Efficacy of Photon-induced Photoacoustic Streaming in the Reduction of *Enterococcus faecalis* within the Root Canal: Different Settings and Different Sodium Hypochlorite Concentrations

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Abstract

Introduction: The purpose of this study was to determine the effectiveness of laser-activated irrigation by photon-induced photoacoustic streaming (PIPS) in the reduction of *Enterococcus faecalis* in root canal disinfection, varying laser energy output, and sodium hypochlorite (NaOCl) concentration. For effective removal of the smear layer, the sequence and resting time of the final irrigation steps were modified compared with the standard PIPS protocol. **Methods:** Eighty-six extracted single-rooted teeth were mechanically prepared, sterilized, and inoculated with *E. faecalis* for 4 weeks. Teeth were divided into 9 groups and treated with an Er:YAG laser using a PIPS 600/9 tip at the following parameters: 10 mJ or 20 mJ, 15 Hz, and a 50-microsecond pulse duration at 0.15 W or 0.3 W average power, respectively. Root canals were irrigated with different concentrations of NaOCl (ie, 1%, 3%, and 5% and activated using the adjusted PIPS protocol). The bacterial count was performed immediately after and 48 hours after decontamination and new incubation on an agar plate. **Results:** A statistically significant difference in bacterial counts ($P < .05$) was detected in all groups before and directly after the treatment and in groups treated with 5% NaOCl 48 hours after treatment. Scanning electron microscopic imaging showed an absence of bacteria and biofilm in the scanned areas after treatment with 5% NaOCl. **Conclusions:** Laser-activated irrigation using 5% NaOCl and a modified PIPS protocol resulted in effective eradication of the bacterial biofilm and removal of the smear layer. *(J Endod 2017;■:1–6)*

Key Words

Disinfection, *Enterococcus faecalis*, irrigation, laser, photon-induced photoacoustic streaming

Significance

Laser-activated irrigation by photon-induced photoacoustic streaming is effective in the reduction of *Enterococcus faecalis* in root canal disinfection with widely used sodium hypochlorite.

The main cause of pulpitis and apical periodontitis is bacteria in planktonic and/or biofilm form (1). Biofilm provides protection for microorganisms against immune responses, stress, antibacterial agents, and antibiotics (2) and enables better resistance to external influence (3).

Until recently, mechanical instrumentation was the most important part of endodontic treatment. Despite different file systems and improved metallurgical properties of materials, more than 35% of the root canal surface remained untouched by instrumentation (4). Furthermore, root canal instrumentation has several disadvantages including dentin debris and smear layer production, iatrogenic errors (eg, apical transportation, crack formation, and stripping), and weakening of the root structure (5–7). Long-term success and resistance to fracture are related to the amount of residual tooth structure (8). Increasing diameter and taper size are counter to the concept of minimally invasive dental treatment.

Root canal irrigation enables chemical dissolution of organic and inorganic tissue, mechanical detachment and flushing of microorganisms and their products, dentin debris, and the smear layer. Different irrigation techniques have been developed. Laser-activated irrigation ($LAI_{s.l.}$) is based on the activation of liquid irrigants by medium-infrared lasers (2780 nm and 2940 nm). Two different techniques are suggested: the tip is placed inside the root canal ($LAI_{s.s.}$) or located inside the pulp chamber only (photon-induced photoacoustic streaming [PIPS]). The latter technique uses a radial firing and stripped tip, allowing lateral emission of laser energy in the liquids. The use of subablative energy (20 mJ) delivered in a very short time (pulse duration of 50 microseconds) produces a high peak power of 400 W, causing an explosion-implosion phenomenon within the irrigant solution. The result is a strong photoacoustic shock wave that induces irrigant streaming 3-dimensionally throughout the entire root canal system while avoiding any direct laser irradiation on the dentin and consequent unwanted thermal effects (9, 10).

