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Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment

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Abstract

Bago I, Plečko V, Gabrić Pandurić D, Schauperl Z, Baraba A, Anić I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. *International Endodontic Journal*.

Aim To evaluate the antimicrobial effect of a diode laser irradiation, photo-activated disinfection (PAD), conventional and sonic activated irrigation with 2.5% sodium hypochlorite (NaOCl) on *Enterococcus faecalis*.

Methodology Root canals of 120 human extracted teeth with single straight canals were prepared with ProTaper files, sterilized, contaminated with an *E. faecalis* suspension and incubated for 7 days. They were then randomly distributed into six groups: G1, diode laser irradiation (2 W, 3 × 20 s); G2, PAD (100 mW, 60 s); G3, PAD with 3D Endoprobe (100 mW, 60 s); G4, 30-gauge syringe irrigation with NaOCl (60 s); G5, sonic agitation of NaOCl with the EndoActivator system (60 s); G6, 30-gauge syringe irrigation with NaCl (60 s). The pattern of colonization was visualized by scanning electron microscopy. The root canals were sampled by flushing with saline solution at baseline and after the treatments. The number of bacteria in each canal was determined by plate count. The presence and the absence of *E. faecalis* in root canals were also demonstrated by polymerase chain reaction (PCR).

Results There was a significant reduction in the bacterial population after all treatments ($P < 0.001$). The PAD, using both laser systems, and the sonic activated NaOCl irrigation were significantly more effective than diode irradiation and single NaOCl irrigation in reducing CFUs ($P < 0.05$). High-power diode laser and single NaOCl irrigation had an equal antibacterial effect ($P > 0.05$).

Conclusions The PAD and EndoActivator system were more successful in reducing the root canal infection than the diode laser and NaOCl syringe irrigation alone.

Keywords: disinfection, *Enterococcus faecalis*, laser, root canal, sodium hypochlorite.

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Introduction

The outcome of root canal treatment is based on efficient disinfection of the root canal system and prevention of reinfection (Byström & Sundqvist 1985, Rossi *et al.* 2005). Traditionally, it is accomplished by a combination of mechanical instrumentation, the use of disinfecting solutions for irrigation and placement of intracanal medicaments between appointments. After using mechanical instrumentation, large areas of the root canal system remained untouched, regardless of the rotary or manual technique used (Peters *et al.* 2001, Paqué *et al.* 2010). This is the reason

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why much has been expected from using various combinations of disinfecting solutions and irrigation devices.

Irrigants have been traditionally delivered using a syringe and needle (Haapasalo *et al.* 2010). The problem with this irrigation technique is inadequate replacement of the irrigant throughout the root canal system because the highest streaming velocity is present only in the lumen of the needle and around the tip of the needle (Boutsioukis *et al.* 2007). Furthermore, the high surface tension of sodium hypochlorite (NaOCl) prevents direct contact of the irrigant with the dentinal walls of the anatomical complexities (Zehnder 2006). Paque *et al.* (2009) reported that after NaOCl syringe/needle irrigation and instrumentation, 40–60% of the canals still contained cultivable bacteria. Over the last few decades, several mechanical devices have been developed to improve the penetration and effectiveness of irrigation in peripheral areas of the root canal space. The efficiency of sonic and ultrasonic devices is based on the creation of hydrodynamic phenomenon in well-shaped canals filled with an irrigant (Ahmad *et al.* 1987, Ruddle 2008). Such active root canal irrigation has been shown to facilitate the disruption of biofilms and make cell the membrane of bacteria more permeable to NaOCl (Plotino *et al.* 2007). The EndoActivator (Dentsply Tulsa Dental, Tulsa, OK, USA) is a sonic device that uses noncutting polymer tips to vigorously agitate irrigant solution in the root canal. It has been recommended for final disinfection protocols with NaOCl and EDTA (Ruddle 2008). However, the superiority of the EndoActivator to standard syringe irrigation in the root canal remains controversial because it did not provide higher bacteria elimination in some studies (Brito *et al.* 2009, Huffaker *et al.* 2010).

