statistically significant reduction in the microbial population after all treatments was observed ($P < 0.001$). Groups 2 and 3 showed similar results, both better than Group 1. Group 4 produced the best results.

Conclusions: The 445 nm PAD protocol has a stronger antimicrobial effect than the 445 nm PT protocol. Prolonged exposure time to laser light and a combination of wavelengths (445/970 PT protocol) helps in the reduction of microbes. *C. albicans* appears to be more sensitive to laser irradiation than the other bacteria tested in this study. Following current results, tested laser protocols could be recommended for clinical usage but only as an adjunct to "classic" NaOCl rinse since alone they are not able to completely eradicate all microorganisms.

1. Introduction

In endodontics, an ideal antimicrobial protocol should eliminate all microbes from the infected root canal and achieve a sterile environment which is normally present in the canal of a healthy tooth. [1] Proper root canal instrumentation is the first step towards this goal [2]. It has been shown that manual and mechanically driven instrumentation reduces bacterial loads, but is not able to completely remove all bacteria [3,4]. Chemical treatment of the root canal, mostly with sodium hypochlorite (NaOCl) which is considered to be the "golden standard" is also unable to eliminate all microbes [5,6]. Thus there is a need for additional antimicrobial tools, for example lasers [7,8]. Due to the recent rapid development of the technology, dental lasers are becoming available to a broad population of dental practitioners [9]. Diode lasers are a subgroup of lasers. They create energy via a semiconductor diode inside the device and can emit light in pulsed or continuous wave mode, with average power output of up to several Watts. The emitted light is usually in the visible or near-infrared spectrum [9]. Diode lasers are relatively cheap, easy to use, mobile and quiet. Their wavelengths show negligible affinity towards hard tissues (water and hydroxyapatite components) and high affinity for soft tissues (tissue pigments like melanin and hemoglobin) [10]. Because of their antimicrobial properties, usage in endodontic procedures is often explored [11–14]. Studies showed that bacteria can be found even 1100 µm deep inside the root

* Corresponding author at: Švarcova 22, 10000, Zagreb, Croatia.
E-mail addresses: ikatalinic87@gmail.com (I. Katalinić), abudimir@kbc-zagreb.hr (A. Budimir), zrinkabosnjak@gmail.com (Z. Bošnjak), suzana.jakovljevic@fsb.hr (S. Jakovljević), anic@sfzg.hr (I. Anić).

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canal walls [15,16]. Diode laser light is able to penetrate the dentine much deeper than the usual chemical irrigants (also up to 1100 μm). This penetrating effect is described as the “light fog” and is able to reach the bacteria hidden deep inside the dentine.16]

In general, the diode laser effect on microorganisms can be described as photo-thermal (PT) and photo-activated (PAD). The PT effect is based on a rise in temperature (heat creates damage). The energy is transferred directly to the pigmented bacteria and indirectly to the transparent bacteria, via the microenvironment. [17] In contrast to PT, PAD is a mostly indirect effect with little or no heating. PAD requires photo-activated substance (the dye). Once applied, the dye is absorbed by the microorganisms. Next, the microorganisms are irradiated with laser light of a specific wavelength. The absorbed dye gets activated by the light and intracellular production of the reactive oxygen species occurs. These highly reactive molecules disrupt normal metabolic mechanisms [18]. Although many articles on the PAD and PT effects of diode lasers in endodontic treatment can be found via relevant on-line search engines (e.g. PubMed), to the best of the authors` knowledge there is no information on antimicrobial activity of 445 nm diode laser light, especially with riboflavin as a photosensitizer.

The purpose of this study was to *in vitro* determine the photothermal and photo-activated effect of the novel diode laser with three wavelengths (445, 660, 970 nm) on three different microbial cultures/biofilms (*E. faecalis*, *S. aureus*, *C. albicans*) grown together inside human root canals and to investigate the efficiency of four different protocols designed for endodontic usage: (1) the 445 nm PT effect, (2) the 445 nm + 970 nm PT effect, (3) the 445 nm PAD effect with 0.1% riboflavin and (4) the combination of 3% NaOCl and the 445 nm PAD effect. The null hypothesis is that there is no difference between groups and that there is no significant antimicrobial effect overall.