Compared with other techniques, many studies reported better cleaning and more efficient smear layer removal using the PIPS technique as confirmed by scanning

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electron microscopy (SEM). A clean dentin surface and open tubules without smear layer or debris remnants are reported (11, 12).

In this study, we investigate the efficacy of the PIPS technique using lower energy and different concentrations of sodium hypochlorite (NaOCl) than suggested elsewhere to increase the overall safety of the procedure. Additionally, a modified sequence of irrigant application was introduced in the final debridement stage to achieve enhanced reduction of *Enterococcus faecalis* in root canal disinfection.

Materials and Methods

Selection of Samples

Eighty-six single-rooted teeth including maxillary incisors, canines, and premolars together with mandibular canines and premolars were used. Teeth extracted for periodontal reasons had external root surfaces cleaned with curettes to remove calculus and periodontal soft tissues. Teeth were stored in physiological solution at 4°C until use.

Root Canal Preparation

Cavity access was prepared using tapered diamond burs in a high-speed handpiece with water cooling and finished with an ultrasonic device (START X nos. 1 and 3; Dentsply DeTrey, Mantova, Switzerland) under microscopic control (Zeiss, Oberkochen, Germany). Using a stainless steel ISO 10 C-PILOT File (VDW, Muenchen, Germany), a glide path to the working length was created. Root canals were prepared with Reciproc R25 (VDW), and the apical preparation was manually completed using K-Reamers ISO30/06 (VDW). Copious irrigation with 3% NaOCl during preparation and a final flush with sterile distilled water were performed.

Bacterial Infection of the Root Canal

Three randomly selected teeth were not infected and served as the negative control group (Table 1). The root canals of the remaining 83 teeth were dried using sterile paper points. Their apical foramina were sealed with a 2-step bonding system (FL-Bond II; Shofu Inc, Kyoto, Japan) and Beautiful Flow Plus F00 composite (Shofu Inc). The entire external root surface of each tooth was isolated with 2 layers of nail polish to prevent lateral and apical bacterial leakage. A pure culture of vancomycin-resistant *E. faecalis*, originating from a single colony and grown in brain-heart infusion broth, was used. Using a micropipette, root canals were inoculated with 10 μL of a bacterial suspension (approximately 5×10^8 colony-forming units [CFUs] per milliliter). The specimens were incubated in individual test tubes for 4 weeks at 37°C, and fresh bacterial suspension was provided every 24 hours. After the incubation, 10 μL suspension fluid from the root canal was withdrawn using a micropipette and serially diluted in 0.9 mL physiological solution. To enumerate bacteria, 0.1-μL aliquots of appropriate dilutions of each specimen were spread onto Columbia agar plates supplemented with vancomycin (40 mg/L). The consequent incubation lasted for 24 hours at 37°C and was kept in an atmosphere of 5% carbon dioxide. The detection limit for bacterial growth was approximately 10^2 CFU/mL sampling fluid. Three from 83 infected teeth were randomly selected for the positive control group and were not treated. The remaining 80 teeth were divided into 9 groups (Table 1).

Canal Disinfection

Disinfection was performed with $LAI_{s.l.}$ at different power settings and concentrations of NaOCl (Table 1). Two Er:YAG lasers (2940 nm) were used following a modified PIPS protocol (13). A proprietary PIPS tip (600/9) was placed inside the pulp chamber and kept stationary. During laser activation, the tip was submerged in irrigant that was continuously applied with a syringe (double side vent Luer Lock 27-G needle). PIPS disinfection was performed using 2 different power settings while water and air on the laser system were turned off. Details of the performed treatment are provided in Table 1. The modified irrigation protocol was as follows:

1. 3 mL NaOCl, PIPS activation for 30 seconds, and rest for 60 seconds
2. 3 mL 17% EDTA, PIPS activation for 30 seconds, and rest for 60 seconds
3. 2 cycles of 3 mL NaOCl, PIPS activation for 30 seconds, and rest for 60 seconds between each cycle
4. 3 mL sterile water and PIPS activation for 30 seconds

For maximum sampling efficacy, the specimens were sealed with Parafilm (Genova, Italy) and vortexed for 10 seconds (MSE Clinomixer; MSE Scientific Instruments, Milano, Italy). After introducing a sterile Ringer solution into the root canal, 10-μL aliquots of solution from the root canal of each specimen were obtained, and a bacterial count was performed immediately.