Relatively, new approaches to disinfecting the root canals include the use of high-power diode lasers as well as photo-activated disinfection (PAD). The laser light is thought to be able to reach areas that are impossible with the traditional techniques (Odor *et al.* 1996). The bactericidal effect of high-power lasers is based on dose-dependent heat generation. Its antimicrobial effectiveness against diverse microorganisms has already been demonstrated in previous studies (Gutknecht *et al.* 2000, 2004). Nevertheless, according to some, it was not more effective than NaOCl irrigation (Piccolomini *et al.* 2002, Gerek *et al.* 2010). Moreover, high-power lasers have the potential to cause dentine charring, ankylosis, root resorption and periradicular necrosis (Bachall *et al.* 1992). PAD is an antimicrobial strategy in which low laser energy is used to activate a nontoxic photosensitizer, and the singlet oxygen released from these dyes causes damage to the membrane and DNA of microorganisms (Demidova & Hamblin 2004). It has been recommended in root canal treatment as an alternative or a supplement to currently used disinfection methods (Lee *et al.* 2004, Rios *et al.* 2011). The photosensitizers have a high degree of selectivity for killing microorganisms without affecting host cell viability (Lee *et al.* 2004). In an *in vivo* study (Garces *et al.* 2010), it was used succesfully for the eradication of multi-drug resistant microorganisms.

The aim of this *ex vivo* study was to compare the antibacterial action of a high-power diode laser irradiation, the PAD, conventional and sonic activated irrigation during root canal treatment. The null hypothesis was that there were no differences between antimicrobial efficacies of these experimental root canal disinfection techniques.

Materials and methods

Selection and preparation of specimen

The study sample consisted of 120 extracted human mandibular incisors and maxillary second premolars. All teeth were extracted because of periodontal disease or extensive cariuos lesions, and approval was obtained by the Ethics Committee of the School of Dental Medicine, University of Zagreb, Croatia. All teeth had completely developed roots and were without root caries or previous endodontic treatment. The presence of a single canal was determined by radiographs taken in both mesiodistal and buccolingual directions.

Following extraction, each tooth was stored in 0.5% chloramine-T solution at 4 °C. The external root surface was cleaned with curettes to remove periodontal soft tissue. Teeth were decoronated with a water-cooled diamond fissure bur number 016 (Komet, Rock Hill, SC, USA). The working length (WL) of 12 mm 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 through a stereomicroscope (Olympus SZX10, DF PL1.5, Hamburg, Germany) and subtracting 1 mm. If initial instrumentation to the apical foramen could not be performed with a size 08 K-file (Dentsply Maillefer), or could be easily passed with a size 20 K-file, those teeth (10) were excluded. All root canals were instrumented with the conventional sequence of rotary ProTaper Universal NiTi (Dentsply Maillefer)

according to the manufacturer's instructions at a rotational speed of 300 rpm. The coronal two-thirds of the canals were prepared with the shaping files SX and S1. Subsequently, rotary instrumentation was accomplished using S1, S2, F1, F2 and F3 (master apical file, MAF) to WL. Each canal was irrigated with 1 mL of 2.5% NaOCl between each instrument using a disposable 2-mL syringe and 30-gauge needle (BD Microlance, Becton Dickinson, Madrid, Spain). After the instrumentation, canals were filled with 1 mL 15% ethylenediaminetetraacetic acid (EDTA) for 2 min followed by a final rinse with 1 mL 2.5% NaOCl and 1 mL saline solution. Finally, the canals were dried with the sterile F3 paper points (ProTaper Universal, Dentsply Maillefer). Each apical foramen was sealed with a composite resin (Gradia, GC, Tokyo, Japan), and the root surface covered with the bonding agent (G-aenial Bond, GC, Tokyo, Japan) to prevent leaking of bacteria and the passage of irrigant through the apical foramen (Meire *et al.* 2009). To simplify the manipulation during contamination and irrigation procedures, specimens were fixed in a 1.5-mL Eppendorf tube (Eppendorf, Hamburg, Germany) with composite resin.

