

Disinfection of Root Canals with Photon-initiated Photoacoustic Streaming

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Abstract

Introduction: This study set out to compare the efficacy of laser-activated and ultrasonically activated root canal disinfection with conventional irrigation, specifically its ability to remove bacterial film formed on root canal walls.

Methods: Seventy human premolars were shaped to an apical size #20, taper .07, sterilized, and contaminated *in situ* with oral bacteria for 1 week and incubated for 2 more weeks. Irrigation was done with 6% NaOCl (group 1), NaOCl ultrasonically activated with blunt inserts (group 2), or a pulsed erbium:YAG laser at non-ablative settings (group 3) for a total of 60 seconds each. Positive and negative controls were also included. Aerobic bacterial sampling was performed, and the incidence of positive samples after 24 and 48 hours as well as bacterial counts (colony-forming units) were determined. Fixed and demineralized sections 1 mm and 4 mm off the apex were Brown-Brenn stained and assessed for remaining intracanal bacteria/biofilm and dentinal tubule penetration. **Results:** All 3 canal disinfection protocols significantly reduced bacterial counts ($P < .001$). None of the 3 techniques predictably generated negative samples, but laser-activated disinfection was superior to the other 2 techniques in this aspect ($P < .05$). Histologic sections showed variable remaining bacterial presence in dentinal tubules at the 4-mm level and significantly less bacterial biofilm/necrotic tissue remaining at the 1-mm level after laser-activated irrigation ($P < .05$). **Conclusions:** Under the conditions of this combined *in situ/in vitro* study, activated disinfection did not completely remove bacteria from the apical root canal third and infected dentinal tubules. However, the fact that laser activation generated more negative bacterial samples and left less apical bacteria/biofilm than ultrasonic activation warrants further investigation. (*J Endod* 2011;37:1008–1012)

Key Words

Biofilm, laser, root canal disinfection

Root canal treatment aims at the elimination or prevention of periradicular periodontitis; bacteria and their toxins are the cause of this disease (1), and therefore the eradication, or reduction to a biologically acceptable number, of intracanal microorganisms is required (2). Enlargement of root canals with modern root canal instruments reduces bacterial counts even in the case of buccolingually wide root canals (3). However, preparation does not eliminate all microorganisms from the root canal system. Therefore, antimicrobial irrigants are commonly used, and it is believed that enhancement of the flushing action is effective in improving root canal cleanliness (4, 5). Different agitation techniques have been proposed to improve the efficacy of irrigation solutions, including agitation with hand files, gutta-percha cones, plastic instruments, and sonic and ultrasonic devices (6).

One of the more recent suggestions is the use of laser energy to enhance irrigation. Lasers are used to activate photosensitizers that associate with bacteria (7, 8) and more recently to activate irrigation solutions by the transfer of pulsed energy (9, 10). It appears that irrigation enhanced by erbium:YAG laser light is effective in removing dentin debris (9) and also in smear layer removal (11). It appears that direct laser irradiation is less effective in killing *Enterococcus faecalis* than 2.5% NaOCl (12), but laser activation of conventional irrigants might aid in debriding root canals (9). The latter might be achieved by the action of a pulsed erbium:YAG laser via photon-initiated photoacoustic streaming (PIPS) (11).

However, to the best of our knowledge, the capacity of PIPS to disinfect root canals has not been established. Therefore this study aimed at comparing the efficacy of root canal disinfection with this technique with conventional syringe irrigation and ultrasonic activation, specifically regarding the ability to remove bacterial biofilm formed on root canal walls.