2. Materials and methods

The study sample consisted of 100 single-rooted, single-canal, endodontically untreated human teeth with a fully developed apex, extracted for periodontal or orthodontic reasons in a private dental office in Zagreb, Croatia. Following extraction, each tooth was stored in 0.5% chloramine-T solution at 4 °C. The external root surface was cleaned with hand curettes (Hu-Friedy, Chicago, USA) to remove periodontal soft tissue. The teeth were decoronated with a water-cooled diamond fissure bur number 016 (Komet, Rock Hill, SC, USA) to make all roots 12 mm long. The working length was established by passing a size 10 or 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) in the canal until it was visible at the apical foramen under a stereomicroscope (Olympus SZX10, DFPL1.5, Hamburg, Germany) and subtracting 1 mm. All root canals were instrumented with the X1-X3 sequence of rotary ProTaper NEXT instruments (Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer’s instructions. Each canal was irrigated with 1 ml of 3% NaOCl between each instrument using a disposable 2-mL syringe and a 30-gauge needle (BD Microlance, Becton Dickinson, Madrid, Spain). After the instrumentation, the canals were filled with 1 ml 15% ethylenediaminetetraacetic acid (EDTA) for 2 min followed by a final rinse with 1 ml of 3% NaOCl and 1 ml of saline solution. The canals were dried with sterile X3 paper points (Dentsply Maillefer, Ballaigues, Switzerland). Each apical foramen was sealed with a composite resin (Universal Flo, GC, Tokyo, Japan), and the root surface covered with the bonding agent (G-aenial Bond, GC, Tokyo, Japan) to prevent leakage of bacteria through the apical foramen. [19] To simplify the manipulation of samples, they were fixed in microtiter plates with a composite resin. The samples were sterilized in hydrogen peroxide gas plasma (Neuster H2O2 Plasma sterilizer, PMS Healthcare Technologies, Turkey).

2.1. Cultivation of the microorganisms and root canal contamination

Three microbial strains of *Enterococcus faecalis* (EF), *Staphylococcus aureus* (SA) and *Candida albicans* (CA) were used for the experiment. EF was isolated from the patient's root canal prior to endodontic treatment, and preserved in a freezer at −80 °C until further use (School of Dental Medicine, Zagreb, Croatia). SA and CA were isolated from oral cavity samples taken from patients at the University Hospital Centre Zagreb, Croatia, shortly before the experiment was performed. Microorganism identification was performed with MALDI-TOF MS (Bruker Daltonics, USA). Two bacteria species were grown on the blood agar plate (5% horse blood) at 35 °C for 24 h, and CA was cultivated at 35 °C during 48 h on the Saburaud agar plate. A suspension density of 0.5 McFarland (Densimat, BioMerieux, France) in a Brain Heart broth was prepared from all three microorganisms and mixed together with the equal volume share. Ten microliters of mixed suspension was inoculated in the root canals and incubated during a period of 15 days in 100% humidity at 35 °C A freshly prepared suspension mixture was added every 48 h into the root canals in order to enable microorganism viability.

2.2. The treatment

The prepared samples were randomly distributed into four groups (n = 20). Four samples were used as a positive control and four as a negative control. The groups were treated as follows:

Group 1 (G1). A diode laser (SiroLaser Blue, Dentsply Sirona, Bensheim, Germany) optic fiber 200 μm in diameter was inserted in the root canal to the apex and activated (445 nm, 3 W, 20 Hz, duty cycle 50%, Ø - power = 1.5 W). A 1 mm/s circular movement from the apex towards the coronal part of the root was used according to the manufacturer`s instructions. The action was repeated 5 times with a pause of 5 s between each action. Total intracanal active treatment time was 60 s.