Samples were placed in envelopes and sterilized in hydrogen peroxide gas plasma (PLASMA). As plasma sterilization has limitations and the success can be questionable for items with a narrow internal diameter and complex structure (Kanemitsu *et al.* 2005), sterilization control was performed on six samples. Root canals were filled with 1 mL sterile broth culture (Brain Heart Infusion, Beckton Dickinskon, Madrid, Spain) using insulin syringes (BD Plastipak, Becton Dickinson) and incubated for 24 h in 100% humidity. Samples from the root canals were spread on plates containing blood agar with 7% horse blood (211037; Becton Dickinson) and immersed in tubes containing sterile broth. Sterilization was confirmed when, after 48 h, there was no growth of bacteria on the agar plates and when the content of the test tubes was without turbidity.

Cultivation of *Enterococcus faecalis* and root canal contamination

A suspension was prepared by mixing a pure culture of *E. faecalis* ATCC 29212, grown in blood agar plates containing 7% horse blood for 24 h, with 2 mL of sterile 0.85% saline solution. The density of 0.5 McFarland was measured by the densitometer (Densimat, BioMérieux, Marcy l'Etoile, France).

Overall, 114 root canals were filled with 10 μL of the bacterial suspension using sterile 1-mL insulin syringes without overflowing. The suspension was carried to the entire root canal length with a size 15 K-file. The samples were incubated at 37 °C for 7 days in 100% relative humidity. Reinoculation was performed on the 1st, 4th and 6th day after initial inoculation. After the incubation period, any residual medium was removed with sterile paper points (Meire *et al.* 2009). The samples were then randomly divided into five experimental groups of 20 teeth each, and 10 samples served as positive controls.

Four samples were stored in 10% buffered formalin and subjected to scanning electron microscopy to visualizate the pattern of colonization. They were split longitudinally using a diamond fissure bur (Dentsply Maillefer) and a chisel. The samples were dehydrated in ascending aqueous ethanol solutions (25%, 50%, 75%, 95% and absolute alcohol twice), for 20 min, mounted on aluminium scanning electron microscopic stubs and sputter coated with a gold–palladium alloy under a vacuum. Examination was performed using a scanning electron microscope (Tescan Vega TS5136LS, Tescan, Brno, Czech Republic).

Experimental procedures

The remaining 110 samples were randomly allocated to five experimental groups (n = 20 per each) and the positive control group (n = 10).

Group 1

The root canals were irrigated with 5 mL 2.5% NaOCl for 60 s using 5-mL syringe and 30-G needle, which was placed 2 mm short of the WL.

Group 2

The root canals were rinsed with 5 mL 2.5% NaOCl for 30 s followed by the NaOCl activation for another 30 s, using the EndoActivator device (10 000 cpm) (Ruddle 2008). The red tip instrument size 25, 0.04 taper was placed 2-mm short of the WL and moved up and down in short vertical strokes.

Group 3

Root canals were irradiated with a pulsed diode laser (LaserHF, Hager Werken, Duisburg, Germany) for 20 s, repeated three times at intervals of 10 s between

each one. The physical parameters of the laser were as λ=975 nm, peak power = 2 W, *t*-on (time on, laser beam operative) = 5 ms, *t*-off (time off, laser beam inoperative) = 25 ms, with continuous timer (laser beam operated through foot switch). The 320-μm optical fibre was introduced 1 mm short of the WL and was withdrawn from apical to coronal according to the recommendations of Gutknecht *et al.* (2004).

Group 4

Root canals were filled with toluidine blue (155 μg mL^{-1}) to the level of the access cavity. The solution was agitated with a size 15 K-file and left undisturbed in the canal for 1 min. Irradiation of the root canals was accomplished with a diode laser (LaserHF, λ = 660 nm, total power = 100 mW). The 320 μm optical fibre was placed to the WL, and spiral movements from apical to cervical were performed for 60 s.