Materials and Methods

In Situ Inoculation of Teeth with Oral Bacteria

From teeth that had been extracted for reasons unrelated to the current study, 70 human mandibular premolars were collected and stored in 0.1% thymol solution at 4°C until further use. Teeth were then decoronated and trimmed to a uniform length of ~14 mm. Canals were checked for patency, and working length (WL) was determined by placing a size #10 K-file so that it was just visible and then reducing 0.5 mm from that length. Subsequently, canals were shaped with ProTaper rotaries (Dentsply Tulsa Dental, Tulsa, OK) to an apical size #20, taper .07 (Finishing File 1), according to manufacturer's instructions. A coronal reservoir for irrigant placement was created with a Gates Glidden drill #5 placed 5 mm into the canal. Between every instrument, canals were irrigated with 6% NaOCl deposited with a 30-gauge Maxiprobe needle

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This manuscript was prepared with financial support by Medical Dental Advance Technologies Group, of which Dr DiVito is a partner.

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doi:10.1016/j.joen.2011.03.016

(Dentsply Tulsa Dental). After shaping was completed, teeth were irrigated with a sequence of 17% ethylenediaminetetraacetic acid (EDTA) for 1 minute and 6% NaOCl for 1 minute and then placed in an ultrasonic bath in 17% EDTA for 2 minutes for smear layer removal. Teeth were sterilized in an autoclave and then, with approval from the universities' Internal Review Board, were prepared for *in situ* contamination.

An *in situ* method to establish root canal infection was described earlier (13, 14); it involves individual sectional specimen holders that house 3–4 roots each. In brief, after impression taking from volunteers' maxillary arches and pouring of plaster models, polymethylmethacrylate appliances were fabricated and fitted so that they could be placed buccally in the maxillary molar region without interfering with occlusion and articulation. The accesses remained open to the oral cavity.

A total of 20 devices were prepared and continuously carried by volunteers for 6–8 days each. The appliances were incubated for a further time period of up to 15 days in tryptic soy broth (TSB) (MoBio, Carlsbad, CA) so that bacterial contamination for all teeth was equilibrated at 3 weeks. Then the 70 roots were removed from the appliances and individually mounted in heavy body silicone blocks to expose only the occlusal surface with the access cavity.

Bacterial Sampling and Enumeration

Sampling followed established methods for *in vitro* root canal disinfection studies (3, 14). In brief, accessible outer root surfaces were carefully cleaned by swiping them with sterile gauze and blotted dry, thus removing any remaining liquid from around the canal orifice; 100 μ L sterile saline was then deposited into the root canals. After agitation with a gently precurved sterile K-file size #10 and removal of excess fluid with sterile gauze, a sterile paper point was placed to WL, allowed to saturate, and then placed in sterile vials containing 2 mL of TSB. A second paper point was then placed in each root canal and also transferred to the vial, which was immediately vortexed with a sterile stainless steel bead and preincubated for 15 minutes. Finally, the cutting part of the K-file was cut with sterile wire cutters and also placed in the same vial. Preliminary experiments had indicated that this approach would generate the highest yield of positive samples.

Of each vial, 1 mL was used for serial dilution to $1:10^{5-6}$ in sterile saline, and the other half was frozen at -20°C for future analyses. Dilutions were determined from pilot studies indicating a bacterial load of $\sim 2 \times 10^8$ colony-forming units (CFUs) after at least 2 weeks of contamination. Further samples were retrieved after canal disinfection. Diluted bacterial samples were placed on TSB agar in duplicate and incubated aerobically; CFUs were then counted out after 24 and 48 hours. In addition, 20 μ L diluted sampling fluid was added to sterile vials with 1 mL TSB broth and grown for 24 and 72 hours, at which point vials were visually checked for presence and absence of turbidity.