Group 2 (G2). A diode laser optic fiber 200 μm in diameter was inserted in the root canal to the apex and activated (445 nm, 3 W, 20 Hz, duty cycle 50%, Ø - power = 1.5 W). 1 mm/s circular movement from the apex towards the coronal part of the root was used according to the manufacturer`s instructions. The action was repeated 5 times with a pause of 5 s between each action. Total intracanal active treatment time was 60 s. Next, a diode laser optic fiber 200 μm in diameter was inserted in the root canal to the apex and activated (970 nm, 2 W, 20 Hz, duty cycle 75%, Ø - power = 1.5 W). A 1 mm/s circular movement from the apex towards the coronal part of the root was used according to the manufacturer`s instructions. The action was repeated 5 times with a pause of 5 s between each action. Total intracanal active treatment time was 60 s.

Group 3 (G3). The root canals were filled with the photoactive substance (a 0.1% riboflavin solution). The solution was left untouched in the canals for 60 s prior to treatment. A diode laser optic fiber 200 μm in diameter was then inserted in the root canal to the apex and activated (445 nm, 200 mW, 100 Hz, duty cycle 50%, Ø - power = 100 mW). A 1 mm/s circular movement from the apex towards the coronal part of the root was used according to the manufacturer`s instructions. The action was repeated 5 times with a pause of 5 s between each action. Total intracanal active treatment time was 60 s.

Group 4 (G4). The root canals were each rinsed with 5 ml of 3% NaOCl and dried with the sterile X3 paper points. 5% sodium thiosulphate was used to inactivate any residual NaOCl. Next, the root canals were filled with the photoactive substance (a 0.1% riboflavin solution). The solution was left untouched in the canals for 60 s prior to treatment. A diode laser optic fiber 200 μm in diameter was then inserted in the root canal to the apex and activated (445 nm, 200 mW, 100 Hz, duty cycle 50%, Ø - power = 100 mW). A 1 mm/s circular movement from the apex towards the coronal part of the root was used according to the manufacturer`s instructions The action was repeated 5 times with a pause of 5 s between each action. Total intracanal active treatment time was 60 s.

12 aditional samples were used as a control for the G4 (3% NaOCl

rinse without laser). The root canals were irrigated using a 20 ml syringe and a 30-gauge needle for approximately 60 s. The needle was inserted to 1 mm short of the working length.

The power density for 1.5 W average output (PT tretaments) was 4775 W/cm^2 (per treatment) and for 100 mW (PAD treatments) output 318 W/cm^2 (per treatment). The energy density for 1.5 W (PT tretaments) average output was 286,479 J/cm^2 (per treatment) and for 100 mW (PAD treatments) output 19,099 J/cm^2 (per treatment).

2.3. Sample analysis

After the procedure the root canals were manually agitated with sterile size 15# Hedstrom files (Dentsply Maillefer, Ballaigues, Switzerland), washed out with 10 µl sterile water and serially diluted 10 times each in 96 microwell plates. The final dilution was 10^{-12}. The content of each microwell was transferred to the blood agar plate and incubated for 24 h at 35 °C After the incubation, the bacterial and fungal growth was observed and the colony forming units count (CFU) was performed. The identification of different colonies was confirmed by the use of MALDI-TOF MS (Bruker Daltonics, USA).

2.4. SEM analysis

Four types of samples were prepared for the SEM analysis; a sterile canal sample (Fig. 1), an infected canal sample in order to confirm biofilm creation (Fig. 2), an infected canal sample treated with 445 nm PAD (Fig. 3) and an infected canal sample treated with a combination of 3% NaOCl and 445 nm PAD (Fig. 4). The last two samples were chosen for SEM analysis because of the superior CFU results for G3 and G4. Prior to the SEM analysis, the samples were stored in 10% buffered formalin. They were split longitudinally using a fine diamond fissure burr (Komet, Germany) and a chisel. The samples were then dehydrated in ascending aqueous ethanol solutions (25%, 50%, 75% and an absolute alcohol - 45 min. bath each), mounted on aluminium scanning electron microscopic stubs and vacuum-coated with a gold–palladium alloy. Examination was performed with a scanning electron microscope (Tescan Vega TS5136LS, Tescan, Brno, Czech Republic) targeting medium portion of the root canals.