Group 5

According to the manufacturer's instructions, root canals were filled with a phenothiazine chloride (10 mg mL^{-1}) (Helbo Endo Blue, Grieskirchen, Austria) to the level of the access cavity, agitated with a size 15 K-file, left in the canal for 2 min and irradiated with a diode laser (Helbo, Bredent, Senden, Germany, λ=660 nm, total power = 100 mW). The 3D EndoProbe was placed to the WL for 60 s.

Positive control

The root canals were rinsed with 5 mL of sterile 0.85% saline solution using a 30-gauge needle, for 60 s. After each protocol, the root canals were rinsed with 1 mL of 5% sodium thiosulfate ($Na_2S_2O_3$) for 30 s, to neutralize any NaOCl used, and with 1 mL of sterile saline for 30 s, to standardize all experimental treatments to the same number of rinse procedure (Rios *et al.* 2011).

Microbiological procedures

The root canals were sampled twice, at baseline and after each protocol. The canals were filled completely with 10 μL sterile 0.85% saline solution. After three aspiration-delivering cycles with a sterile insulin syringe, the canal content was finally aspirated and transferred to the first 0.5-mL Eppendorf tube, which contained 90 μL sterile saline, to achieve a 10^1 dilution rate. During the sampling procedure, the teeth were held upside down to collect all the sampling fluid. After 10-fold serial dilutions and agitation in vortex for 1 min, aliquots of 10 μL were plated onto blood agar plates (211037, Becton Dickinson, NJ, USA) and incubated for 48 h at 37 °C in 100% humidity. CFUs grown were counted and finally transformed into actual counts based on the dilution factor. Before the second sample was taken, a size 30 Hedström file (Dentsply Maillefer) was used to file vigorously the dentinal walls (Brito *et al.* 2009).

The antimicrobial efficacy of the used 2.5% NaOCl was confirmed by the culture method and the turbidity test.

PCR detection of *E. faecalis*

All samples were also examined by the polymerase chain reaction (PCR), to confirm the presence of *E. faecalis* and to exclude the possibility of false-negative results because of the low number of *E. faecalis* that may not have been cultivated, or were in a stationary phase (Molander *et al.* 2007).

Liquid culture was centrifuged (4007 ***g***) for 1 min to break the cells (Eppendorf 541SD, Hamburg, Germany). The sediment was resuspended in 100 μL 1×PCR buffer (10 mmol L^{-1}, KCl 50 mmol L^{-1}, pH 8.0) (Merck, Darmstadt, Germany). The suspension was heated at 95 °C for 15 min and then centrifuged (8000 rotation) for 1 min. The supernatant was stored at −20 °C until use. Conditions for PCR reaction were optimized by repeated reactions. Standard isolate of *E. faecalis* (ATCC 29212) was used as positive control. The reaction mixture was composed of 1.0×PCR buffer, 2.0 mmol L^{-1} $MgCl_2$, 0.2 mmol L^{-1} dNTP (Fermentas, Vilnijus, Lithuania), six primers (each 0.5 μmol L^{-1}) and 2 units of recombinant Taq DNA polymerase (Cinnagan Inc, Tehran, Iran). Primers for PCR were used as described by Mahmoudpor *et al.* (2007). Primer sequences were designed based on the whole *E. faecalis* V583 genome:

E16F (AGAGTTTGATCCTGGCTCA) and Ef16R (GGTTACCTTGTTACGACTTC); product 1522 bp;

EfisF (ATGCCGACATTGAAAGAAAAAATT) and EfisR (TCAATCTTTGGTTCCATCTCT); product 803 bp;

EfesF (GTGTTAAAACCATTAGGCGAT) and EfgsR (AAGCCTTCACGAACAATGG); product 650 bp.