Assessment of Further Bacterial Growth

After irrigation and the bacterial count, all specimens were incubated for 48 hours at 37°C and kept in an atmosphere of 5% carbon dioxide. Afterward, 10-μL aliquots of solution were obtained as described previously, and a new bacterial count was performed.

TABLE 1. A List of the Analyzed Teeth and Treatment Conditions

Sample group	Number of teeth	Infection (Y/N)	Treatment (Y/N)	Laser energy (mJ)	Average power (W)	NaOCl (%)	Type of laser*
Infected teeth							
$PIPS_1$ 20-5	6	Yes	Yes	20	0.30	5	Laser 1
$PIPS_1$ 20-3	8	Yes	Yes	20	0.30	3	Laser 1
$PIPS_1$ 20-1	6	Yes	Yes	20	0.30	1	Laser 1
$PIPS_2$ 20-5	10	Yes	Yes	20	0.30	5	Laser 2
$PIPS_2$ 20-3	10	Yes	Yes	20	0.30	3	Laser 2
$PIPS_2$ 20-1	10	Yes	Yes	20	0.30	1	Laser 2
$PIPS_2$ 10-5	10	Yes	Yes	10	0.15	5	Laser 2
$PIPS_2$ 10-3	10	Yes	Yes	10	0.15	3	Laser 2
$PIPS_2$ 10-1	10	Yes	Yes	10	0.15	1	Laser 2
Positive control group	3	Yes	No	—	—	—	—
Negative control group	3	No	No	—	—	—	—
Sum	86	—	—	—	—	—	—

*Two laser models were used: laser 1 (AT Fidelis) in which energy can be reduced a minimum to 20 mJ and laser 2 (Light Walker) in which energy may be reduced all the way to 10 mJ. In all cases, the frequency was set to 15 Hz and the pulse duration to 50 microseconds. $PIPS_1$ 20-5, etc, are sample group names, where subscript 1 is referring to the type of laser (1 or 2), and the number 20-5 is a combination of laser energy and concentration of NaOCl that was used during treatment.

TABLE 2. The Mean Colony-forming Units per Milliliter Results for 9 Experimental Groups

Sample group*	Number of teeth	Laser energy (mJ)	NaOCl (%)	Colony-forming unit/mL			*P* value	
				Pretreatment	Posttreatment	48 h posttreatment	Before and after	Before and after 48 h
$PIPS_1$ 20-5	6	20	5	1.9×10^7	$<1.0 \times 10^2$	$<1.0 \times 10^2$	<.05	<.05
$PIPS_1$ 20-3	8	20	3	1.9×10^7	9.0×10^2	2.9×10^7	<.05	.70
$PIPS_1$ 20-1	6	20	1	4.0×10^7	4.0×10^3	4.4×10^7	<.05	.88
$PIPS_2$ 20-5	10	20	5	1.3×10^7	$<1.0 \times 10^2$	$<1.0 \times 10^2$	<.05	<.05
$PIPS_2$ 20-3	10	20	3	1.2×10^7	$<1.0 \times 10^2$	1.3×10^7	<.05	.91
$PIPS_2$ 20-1	10	20	1	1.4×10^7	$<1.0 \times 10^2$	1.0×10^7	<.05	.37
$PIPS_2$ 10-5	10	10	5	1.3×10^7	$<1.0 \times 10^2$	$<1.0 \times 10^2$	<.05	<.05
$PIPS_2$ 10-3	10	10	3	2.7×10^7	$<1.0 \times 10^2$	1.3×10^7	<.05	.19
$PIPS_2$ 10-1	10	10	1	1.8×10^7	$<1.0 \times 10^2$	9.0×10^6	<.05	.09

*$PIPS_1$ 20-5, etc, are sample group names, where subscript 1 is referring to the type of laser (1 or 2), and the number 20-5 is a combination of laser energy and concentration of NaOCl that was used during treatment.