Canal Disinfection with Needle Irrigation, Ultrasonic or Laser Activation

After the initial microbiological sample, roots were divided randomly into 3 experimental groups ($n = 20$ each) and 2 control groups ($n = 10$ each), making sure that there was an even distribution with respect to the volunteers who had carried the appliances. In group 1, canal disinfection was accomplished by placing a 30-gauge Maxiprobe needle as close to WL as possible without binding and depositing 5 mL of 6% NaOCl during 30 seconds and allowing solution to remain in the canal for an additional 30 seconds. In group 2, NaOCl was deposited as before, but the solution was placed over 30 seconds and then activated during 30 seconds by using a non-cutting insert

(Endosoft ESI; EMS, Nyon, Switzerland) and an EMS 600 ultrasonic unit. Stainless steel inserts were placed 1 mm short of WL, and power setting was 5/10 on the power dial. There was no additional irrigation solution deposited except when it was noted that the coronal reservoir was depleted; in these cases care was taken to place supplemental irrigant in the coronal reservoir only. In group 3, 6% NaOCl was deposited during 30 seconds as before, but the solution was then activated by a 2940-nm wavelength Er:YAG laser (Fidelis; Fotona, Ljubljana, Slovenia) at 10 Hz and 50 mJ and fitted with a newly designed 21-mm-long, 400- μ m endodontic fiber. The tip was placed into the coronal reservoir only and activated for 30 seconds. Again, additional irrigant was deposited only in cases in which the coronal reservoir was depleted. After canal irrigation had been completed, remaining intracanal NaOCl was neutralized with 5 mL 2M sodium thiosulfate for 30 seconds, and the postdisinfection sample S2 was retrieved as described before.

Histology

After canal preparation, specimens in positive (contamination) and negative (sterility) control groups were processed along with the experimental roots for histologic evaluation. All samples were placed in buffered neutral formalin for 7 days and then decalcified in 17% EDTA for 90 days. During decalcification, samples were agitated continuously and washed in tap water once per week. After decalcification was complete as judged from radiographs, roots were trimmed with a scalpel to produce 4-mm-long apical segments, which were then infiltrated with paraffin and processed. Cross sections from the apical-most portion, as well as from the 4-mm level, were subjected to Brown-Brenn staining by using a commercial kit (American Mastertech, Lodi, CA) according to the manufacturer's guidelines. Sections were photographed at 40–400 \times magnification in an Eclipse 500 microscope (Nikon, Melville, NY), and files were saved in jpeg format for evaluation in ImageJ software. Sections were assessed for the presence of bacterially infected matter (expressed as percentage of the covered portion of the canal perimeter) as well as for the presence of bacteria in canal cross section and dentinal tubule penetration.

Statistical Analysis

After calculation of percentages for bacterial reduction from duplicate bacterial counts, percentage reduction data were expressed as means and median values. Because of non-normal distribution, counts were submitted to log transformation, and those values were then contrasted with repeated-measures analysis of variance. The incidence of negative cultures (yes/no) was analyzed by using χ^2 tests. Histology data were not normally distributed, and means were compared by using Kruskal-Wallis and Mann-Whitney post hoc tests.

Results

Microbiological Analysis

Bacterial samples from positive control and initial samples from experimental root canals were all positive, as evidenced by turbidity after 24-hour incubation. Samples from the negative controls (noncontaminated teeth) were all negative. Table 1 shows initial bacterial counts for the 3 experimental groups; there was no significant difference in initial bacterial load after the 3-week contamination period ($P < .001$). Irrigation with NaOCl alone significantly reduced bacterial counts by 96.6% ($P < .001$); however, only 1 of 20 samples was negative at the conclusion of the 72-hour observation period (Table 1). Passive ultrasonic activation also resulted in significant elimination of bacteria by 98.5% ($P < .001$; Table 1), and only 2 of 20 samples were negative after 72 hours. Finally, activation with PIPS led to significant reduction in bacterial contamination by 99.5% ($P < .001$;