The final volume of the reaction mixture was 25 μL, including 2–5 μL of primers. Conditions for

PCR were starting denaturation during 4 min on 95 °C, 35 cycles 95 °C per 30 s; 55 °C per 30 s; and 72 °C per 90 s. Gel electrophoresis reaction was performed on 1% agarose gel (Cinnagen, Tehran, Iran) for gel electrophoresis reaction (Akhtarian, Tehran, Iran) for 1–1.5 h in 1× TEA buffer. After the electrophoresis was completed, the molecules in the gel were stained with ethidium bromide (Merck), which when intercalated into DNA, fluoresce under ultraviolet light (UVP Gel Documentation, Upland, CA, USA). Samples that contained *E. faecalis* DNA showed positive amplification of 1522, 803, 650 pairs base (Fig. 1).

Statistical analysis

The Mann–Whitney *U* test was used for intragroup analyses (before and after certain disinfection protocol). Results after the treatment were presented graphically (Box and Whisker plot). The Kruskal–Wallis test was used for the intergroup comparative analysis of data of second samples. The significance level was set at 5%. Analyses were performed using SPSS 11. 0 (SPSS, Chicago, IL, USA).

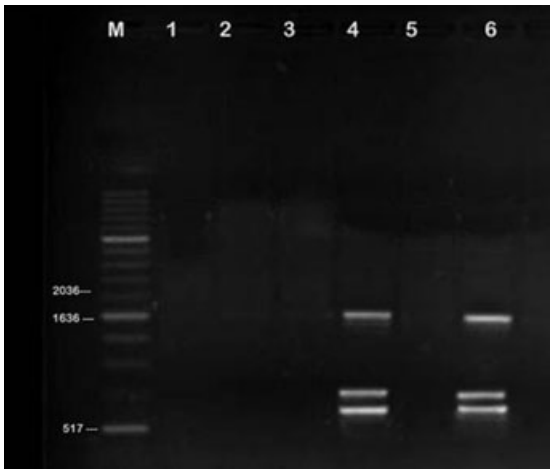

Figure 1 Agarose gel electrophoresis for PCR identification of *Enterococcus faecalis* DNA using 150 0 bp DNA ladder.

Results

The scanning electron observation revealed colonization of *E. faecalis* on the canal surface (Fig. 2).

Table 1 presents the distribution of the results (mean, median, range, reduction rate of CFUs) before and after treatment protocols. The reduction in the number of CFUs after the treatment protocol was highly significant for all groups ($P < 0.001$). Almost all experimental techniques were significantly superior over the positive control ($P \leq 0.001$) except the high-power diode laser ($P = 0.271$) and conventional NaOCl syringe irrigation ($P = 0.795$). EndoActivator and PAD, using both Helbo and LaserHF, were equally effective in reducing *E. faecalis* populations ($P > 0.05$) and statistically more effective than the high-power diode laser and conventional NaOCl syringe irrigation ($P < 0.05$). PAD and EndoActivator also achieved equal number of negative cultures in the second samples (six of 20). The high-power diode laser and conventional NaOCl syringe irrigation did not differ significantly between each other ($P = 0.131$).

Discussion

Repeated appointments and inadequate coronal sealing during root canal treatment can lead to the recolonization of the root canal system and reinfec-

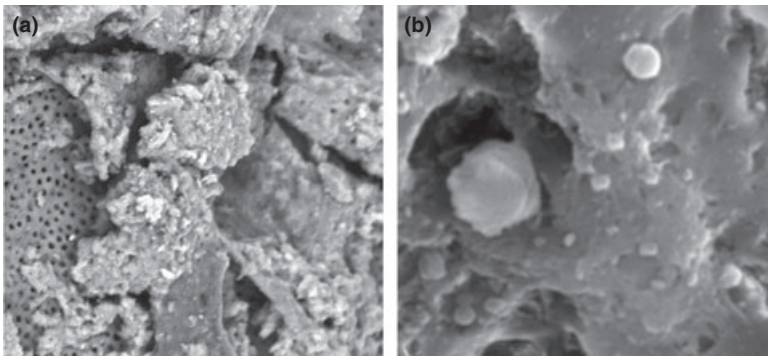

Figure 2 Scanning electron micrograph of *Enterococcus faecalis* colonization on root canal surface, magnification of 1000 (a) and 15 000 (b).