SEM

Scanning electron microscopic analyses were used to determine the presence of bacterial infection in canal cross sections and bacterial penetration inside dentin tubules. For sample preparation, root canals were dried for 24 hours at 21°C, longitudinally sectioned using a carbon disk without water spray to make a guiding line, and split into 2 halves with a hammer. After drying for 24 hours at 21°C, they were sputter coated with gold. Using SEM (Vega3; Tescan, Brno, Czech Republic), the entire root canal area (1–8 mm from the apex) in each specimen was examined.

Statistical Analysis

Bacterial counts in CFUs were measured using log calculations to conform to normal distribution. Statistical analyses using SPSS software (Version 14.0 for Windows; SPSS Inc, Chicago, IL) were performed. One-way analysis of variance testing for repeated measures was used to test for statistical differences in normally distributed variables. Post hoc Sheffé tests were used when 2 or more mean data points were compared. Data were summarized as the mean depending on the variable. *P* values (2-sided) <.05 were considered statistically significant.

Results

Results of Statistical Analysis

Analysis of variance was used to evaluate the decontamination of the root canal after treatment with different concentrations of NaOCl and different power settings following a modified PIPS protocol. Measurements were performed before, immediately after, and 48 hours after treatment (Table 2). Statistically significant differences in bacterial counts ($P < .05$) were obtained before and directly after treatment for all sample groups regardless of the NaOCl concentration and the applied laser energy. Before and 48 hours after treatment, a statistically significant difference was noticed only in groups $PIPS_1$ 20-5, $PIPS_2$ 20-5, and $PIPS_2$ 10-5 where 5% NaOCl was used. In groups treated with 1% and 3% NaOCl, bacteria regrew 48 hours after the procedure regardless of the applied laser power. Post hoc analysis confirmed that decontamination is effective and durable only when using 5% NaOCl (Fig. 1*A* and *B*).

Results of SEM

Using SEM examination, the area 1–8 mm from the apex was examined. In negative control specimens, remnants of some debris, smear layer without bacteria, and a few open dentin tubules were observed (Fig. 2*A*). Positive control specimens showed a thick biofilm of *E. faecalis*. Dentinal tubules were occluded by the smear layer (Fig. 2*B*), and numerous *E. faecalis* bacteria were colonizing dentinal tubules (Fig. 2*C*).

After using NaOCl and 17% EDTA, the smear layer, debris, and bacteria were absent, and open dentinal tubules were exposed (Fig. 2*D–F*). Results of treatment with different concentrations of NaOCl are shown in Figure 2. The decontamination was only efficient when using 5% NaOCl.

Discussion

Teeth used in our experiment were incubated with *E. faecalis*, which is the most common isolated bacteria connected to failed endodontic treatment (14). The incubation time, which is an important factor for biofilm development, was set to 4 weeks. During the first hour, bacteria are mostly planktonic. During the first 2 weeks, biofilm bacteria are