TABLE 1. Microbiologic Counts before and after Root Canal Irrigation with Needle-delivered NaOCl, Ultrasonic Activation, and PIPS Activation (n = 20 each)

| | Counts before | | | Counts after | | | % Reduction | | Negative culture |
|-----------------------|--------------------|--------------------|--|--|--------------------|--------------------------|--------------------------|-------|------------------|
| | Mean | Median | Range | Mean | Median | Range | Median | | |
| | NaOCl | 2.50×10^8 | 1.95×10^8 | 2.5×10^7 – 9.30×10^8 | 2.93×10^6 | 3.00×10^6 | 0 – 3.75×10^7 | 96.6 | |
| Ultrasonic activation | 2.35×10^8 | 4.5×10^7 | 1.5×10^7 – 1.5×10^9 | 4.87×10^6 | 2.50×10^6 | 0 – 8.50×10^6 | 98.5 | 2/20 | |
| PIPS | 3.13×10^8 | 2.63×10^8 | 3.0×10^7 – 1.13×10^9 | 2.22×10^6 | 1.75×10^6 | 0 – 1.25×10^6 | 99.5 | 10/20 | |

Table 1), albeit with 10 of 20 samples negative at the end of the observation period. Laser-activated irrigation was significantly superior to irrigation alone as well as passive ultrasonic activation in generating negative samples ($P < .01$); however, the difference among the 3 groups in bacterial reduction did not reach the level of significance ($P = .071$).

Histologic Analysis

Brown-Brenn stained specimens showed variable penetration of bacteria into dentinal tubules, with some bacteria present at the 4-mm section in most cases (Fig. 1); however, less and more irregular colonization of bacteria was seen in the apical sections (Figs. 2, 3). Although there were cases at the 4-mm level with apparent bacteria-free tubules, in particular in the buccal and lingual aspects of the root cross sections, there was no consistent pattern of bacterial removal from dentinal tubules close to the root canal lumen in any of the 3 experimental groups. There was also no difference in canal cleanliness at the 4-mm level, with all 3 irrigation techniques producing canal walls with no evidence of remaining microorganisms in the canal lumen (Fig. 1).

At the 1-mm level, bacterial biofilm was established in positive controls (Fig. 2) and absent in negative controls. There were varying amounts of bacteria/biofilm in the 3 experimental groups (Fig. 3). Quantitative assessment showed that $13.3\% \pm 19.0\%$, $13.4\% \pm 16.2\%$, and $4.3\% \pm 7.9\%$ of cross-sectional areas were covered after irrigation with NaOCl alone, ultrasonic and PIPS activation, respectively ($P < .05$). Laser activation performed significantly better than both other techniques in this aspect ($P < .05$).

Discussion

This combined *in situ/in vitro* study aimed at comparing laser-activated and ultrasonically activated root canal disinfection with conventional irrigation, specifically its ability to remove bacterial biofilm formed on root canal walls. We found that so-called PIPS led to more negative samples, a tendency of greater reduction in bacterial contamination, and less bacterial mass contained in apical canal cross sections compared with ultrasonic activation and syringe irrigation.

In the present experiment, it was attempted to create intracanal bacteria/biofilm by contaminating canals of extracted single-rooted teeth *in situ* with oral bacteria. Barthel et al (14) introduced the use of intraoral appliances containing up to 5 teeth, which are then contaminated with saliva, to test the antimicrobial effect of intracanal medications. However, in contrast to the previously selected 7-day period of intraoral carriage (13, 14), the present experiment used an additional 2-week incubation to enhance bacterial penetration into dentinal tubules and promote bacterial biofilm formation in the apical root canal thirds. Taken together with the aerobic incubation used in the present model and slight modifications in bacterial sampling, this might explain the higher preoperative bacteria yield than seen in the present study.

Alternatively, freshly extracted infected teeth that were associated with periapical lesions could have been used for the present study. However, it was considered difficult to obtain a sufficient number of clinically available teeth with similar intracanal microbial status, and hence, the present established model was preferred.