Table 1 Counts of *Enterococcus faecalis* CFUs before and after four disinfection protocols

Group	Baseline sample			Second sample			% Reduction median (range)
	Mean	Median	Range	Mean	Median	Range	
Diode laser	6.59×10^8	7.00×10^7	1.00×10^7 to 8.00×10^9	4.5×10^5	8.00×10^4	2.00×10^3 to 5.00×10^6	99.89 (99.93–99.98)
PAD (HFLaser)	5.43×19^8	4.00×10^8	3×10^6 to 2.20×10^8	2.00×10^4	7.50×10^2	0 to 1.80×10^5	99.99 (99.99–100)
PAD (Helbo)	2.55×10^8	2.00×10^8	1.80×10^6 to 8.00×10^8	2.02×10^3	7.50×10^2	0 to 8.00×10^3	99.99 (99.99–100)
NaOCl (30G needle)	4.30×10^8	3.00×10^8	1.00×10^7 to 9.00×10^9	2.09×10^6	6.00×10^5	1.00×10^4 to 2.00×10^7	99.80 (97.78–99.90)
NaOCl (EndoActivator)	5.38×10^8	3.50×10^8	4.00×10^6 to 2.40×10^8	1.12×10^3	5.00×10^2	0 to 6.00×10^3	99.99 (99.99–100)
Control	1.45×10^8	1.00×10^8	1.00×10^7 to 4.00×10^8	6.10×10^5	3.00×10^5	1.00×10^5 to 2.00×10^6	99.70 (99.00–99.50)

tion, which may consequently hinder the healing of periradicular tissues (Siren *et al.* 1997). Therefore, the main goal is to complete treatment in as few visits as possible using protocols that can achieve root canal disinfection effectively (Siqueira 2002). Over the years, an increasing number of studies on root canal disinfection techniques have been published but with contradictory results. Therefore, this subject merits additional study to determine the most effective root canal disinfection protocols.

This laboratory study evaluated the antimicrobial effect of four disinfection techniques, which could be used as an adjunct to chemomechanical canal preparation. *E. faecalis* was chosen as the microbiological marker because it has the ability to colonize the root canal in biofilms, representing the *in vivo* growth condition (Love 2001). In addition, it often survives chemomechanical preparation (Gomes *et al.* 2006) because of its resistance to antimicrobial agents and its ability to cause a monoinfection in the root canals (Portenier *et al.* 2003, Nakajo *et al.* 2006).

The results clearly showed the superiority of PAD and sonic activated irrigation. What is more, only these two techniques succeeded in the eradication of *E. faecalis* from the root canals of six samples. The good antimicrobial efficacy of the PAD is in accordance with the results of other studies, which also evaluated its use (Garces *et al.* 2007, Bergmans *et al.* 2008). Fonesca *et al.* (2008) reported a large reduction rate (99.9%) after treating intracanal *E. faecalis* with toluidine blue and a 50-mW diode laser. When comparing PAD protocols variables such as light parameters, photosensitizers and light delivery technique have to be considered (Soukos *et al.* 2006, Foschi *et al.* 2007). It was expected that the 3D EndoProbe of the Helbo laser would achieve greater antimicrobial effects than the 2D Spot Probe of LaserHF. However, both systems achieved the same *E. faecalis* reduction rate, regardless of the different photosensitizer and concentration used. Differently from the present study, Meire *et al.* (2009) reported the greater efficacy of 2.5% NaOCl compared to the PAD. It is well known that biofilm maturity influences its tolerance to killing by antimicrobial agents (Portenier *et al.* 2003). Thus, it is quite possible that 7-day-old *E. faecalis* biofilm, used in this study, was more resistant to NaOCl than the 24-h-old biofilm in the study of Meire *et al.* (2009). In addition, the time of exposure to 2.5% NaOCl was 1 min in the present study, whereas in the study of Meire *et al.*(2009), it was 15 min and without subsequent NaOCl inactiva-

tion with sodium thiosulfate. On the other hand, regardless of the previously mentioned factors, the PAD may be actually more effective than single NaOCl irrigation in the eradication of intracanal *E. faecalis*.