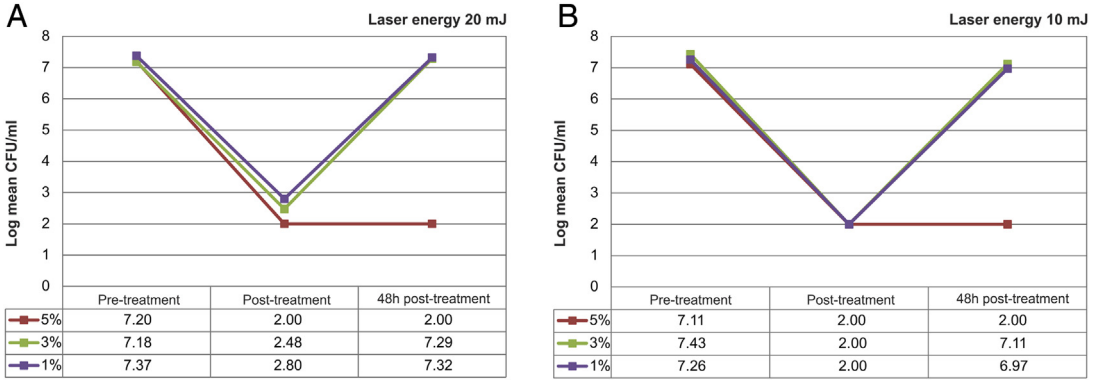

Figure 1. A variation of the decontamination procedure with time for the different laser power settings, (*A*) 20 mJ and (*B*) 10 mJ, and the different concentrations of NaOCl used during the modified PIPS irrigation procedure.

