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CLIFFORD J. RUDDLE, DDS
Founder & Director

PHOTON-INDUCED PHOTOACOUSTIC STREAMING (PIPS) *

In the 40+ years that I have practiced and taught clinical endodontics, I have observed an awakening with the advent of the dental operating microscope, ultrasonically-driven instrumentation, NiTi files, and MTA. Recently, the renaissance has continued with the emergence of CBCT, 3D disinfection methods and the promise of regenerative endodontics. Today, Photon-Induced Photoacoustic Streaming (PIPS) represents a leading advancement in laser-activated irrigation and disinfection. Any dentist who is committed to exquisitely cleaning root canal systems will definitely appreciate PIPS. This technology will not only send photoacoustic shockwaves through both minimally and fully prepared canals, but will also propagate shockwaves through our profession by promoting predictably successful results.

-- Clifford J. Ruddle, DDS

* Summary of PIPS references and articles attached:
Organized alphabetically
Post date: September 2016

122 S. Patterson Avenue, Ste. 206 Santa Barbara, CA 93111 (805) 964-8838 • (800) 753-3636 • Fax (805) 965-8253
www.endoruddle.com • email: ruddlec@aol.com

SUMMARY OF PIPS REFERENCES

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Post Date: September 2016

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Dentinal Tubule Penetration of AH Plus, iRoot SP, MTA Fillapex, and GuttaFlow Bioseal Root Canal Sealers After Different Final Irrigation Procedures: A Confocal Microscopic Study

Merve Akcay,[1] Hakan Arslan,[2] Nazli Durmus,[1] Merve Mese,[1]* and Ismail Davut Capar[3]
[1]*Faculty of Dentistry, Department of Pedodontics, Izmir Katip Celebi University, Izmir, Turkey*
[2]*Faculty of Dentistry, Department of Endodontics, Ataturk University, Erzurum, Turkey*
[3]*Faculty of Dentistry, Department of Endodontics, Izmir Katip Celebi University, Izmir, Turkey*

Background and Objective: Varied physical and chemical characteristic of root canal sealers and different irrigant agitation systems can influence the depth of penetration. The aim of this *in vitro* study was to use a laser scanning confocal microscope in order to assess the dentinal tubules penetration of various sealers after the application of different final irrigation techniques.

Study Design/Materials and Methods: A total of 156 single-rooted extracted mandibular premolars were prepared up to size 40 and randomly distributed into four groups according to the sealer type ($n = 39$): AH Plus, iRoot SP, MTA Fillapex, and GF Bioseal. Each group was randomly subdivided into three groups according to the final irrigation protocol ($n = 13$): conventional needle irrigation (CI), photon-induced-photoacoustic streaming activation (PIPS), and passive ultrasonic irrigation (PUI). After the final irrigation procedures, the root canals were obturated with single gutta-percha and labeled sealer mixed with 0.1% fluorescent rhodamine B isothiocyanate. Specimens were sectioned at 2, 5, and 8 mm from the apex, and all the sections were examined under confocal microscope to calculate the dentinal tubule penetration area. Data were analyzed using three-way analysis of variance and Tukey's *post hoc* tests ($P = 0.05$).

Results: iRoot SP exhibited a significantly higher penetration area than the other groups ($P < 0.001$), although there were no statistically significant differences between AH Plus, MTA Fillapex, and GF Bioseal ($P > 0.05$). Er:YAG laser activation with PIPS and PUI had significantly higher penetration than CI ($P < 0.001$). Statistically significant differences were also determined at each root canal third (coronal > middle > apical; $P < 0.001$).

Conclusions: The dentinal tubule penetration area was significantly affected by the selection of root canal sealer, final irrigation procedure, and root canal third. Use of iRoot with PIPS tip or PUI seems advantageous in dentinal tubule penetration. Lasers Surg. Med. 48:70–76, 2016.