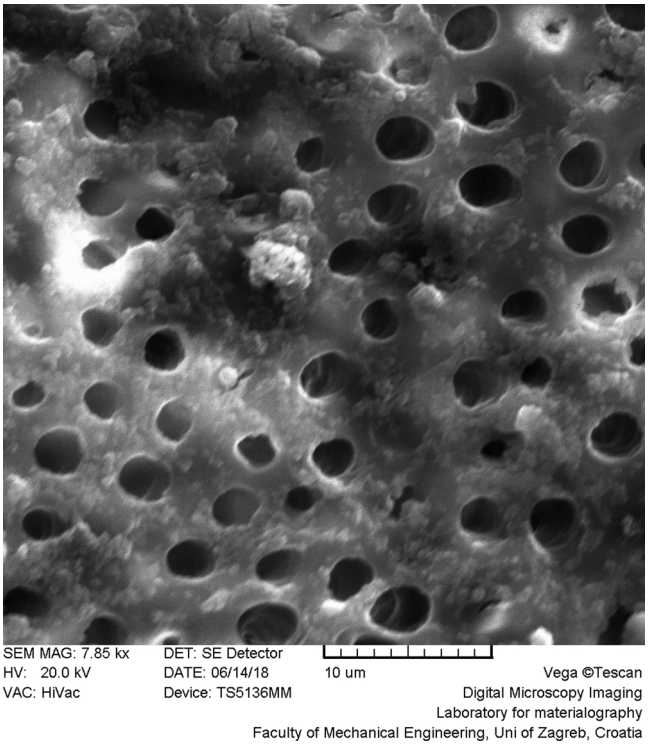

Fig. 1. SEM image shows root canal walls free of bacteria. Dentine tubules are clean and open.

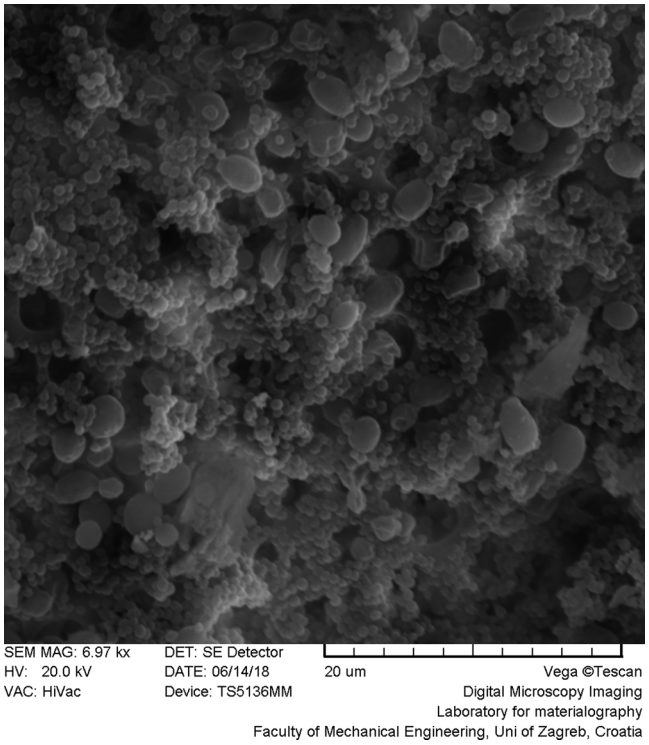

Fig. 2. SEM image shows thick microbial biofilms on the root canal surface and inside dentine tubules.