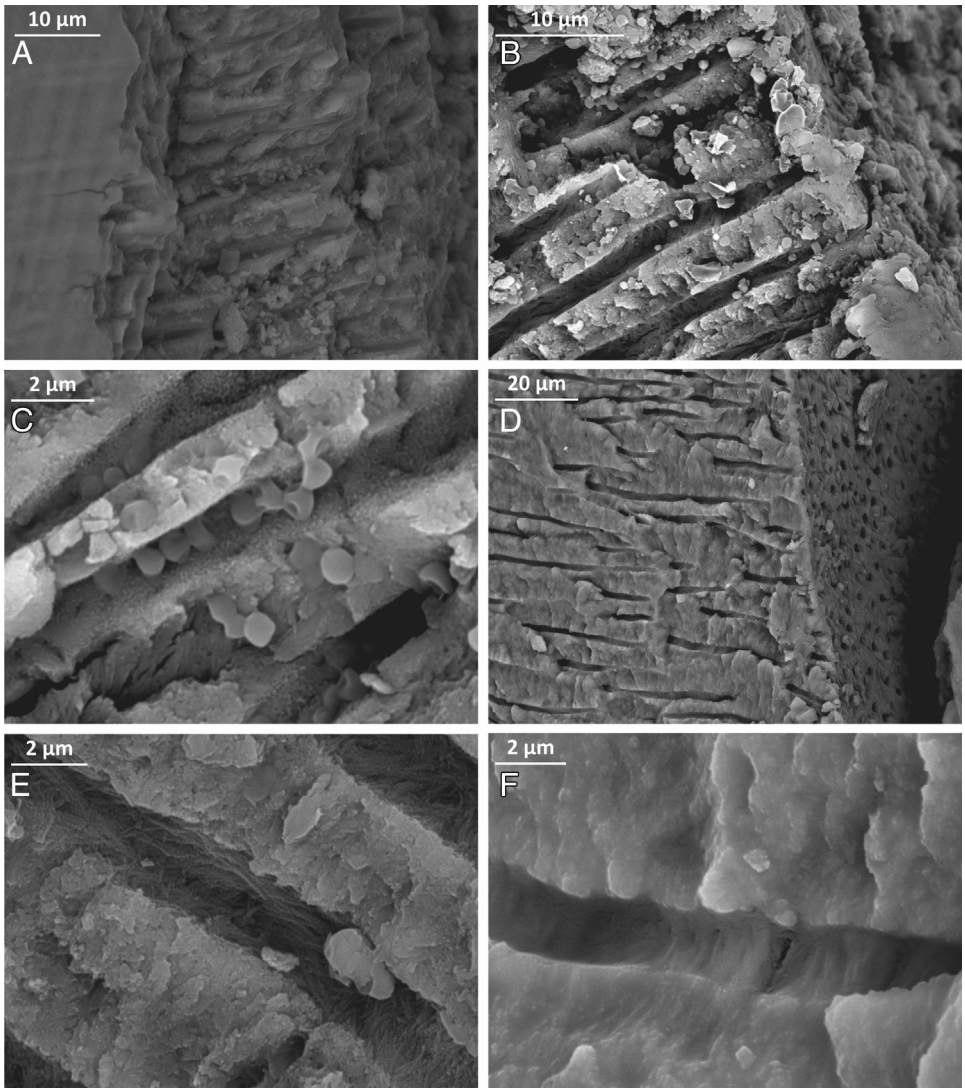

Figure 2. SEM images. (*A*) The negative control group after 3 days of sterilization with 5% NaOCl shows debris, the smear layer, and some open dentinal tubules without bacteria (5110× magnification). (*B*) The positive group after 4 weeks of incubation with *E. faecalis*. The smear layer and dental plug occluding dentinal tubules, which are infected by bacteria (7920× magnification). (*C*) A high concentration of *E. faecalis* colonizing the dentinal walls of tubules (24,400× magnification). (*D*) Treatment with 17% EDTA and 1% NaOCl. Clean open dentinal tubules with few remnants of debris are present (2400× magnification). (*E*) Treatment with 17% EDTA and 3% NaOCl. High magnification shows clear dentinal tubules with collagen fibers without bacteria (22,800× magnification). (*F*) Treatment with 17% EDTA and 5% NaOCl. The longitudinal section along the clean dentinal tubule without any bacteria (20,900× magnification).

sensitive to NaOCl (1%), chlorhexidine (2%), and iodine (0.2/0.4%); after 3 weeks, they become very resistant to the same agents (15).

In endodontic therapy, the combination of NaOCl and EDTA irrigants is most commonly used. Both weaken dentin by dissolution of its organic and inorganic components. The elastic modulus and flexural strength of dentin are dependent on the NaOCl concentration and time of exposure (5% NaOCl at 37°C: reduction by half in 1 hour [16], 3% NaOCl: significant reduction after 2 hours [17] only). We used 3% NaOCl during the preparation of the canal and time of instrumentation, and irrigation was extended for teeth with more than 1 root canal. For the final irrigation, a statistically significant difference in the reduction of *E. faecalis* by PIPS, before and 48 hours after treatment, was obtained only when using 5% NaOCl.

The classic final irrigation sequence for the PIPS technique is as follows (18): 3× NaOCl followed by a cycle of distilled water and finished with EDTA (Fig. 3*A*). Each active cycle of agitation/activation for 30 seconds is followed by a 30-second resting phase that allows the irrigant to react. The most effective sequence of irrigant used is reported as follows: 5.25% NaOCl during instrumentation, 17% EDTA during final irrigation, and again 5.25% NaOCl (19, 20). When the last irrigant in the sequence is EDTA, collagen is exposed on the surface of the root canal lumen (19, 21). The remaining *E. faecalis* inside the dentinal tubules later binds on collagen and causes failure of the endodontic treatment (22, 23). In our study, a modified sequence of irrigants during the final flushing was shown to be effective, and the resting phase was prolonged to 1 minute (Fig. 3*B*). After 1 minute of irrigation with 17% EDTA, the smear layer was completely removed, dentin tubules were opened, and only a slightly erosive effect of EDTA in the peritubular and intertubular dentinal area was observed (24). The total exposure time of 6 minutes, during the full sequential use of EDTA and NaOCl, is too short to affect the mechanical properties of dentin (25). The application of EDTA before