By using conventional methods, microbial sampling, aerobic culturing, and counting of CFUs were performed as the first evaluation method. This approach is similar to recent publications on ultrasonic activation and other methods to enhance irrigation (3, 7, 12, 15, 16). As a second method of evaluation, Brown-Brenn stained root cross sections were used in the present study. This stain in the Taylor-modified

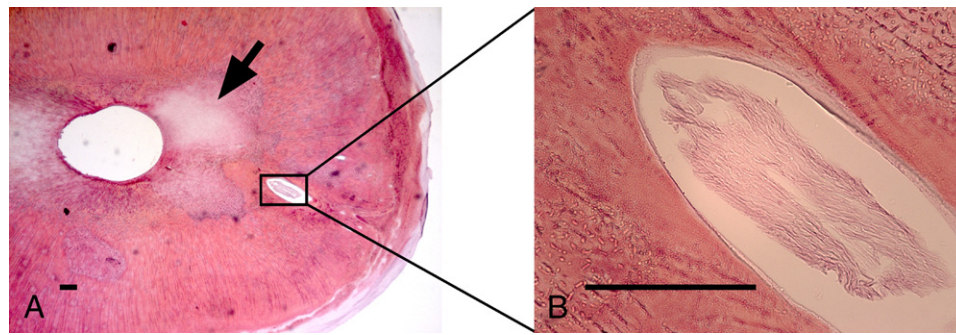


Figure 1. Cross sections at the 4-mm level through the main canal (A) and an accessory canal (B) after laser-activated irrigation. Note deep bacterial penetration and apparent bacteria-free zones buccally and lingually (arrow, A) as well as tissue remnants in the accessory canal (B). Original magnification, $100\times$ (A), $400\times$ (B). For each image, the black bar represents $100\ \mu\text{m}$.

version (17) stains both gram-negative and gram-positive bacteria and has been used previously to visualize bacterial films on root canal walls (18). Moreover, Burlison et al (4) showed morphologically similar masses in Brown-Brenn stained cross section and addressed this material as biofilm/necrotic tissue.

In the present experiment, canals were shaped to an apical size #20, taper .07, which might be considered a minimally invasive preparation size and possibly too small for syringe irrigation to be effective (19). It appears that activation of the delivered irrigant with an ultrasonic unit, termed passive ultrasonic irrigation (PUI) (20), was not effective under the present condition to enhance irrigant activity.

On the other hand, a pulsed Er:YAG laser, while not completely and predictably removing all microbial load, was more effective in debriding apical canal sections in the present study. This effect is nonthermal and does not depend on the canal size because the laser tip is maintained without wall contact in the access cavity (11). One possible explanation for the effect of laser activation in the present study might be increased NaOCl reaction kinetics (21).

Ultrasonic activation has been shown to be effective in debriding root canals, both used as PUI (22) or with continuous irrigant flow (5). Both variants depend on the ability of the activated instrument to oscillate (20) and hence canal size. This might be an explanation why PUI was not effective in the small canal shapes in the present experiment. In contrast, PIPS is believed to function via a direct shock wave (23), which is elicited from the specific model and settings of an Er:YAG laser used in this investigation (11). The unit was equipped with a novel $400\text{-}\mu\text{m}$ diameter radial and stripped tip. Subablative parameters (average power $0.3\ \text{W}$, $20\ \text{mJ}$ at $15\ \text{Hz}$) were used to produce a photomechanical effect seen when light energy is pulsed in liquid (9, 11, 24). When activated in a limited volume of fluid, the high absorption of the

Er:YAG wavelength in water, combined with the high peak power derived from the short pulse duration that was used (50 microseconds), resulted in a photomechanical phenomenon.

The *in situ* contamination used in the current experiment led to variable and partially severe dentinal tubule penetration with a mixed flora of oral bacteria (25). The variability might be explained by the presence of various amounts of sclerotic dentin (26), in correlation with teeth of patients with varying ages (27, 28). In contrast to recent findings (29), bacteria were not effectively removed from dentinal tubules under the conditions of this present study by any of the 3 irrigation regimes. In addition to the small apical size, the total contact time of the irrigant used, 6% NaOCl, and an activation time of 30 seconds might be too short to allow penetration into dentinal tubules (30). A longer time frame, 1 minute, was sufficient in another study to enhance NaOCl reaction rate by laser-activated irrigation (21).