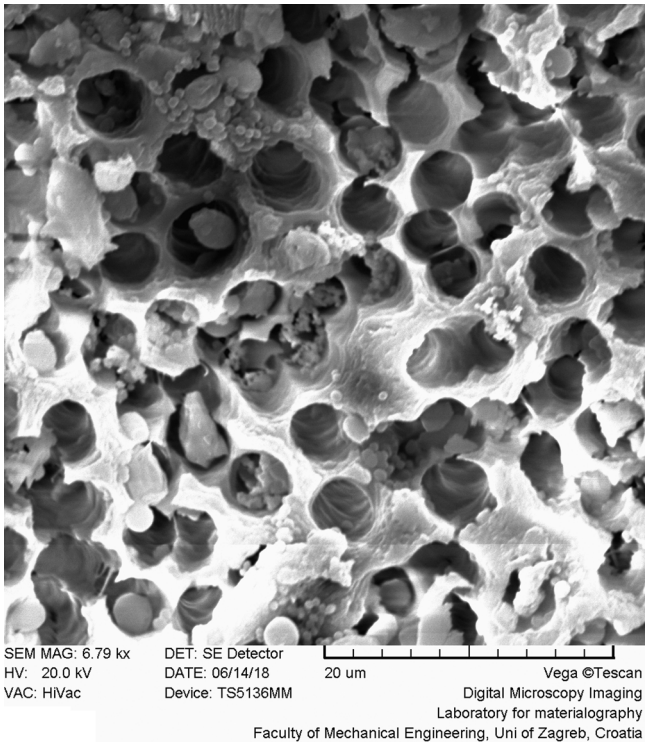

Fig. 3. After the 445 nm PAD treatment, severe reduction of microbes can be observed.

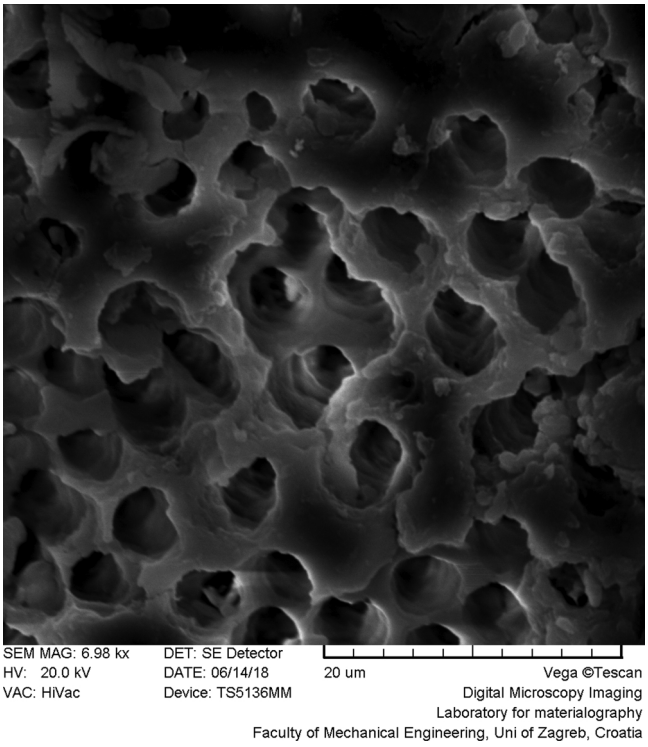

Fig. 4. After 3% NaOCl and the 445 nm PAD treatment, dentinal walls are virtually free of microbes. Some dentinal erosions and cracks have appeared probably due to the sample preparation and storage prior to the SEM microscopy (over-drying and chisel splitting of the samples). NaOCl could have induced some of the erosions.

2.5. Statistical analysis

Due to the sample size and dispersion of quantitative data, the Mann–Whitney U test was used for group-to-group analyses. The significance level was set at 5%. Analyses were performed using the SPSS 24.0 (SPSS, Chicago, IL, USA).

3. Results

Patterns of microbial colonization and the antimicrobial effects of the 445 nm diode laser are visualized on the SEM images (Figs. 2–4).

Tables 1–3 present the distribution of results for every tested microbial culture (*E. faecalis* – Table 1, *S. aureus* – Table 2, *C. albicans* – Table 3), across the tested groups/protocols (G1-G4 compared to the control group). Numbers are presented as *log* values of number 10. In each table, the difference between the control group and the other four groups can be observed (The Mann-Whitney U test). The reduction in the CFU number was highly significant for all groups ($P < 0.001$). All protocols resulted in a reduction of CFU numbers in comparison to the control group. Group 4 showed the best results overall. An additional control group was formed just to check the G4 results (Table 4).

