ADA CE Program ARTICLE 3

Disinfection efficacy of photon-induced photoacoustic streaming on root canals infected with *Enterococcus faecalis*

An ex vivo study

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ABSTRACT

Background. In 2010, one of the authors proposed that lasers could be used to enhance the decontaminating action of sodium hypochlorite (NaOCl). The authors conducted a study to compare the disinfection efficacy of laser-activated irrigation (LAI) by using a photon-induced photoacoustic streaming (PIPS) tip with conventional irrigation and specifically LAI's ability to remove bacterial film formed on root canal walls.

Methods. The authors shaped 26 human anterior teeth to a master apical file size of International Organization for Standardization 25/06 (size 25 tip and size .06 taper) and then sterilized the teeth, infected them with *Enterococcus faecalis* and incubated them for four weeks. The authors used two irrigation protocols. Group A received two cycles of 30 seconds each of 5 percent NaOCl laser activation and one cycle of 30 seconds with laser activation involving the use of 17 percent ethylenediaminetetraacetic acid (EDTA). The erbium:yttrium-aluminum-garnet (Er:YAG) laser's settings were 20 millijoules, 15 hertz, 50-microsecond pulse duration, and it had a 600-micrometer PIPS tip. Group B received two cycles of 30 seconds each of 5 percent NaOCl and 17 percent EDTA irrigation alone, delivered via a syringe with a 25-gauge needle.

Results. The authors found that group A had significantly better disinfection compared with group B ($P < .05$). The results of cultures obtained after 48 hours showed that disinfection was maintained better in group A compared with group B ($P < .0001$). Scanning electron microscopic images showed absence of bacterial biofilm remaining after LAI using PIPS.

Conclusions. Er:YAG laser activation of 5 percent NaOCl and 17 percent EDTA was more effective than conventional irrigation for eradicating *E. faecalis* and preventing new bacterial growth ex vivo. Additional clinical studies are needed to clarify the effect on endodontic treatment outcomes.

Practical Implications. PIPS appears to be effective in enhancing the effect of the irrigants commonly used in endodontics.

Key Words. Decontamination; lasers; endodontics; irrigation.
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Debridement focusing on removal of pulp remnants, as well as microorganisms and microbial toxins from the root canal system, is considered essential for endodontic success.[1-3] However, current endodontic techniques fall short of the goal to remove all infective microorganisms and debris consistently. This shortfall mainly is due to the complex anatomy of the root canal system,[4-6] the type of bacteria and resistance of bacterial colonization, the limitation of rotary instrumentation to remove all tissue from the surfaces after completion of the preparation[7-9] and the limited potential for commonly used irrigants to penetrate the dentin walls.[10]

Irrigation is an essential part of root canal therapy because it allows for cleaning and decontaminating beyond what might be achieved by instrumentation alone. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant because it dissolves organic tissue, kills microorganisms and acts as a lubricant.[11,12] However, owing to high surface tension, NaOCl penetration is limited to about 130 micrometers into dentin tubules, whereas bacteria can colonize the dentin tubules as deeply as 1,100 µm from the canal lumen.[10]

Warming NaOCl from 20°C to 45°C, as well as agitating it, was shown to enhance its efficacy in killing bacteria.[13] Tissue dissolution was greater when NaOCl was agitated continuously than when only the temperature was increased.[14]

Different agitation techniques have been proposed to improve the efficacy of irrigation solutions, including hand agitation and the use of sonic and ultrasonic devices.[14,15] In 2009, investigators found that lasers activated irrigation solutions via the transfer of pulsed energy.[16] Laser-

activated irrigation (LAI) by means of an erbium laser (2,780 nanometers and 2,940 nm) was more effective in removing dentin debris and the smear layer, respectively, compared with passive ultrasonic irrigation or hand irrigation.[17-19] The use of laser energy also has been shown to enhance the decontaminating action of NaOCl.[20] During the LAI process, photons are emitted in a short time (50 microseconds) to pulse subablative energy levels of laser light (20 millijoules) to generate photoacoustic shockwaves into liquid-filled root canals.

With the irrigant continuously delivered by means of a syringe into the pulp chamber during the laser-activation process, the resultant shock wave travels three dimensionally in the fluid and effectively debrides and removes both vital and necrotic tissue remnants.[21]

We conducted a study involving NaOCl to determine the efficacy of LAI using photon-induced photoacoustic streaming (PIPS) by using an erbium:yttrium-aluminum-garnet (Er:YAG) laser with a quartz radial and stripped tip to disinfect root canals. We compared the results of LAI with those of conventional hand irrigation.