The results of the present study are suggestive of a positive effect of laser-activated irrigation and are in line with recent *in vitro* studies (9, 11, 24). Laser-activated irrigation is effective in a short time frame; 20 seconds of laser activation is equally as effective as 3 ultrasonic activations of the irrigant (24). One possible explanation for this is the higher amount of energy transferred to the irrigant with laser activation compared with PUI (9). Cavitation-based fluid flow appears to be the functional characteristic for laser-activated irrigation at subablation settings (9, 23), in contrast to acoustic streaming found during ultrasonic activation (20).

Changes in the irrigation protocol might be required to increase the number of cases rendered bacteria-free. A longer activation time and possibly changes in the chemical composition of the irrigant, such as adding surface-active agents, might be helpful to enhance deep penetration into dentinal tubules. However, the fact that laser

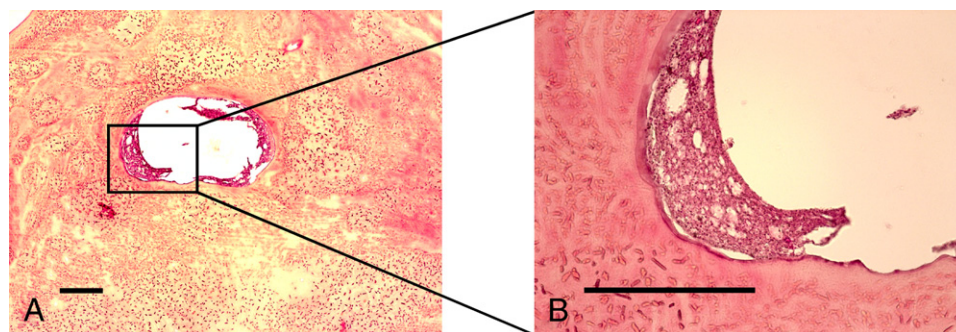


Figure 2. Root canal cross section at the 1-mm level with gram-positive bacteria/biofilm present in a positive control sample. Original magnification, $100\times$ (A), $400\times$ (B). For each image, the black bar represents $100\ \mu\text{m}$.

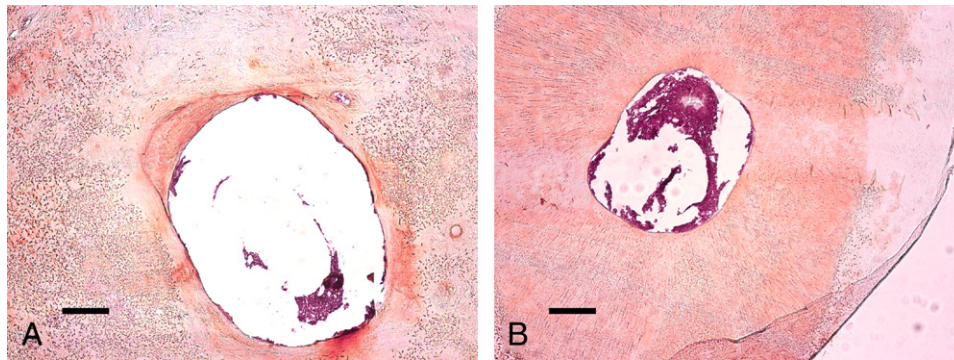


Figure 3. Cross sections at the 1-mm level with variable amounts of bacteria/biofilm after irrigation with PIPS-activated NaOCl (A) and ultrasonically activated NaOCl (B). Original magnification, 100×. For each image, the black bar represents 100 μm.

activation generated more negative bacterial samples and left less apical bacteria/biofilm than ultrasonic activation warrants further investigation. Clinical studies should be undertaken to assess the portability of the present *in vitro* data.

Acknowledgments

Expert technical assistance by Drs Claudia Boessler, Daniel McMillan, and Alex Hanley is gratefully acknowledged. Dr DiVito has advanced training and mastership certification in both hard and soft tissue laser application.

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