4. Discussion

The survival of microorganisms in endodontic treatment or retreatment can severely impair tooth healing. [20–22] Because of this, dentistry is in constant search for new protocols and techniques that can help to remove the bacteria from root canals. Diode lasers have the capacity to eliminate different microorganisms so their use in endodontic protocols has been explored [11–14]. A novel diode laser that offers the power of three different wavelengths (445, 660 and 970 nm) was used in this study. The authors primarily wanted to explore and compare PAD and PT possibilities of blue light (445 nm). Since the mentioned device integrates different wavelengths, a protocol combining two laser wavelengths has also been tested. The PAD treatment with 445 nm blue light can be done with the corresponding dye ranging between the yellow and orange spectrum. Riboflavin (vitamin B2) was chosen for this purpose since it is light-yellow, biocompatible, relatively cheap and produces reactive oxygen species when irradiated with blue light. [23] Dyes used for similar red and near infra-red light PAD treatments stain the tooth which can impair the esthetic outcome of the endodontic treatment. [24,25] Riboflavin does not stain the tooth. This fact could be interesting when considering PAD treatment in the esthetic region (frontal teeth). In the current study the authors observed that riboflavin has poor solubility; it must be well shaken prior to usage, otherwise it quickly precipitates into clot-like formations. The answer to this problem could be the implementation of E101a (riboflavin-5`-phosphate) which is easier dissolved but also more expensive.

The 445 nm blue light was found to be very effective for cutting soft tissues [26,27], but to the best of the authors` knowledge there is no data on the efficiency of this wavelength in endodontic procedures. Bärenfaller et al. [28] tested the blue light PAD treatment with a 0.1% riboflavin photosensitizer and found that it is less effective than PAD red light (660 nm) with a toluidine blue photosensitizer. They used a LED polymerization lamp on multiple species found in periodontal infections, grown on artificial hydroxyapatite discs, so a clear parallel to the current study which tested the laser light on different microorganisms inside human root canals cannot be drawn. Another study showed similar results and the superiority of PAD Red over PAD Blue treatment. [29] Some of the tested microorganisms were *E. faecalis* and *C. albicans*, as in the current study, but treatment was also done with a LED polymerization lamp and microbiological samples placed in Eppendorf tubes so a direct comparison with the current study cannot be made. An interesting thing to notice is that, according to the same study, blue light alone completely eradicated all black pigmented bacteria (*Prevotella*, *Porphyromonas*), probably due to the bacterial endogenous chromophores that attract blue light. These bacteria are often found in endodontic infections. [30,31]

C. albicans is the most commonly found yeast inside infected root canals. [32,33] A strong effect of almost all laser protocols on *C. albicans* was shown in the current study and only the 445 nm PT procedure

Table 1
E. faecalis: differences in the CFU count between the positive control group and other groups.

GROUPS		N	Arithmetic mean	SD	Min	Max	Centile			Percentage of the reduction in comparison with the Control (median)	P value
							25.	Median	75.		
E. faecalis	Positive control	4	6,53E+13	4,62E+13	1,10E+13	1,20E+14	2,08E+13	6,50E+13	1,10E+14		
	(G1) 445 FT	20	9,44E+09	9,74E+09	1,00E+07	3,00E+10	1,00E+09	7,00E+09	2,00E+10	99,9892308%	0,002
	(G2) 445 + 970 FT	20	1,62E+09	4,64E+09	0,00E+00	2,00E+10	1,25E+03	5,50E+05	2,00E+08	99,9999992%	0,002
	(G3) 445 PAD	20	5,51E+08	1,42E+09	1,00E+04	6,00E+09	4,00E+05	1,50E+06	2,50E+08	99,9999977%	0,002
	(G4) 445 PAD + NaOCl	20	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	100,0000000%	< 0,001

Table 2
S. aureus: differences in the CFU count between the positive control group and other groups.