METHODS

The ethics committee at the University of Genoa, Italy, approved the study protocol (protocol GE-15-011).

In the study, we used 26 single-root human anterior central and lateral incisors and canines (with the exception of mandibular central and lateral incisors) extracted for periodontal reasons with patients' written consent. We stored all of the teeth in 0.1 percent thymol solution at 4°C during the collection process and until use.

Root canal treatment. We prepared access cavities on all 26 teeth by using a tapered diamond bur and then created a glide path to working length by using a size 10 K file. We then prepared root canals by using nickel titanium rotary files in a sequential crown-down method to a size International Organization for Standardization 20 tip with a .06 taper (Profile GT, Dentsply Tulsa Dental, Tulsa, Okla.). We manually completed the apical preparation by using a size International Organization for Standardization 25/06 (size 25 tip and size .06 taper) master apical file. We provided copious irrigation by using 5 percent NaOCl during the instrumentation. We performed a final flush by using sterile distilled water. We dried the canals by using air and sterile paper points and placed the teeth individually in sterilizer pouches, autoclaved them at 134°C for 17 minutes and stored them until use. We did not infect three teeth, which constituted the negative control group.

Bacterial sampling and enumeration. We sealed the apical foramina by using a three-step bonding system (Adper Scotchbond, 3M ESPE, St. Paul, Minn.) and a flowable composite (Ena Flow, Micerium, Genova, Italy), and then we isolated the entire root surfaces by using nail polish to prevent lateral and apical bacterial leakage.

We used a pure culture of vancomycin-resistant *Enterococcus faecalis* grown in brain-heart infusion broth. This culture originated from a single colony of a clinical isolate of *E. faecalis* previously identified by means of biochemical tests by using the Vitek 2 AES system (bioMèrieux, Marcy l'Etoile, France). We inoculated root canals with 10 microliters of a bacterial suspension (approximately 5×10^8 colony-forming units [CFUs] per milliliter) by using a micropipette.

We then incubated the specimens at 37°C for four weeks in individual test tubes, while adding fresh tryptic soy broth every 12 hours. After the incubation period, we withdrew 10 µL of suspension fluid from the root canal by means of a micropipette and serially diluted it by using a physiological solution. To enumerate the bacteria, we spread 0.1-mL aliquots of appropriate dilutions of each specimen onto Columbia agar plates supplemented with vancomycin (40 milligrams per liter) and incubated them for 24 hours at 37°C in an atmosphere with 5 percent carbon dioxide. In this study, the detection limit for bacterial growth was approximately 100 CFU/mL sampling fluid.

We did not treat three of the 23 infected teeth, and they constituted the positive control group. We divided the remaining 20 teeth randomly into two groups of 10 each.

Canal disinfection with manual irrigation or laser activation. We performed LAI by following a 2,940-nm Er:YAG laser (LightWalker AT, Fotona, Ljubljana, Slovenia) and PIPS protocol. The laser was equipped with a 9-millimeter, 600-µm quartz tip (PIPS tip) (Figure 1A). The tip was tapered and had 4 mm of the polyamide sheath stripped back from its end. We used laser operating parameters of 20 mJ per pulse at 15 hertz (average power 0.3 watts), 50 µs pulse duration for all of the treatment groups in which we used lasers. We set the coaxial air-water spray feature of the handpiece to off. We placed the tip into the coronal access opening of the pulp chamber only and kept the tip stationary and did not advance it into the root canal system (Figure 1B).

We subjected the root canals of teeth in group A to two cycles of 5 percent NaOCl (3 mL each) irrigation and PIPS laser activation for 30 seconds, with a resting time of 30 seconds between each cycle. After irrigating the teeth for 30 seconds with sterile water, we subjected the root canals to 17 percent ethylenediaminetetraacetic acid (EDTA) irrigation and PIPS laser activation for 30 seconds.

We subjected the root canals of teeth in group B to two cycles of 5 percent NaOCl (3 mL each) for 30 seconds by using a Max-I-Probe needle (Dentsply Rinn, Elgin, Ill.) placed into the middle one-third of the root canal without any activation and with a resting time of 30 seconds

ABBREVIATION KEY. **CFU:** Colony-forming units. **EDTA:** Ethylenediaminetetraacetic acid. **Er:YAG:** Erbium:yttrium-aluminum-garnet. **LAI:** Laser-activated irrigation. **NaOCl:** Sodium hypochlorite. **NG:** No growth detected. **PIPS:** Photon-induced photoacoustic streaming. **SEM:** Scanning electron microscopy.