Furthermore, the findings did not reveal differences between conventional NaOCl irrigation, saline irrigation and high-power diode laser, which can be partially explained with the time of NaOCl exposure. There is still no agreement regarding the time of disinfection with NaOCl required to eliminate *E. faecalis* from the root canals. The one minute of disinfection used in this study was chosen according to the time recommended for the final disinfection protocol (Harrison *et al.* 2010, Alves *et al.* 2011). However, the results suggest that the time was not sufficient for the antimicrobial action of 2.5% NaOCl. In fact, the equal efficacy of saline and the NaOCl irrigation could be attributed to the mechanical action of streaming and fluid replacement as a result of continuous irrigation with a flow rate of 5 mL min^{-1}. It has been demonstrated already that irrigant velocity on the root canal wall is an important fluid mechanic parameter, and shear stress on the canal wall influences on the mechanical detachment of debris, isolated microbes and biofilm (Boutsioukis *et al.* 2010). Regarding the high-power diode laser, the present results are in agreement with previous studies that also demonstrated greater difficulties in eliminating gram-positive *E. faecalis* using diode and Nd:YAG lasers (Schoop *et al.* 2004, Meire *et al.* 2009). Laser-induced bacteria killing is because of thermal heating of the environment above the lethal values and local heating inside bacteria (Meire *et al.* 2009). Survival of *E. faecalis* and the lower reduction rates can be attributed to the high resistance of *E. faecalis* to heat, because of its cell-wall structure (Schoop *et al.* 2004).

Disinfection agents such as NaOCl require direct contact with the bacteria what is often impossible in peripheral areas of the root canal such as anastomoses, fins and the most apical part of the main root canal (Haapasalo *et al.* 2010). The EndoActivator system has been reported to provide deeper penetration of an irrigant to all areas of the endodontic space, and to effectively clean debris from lateral canals, remove the smear layer and dislodge clumps of simulated biofilm (Caron 2007). This was also confirmed in the present study where significantly greater efficiency of the EndoActivator against intracanal *E. faecalis* biofilm compared to the NaOCl irrigation alone was found. Similar bacterial load reduction was reported by Pasqualini *et al.* (2010). However, they did not observe complete *E. faecalis* eradication in any of the tested protocols with the EndoActivator. In both studies, the diameter and taper of the root canal instrumentation, the amount of NaOCl and the time of sonic activation were same. Therefore, it is suggested that the oscillation of the EndoActivator polymer tips of greater size (size 25, 0.04 taper), used in this study, created more powerful hydrodynamic phenomenon and caused complete eradication of *E. faecalis*. The possible influence of the size of sonically or ultrasonically oscillating tip upon the irrigation has been previously indicated (Ahmad *et al.* 1987, Brito *et al.* 2009). For a more reliable conclusion, it is necessary to compare the efficacy of different sizes of the EndoActivator tips during irrigation in the root canal.

In this study, the PAD and the EndoActivator were superior to diode laser and single NaOCl irrigation in eliminating intracanal *E. faecalis*. However, to determine the most effective endodontic disinfection protocol, the efficacy of the techniques should be further determined on multispecies biofilm. Although the time of 1 min caused 99.99% reduction after PAD and sonic activation of NaOCl, it is questionable whether longer exposure time could provide complete eradication in all samples. Finally, it is necessary to evaluate their real contribution to conventional chemomechanical preparation in *in vivo* studies.

Conclusion

The EndoActivator and PAD succeeded in reducing root canal infection and had the capacity to eradicate *E. faecalis*. The high-power diode laser and the conventional NaOCl syringe irrigation had equal and lower antibacterial effect.

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