GROUPS		N	Arithmetic mean	SD	Min	Max	Centile			Percentage of the reduction in comparison with the Control (median)	P value
							25.	Median	75.		
S. aureus	Positive control	4	2,38E+14	1,80E+14	1,00E+14	5,00E+14	1,13E+14	1,75E+14	4,25E+14		
	(G1) 445 FT	20	2,71E+10	3,08E+10	2,00E+07	1,00E+11	4,00E+09	2,00E+10	5,00E+10	99,9885714%	0,002
	(G2) 445 + 970 FT	20	7,91E+08	2,26E+09	0,00E+00	1,00E+10	5,75E+01	1,00E+06	3,75E+08	99,9999994%	0,002
	(G3) 445 PAD	20	6,46E+08	1,45E+09	1,00E+03	4,00E+09	1,25E+05	8,00E+05	1,75E+08	99,9999995%	0,002
	(G4) 445 PAD + NaOCl	20	1,82E+01	5,27E+01	0,00E+00	2,00E+02	0,00E+00	0,00E+00	0,00E+00	100,0000000%	< 0,001

did not efficiently eliminate the yeast. The addition of the 970 nm PT effect in G2 gave much better results, similar to those of the G3 and G4. This indicates that time and/or a combination of wavelengths plays a significant role in the antimicrobial process. Several authors stated the high efficacy of blue light in the elimination of *C. albicans*, although light source was not a diode laser. [34,35] This is probably due to the intracellular porphyrins and flavins which act as photosensitizers and absorb blue light well, similarly to the previously mentioned black pigmented bacteria. [34,35]

E. facealis is often labeled as one of the main microbial reasons for endodontic (re)treatment failures. [36–38] Staphylococci can be found in endodontic lesions, along with other bacteria [39,40]. The literature states that Gram-positive bacteria (both Enterococci and Staphylococci) are less affected by heat (e.g. the laser PT effect) due to the structural characteristics of their cell wall when compared to Gram-negative bacteria [41]. On the other hand, the cell wall of Gram-positive bacteria is more porous that of Gram-negative ones so different photosensitizers can diffuse more easily. Thus, PAD treatment is generally recognized as more effective than PT treatment for the elimination of these bacteria [42,43]. In the current study, the combined PT strength of 445 and 970 nm wavelengths in one protocol gave significantly better results compared to single PT treatment. The most influencing factor could be time; in the combined protocol the total treatment time is longer - 2 min (1 min for each treatment) which means more energy is transferred to the microorganisms. More energy also means more heat, thus a great care should be taken not to create damage to the neighboring tissues. Other researchers have shown as well that longer light exposure produces better antimicrobial results [44]. The other influencing factor could be that the microorganisms are attacked with two different wavelengths, rather than just one.

CFU count has been considered as the" golden standard" when evaluating the efficacy of different disinfection methods. [45,46] It is a cheap and relatively easy method but it is questionable if it realistically presents microbial growth inside the root canal. Microorganisms respond differently to removal from the root canal and subsequent inoculation on the agar plates. Also, only organisms that adhere to the outer root canal walls are usually sampled via mechanical means (the sterile paper point sample collection). Organisms in deeper dentine layers remain mainly untouched [47]. It would be useful to additionally evaluate the results obtained with the CFU; after the disinfection, some microorganisms could be viable but non-culturable. Microbiological quantification methods should be ideally complemented with fluorescent image–based microscopy for an accurate assessment of biofilm [48].

Table 4
G4 control – total number of remaining microorganisms after the 3% NaOCl rinse alone, without the PAD.

Mean CFU	Median CFU	Range CFU	SD
4.68×10^1	3.75×10^1	1.00×10^1 to 1.00×10^2	3.42×10^1

In microbiology a clear antimicrobial activity is suggested when a reduction of viability by 3 log10 (99.9%) is reached. [28] In the current study, the first protocol (G1) showed such an antimicrobial effect, but perhaps this result is not of high clinical importance. Results that could be of significant clinical importance, meaning at least a 6-log bacterial count reduction, as stated by Hoedke et al. [47], were achieved in the G2-G3 protocols, while the best results (almost total eradication of all microorganisms) were observed when PAD treatment was combined with a 3% NaOCl rinse (G4).