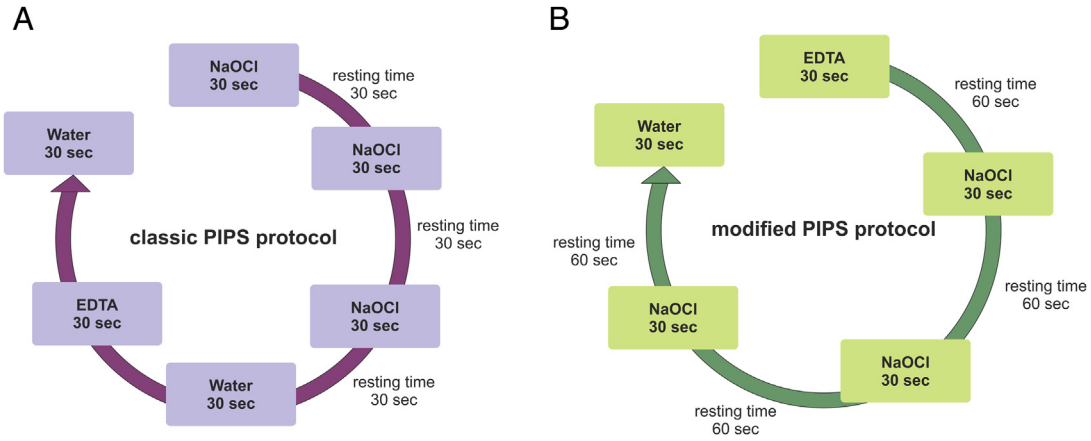

Figure 3. Comparison between (*A*) the classic PIPS protocol (adapted from Jamarillo [18]) and (*B*) the modified PIPS protocol.

NaOCl enables cleaning of the dentin surface and opening of the dentinal tubules as evident from scanning electron microscopic images. Therefore, penetration of NaOCl in the dentinal tubules and destruction of *E. faecalis* are more likely possible.

Water is the main component of NaOCl and EDTA and the main chromophore for the laser with a wavelength of 2940 nm (26). The bactericidal effect of PIPS with continuous activation of NaOCl is based on the following effects: (1) an increased reaction rate of NaOCl (27), (2) 3-dimensional streaming of the fluid with improved penetration of the irrigant into dentinal tubules (28, 29), and (3) shock wave phenomena causing cell lysis and mechanical breakup of debris and the smear layer (30). The primary thermal effect of laser energy absorbed in water leads to superheating of the irrigant up to the boiling point and formation of vapor bubbles. The size and the life cycle of bubble (explosion and successive implosion) depend on the applied energy (31, 32). The bubble energy is proportional to the volume of the bubble at its maximum size and is converted into the mechanical energy of the liquid medium (33). The energy of the bubble collapse (implosion) is concentrated in a short time within a small volume, causing cavitation (34). The ejection of irrigant through the apical foramen during root canal irrigation with NaOCl may cause complications because NaOCl is toxic in vital tissues (35). The extrusion of irrigant occurs because of high pressure in combination with a wide apical foramen. Our results show that the reduction of bacteria in the root canal during PIPS irrigation was equal when using 10 or 20 mJ with 5% NaOCl.

Conclusions

Based on results obtained and in comparison with the published literature, we made the following conclusions:

1. During instrumentation, 3% NaOCl is recommended for irrigation to avoid weakening of dentin and superficial erosion of the dentinal surfaces.
2. To increase the effectiveness of the treatment, we propose the adjustment of the final irrigation sequence in the PIPS protocol as follows:
   a. 17% EDTA for the removal of the mineralized part of the smear layer and opening of dentinal tubules for irrigant
   b. 3 × 5% NaOCl for the reduction of microorganisms in dentinal tubules and the denaturation of collagen, which is important for the bacterial binding, including *E. faecalis*
   c. Distilled water for the inactivation of oxygen before obturation to avoid a chemical interaction of sealer with oxygen
3. To increase the safety of the treatment, we lowered laser energy from 20 to 10 mJ without diminishing the efficacy of PIPS treatment for the reduction of *E. faecalis*.

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The authors deny any conflicts of interest related to this study.

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