The existence of highly organized bacterial biofilms inside root canals diminishes the disinfective potential of most endodontic irrigants. [49,50] Contemporary endodontics is favoring higher concentrations of NaOCl (> 5%) in order to disrupt the biofilm and achieve best possible antimicrobial results. [51–58]. A lower concentration of NaOCl (3%) used in this protocol was sufficient for exceptional antimicrobial activity, which is in consistence with other studies that have tested similar diode lasers (970 nm PT and 660 nm PAD treatments) in protocols with NaOCl [59–62]. A lower NaOCl concentration also means a lower potential for tissue damage and less cytotoxicity, which is an important fact. It is possible that in the G4 NaOCl disrupted the bacterial biofilm and killed most of the microorganisms. This action probably allowed the PAD photoactive substance to reach and eliminate remaining microorganisms hidden inside the biofilm/the dentine. PAD was chosen for the combined protocol because it showed the best antimicrobial results with lowest energy levels and lowest risk of thermal damage. Without 3% NaOCl such results couldn`t be achieved.

NaOCl remains mandatory in endodontic procedures but the laser

Table 3
C. albicans: differences in the CFU count between the positive control group and other groups.

GROUPS		N	Arithmetic mean	SD	Min	Max	Centile			Percentage of the reduction in comparison with the Control (median)	P value
							25.	Median	75.		
C. albicans	Positive control	4	4,55E+11	3,01E+11	2,00E+10	7,00E+11	1,40E+11	5,50E+11	6,75E+11		
	(G1) 445 FT	20	1,67E+09	2,11E+09	0,00E+00	7,00E+09	3,00E+07	2,00E+08	3,00E+09	99,9636364%	0,002
	(G2) 445 + 970 FT	20	5,00E+06	2,24E+07	0,00E+00	1,00E+08	0,00E+00	0,00E+00	1,00E+01	100,0000000%	0,001
	(G3) 445 PAD	20	2,46E+02	6,04E+02	0,00E+00	2,00E+03	0,00E+00	2,00E+01	1,75E+02	100,0000000%	0,002
	(G4) 445 PAD + NaOCl	20	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	0,00E+00	100,0000000%	< 0,001

can be added to further boost antimicrobial results.

The power settings for the PT groups in this research were set to 3 W and gated mode – 20 Hz. This created a final average output of 1.5 W which is within the safe limit proposed by Gutknecht et al. [63] In this way sufficient energy for microbial destruction is produced without irreversible thermal damage to the neighboring tooth structures. Constant moving of the fiber inside the root canal is also important to keep the temperature rise as low as possible in order to avoid potential thermal damage to the neighboring periodontal tissues [63].

Although the current study showed antimicrobial activity of all laser protocols and the superiority of the 60 s PAD therapy over the 60 s PT therapy, additional research is necessary to further investigate and evaluate the potential of blue laser light and different diode laser protocols in root canal disinfection, e.g. testing lower power settings for the PT treatments, lower concentrations of NaOCl in the combined protocol, different combinations of wavelengths/irrigants and the effect of different protocols on the biofilm over time. The use of NaOCl remains mandatory in all root canal procedures.

5. Conclusions

Within the limitations of this study, it can be concluded that the 445 nm PAD protocol has a stronger antimicrobial effect than the 445 nm PT protocol. Prolonged exposure time to laser light and a combination of wavelengths (445/970 nm PT protocol) helps in the reduction of microbes. *C. albicans* appears to be more sensitive to laser irradiation than the other bacteria tested in this study. Following current results, tested laser protocols could be recommended for clinical usage in endodontics but only as an adjunct to a classic NaOCl rinse since alone they are not able to completely eradicate all microorganisms.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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