Key words: confocal; dentinal tubule penetration; GuttaFlow Bioseal; iRoot SP; MTA Fillapex; PIPS

INTRODUCTION

The basic requirements of root canal treatment are effective chemomechanical preparation and three-dimensional obturation of the root canal system [1,2]. Conventional needle irrigation (CI) alone cannot assure effective chemomechanical preparation. Therefore, different manual agitation techniques and machine-assisted agitation instruments have been suggested to enhance the efficiency of chemomechanical preparation, consisting of brushes, hand-activated files, or gutta-percha cones, sonic and ultrasonic systems [3,4], and laser systems [5,6].

Subsequent to sufficient chemomechanical preparation, a hermetic sealing with a biocompatible material is another important objective of root canal treatment. Root canal sealers should seal the canal laterally and apically and have good adaptation to the root canal dentin. The penetration of sealer into dentinal tubules is a required feature, because it improves the connection of sealer and dentine; thus, enhancement of the sealing ability and retention of the sealer may be advanced by mechanical locking [7]. Through its antibacterial effect, the penetration ability of root canal filling materials into the dentinal tubules may also enable avoidance of the colonization of residual bacteria and reinfection of the root canal [8,9].

Epoxy resin-based root canal sealers, especially AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) have been widely used. This sealer is frequently used for comparison

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*Correspondence to: Assistant Professor, Dr. Merve Akcay, Faculty of Dentistry, Department of Pedodontics, Izmir Katip Celebi University, Izmir 35640, Turkey.
E-mail: merve.akcay@ikc.edu.tr
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because of its good physicochemical features and adaptability to the root canal walls [10,11]. However, investigators have demonstrated that it displays higher cytotoxic effects than MTA and iRoot SP sealers [12].

iRoot SP (Innovative BioCeramix Inc., Vancouver, Canada), a new root canal sealer, is a convenient premixed, aluminum-free, ready-to-use, injectable white hydraulic cement paste developed for permanent root canal filling and sealing applications. iRoot SP is composed of calcium silicate, calcium phosphate, calcium hydroxide, and zirconium oxide, and it requires the presence of water to set and harden. With a similar composition to that of white mineral trioxide aggregate (MTA), iRoot SP has both excellent physical properties and biocompatibility [13]. iRoot SP was shown to have an apical sealing ability equivalent to that of AH Plus [13] but with less cytotoxicity [12,14].

MTA Fillapex (Angelus dental solutions, Londrina, PR, Brazil) is another currently available calcium silicate-based root canal sealer. This sealer consists of salicylate resin, diluting resin, natural resin, bismuth oxide, nanoparticulated silica, and MTA. It was developed to utilize the good features of MTA; relatively high levels of biocompatibility, antimicrobial activity, and sealing ability were reported for this material [15].

GuttaFlow 2 (Coltene Whaledent, GmBH+Co KG, Langenau, Switzerland), a new formulation of GuttaFlow, is a silicone-based root canal sealer that combines sealer and gutta-percha in powder form with a particle size of less than 30 μm. It consists of a mixture of gutta-percha powder, poly-dimethylsiloxane, platinum catalyst, zirconium dioxide, and micro-silver. GuttaFlow 2 has been shown to be more biocompatible than AH Plus Jet sealer [16] and less toxic to human gingival fibroblasts cells than AH Plus [17]. Recently, the same manufacturer has launched a new bioactive sealer, GuttaFlow Bioseal (GuttaFlow 3).

To our knowledge, no study has been made of the dentinal tubule penetration of these new root canal sealers with different final irrigation techniques application. Therefore, the aim of this *in vitro* study was to assess the dentinal tubule penetration of the four various sealers after the application of different final irrigation techniques, namely, CI, Er:YAG laser with photon-induced-photoacoustic streaming activation (PIPS), and passive ultrasonic activation (PUI) by using a laser scanning confocal microscope. The null hypothesis was that there would be no difference among the sealer penetration for either (i) the different final irrigation techniques or (ii) the application of different sealers into dentinal tubules.

MATERIALS AND METHODS

A total of 156 human mandibular premolars were selected from a collection of teeth that had been extracted for reasons unconnected to this study. The specimens were then immersed in thymol solution for 48 hours for disinfection and stored in 4°C distilled water until used. Periapical radiographs were taken in the buccolingual and mesiodistal directions in each tooth selected with a long: short diameter ratio of ≥2.5 at 5 mm from the apex. Therefore, only single and round root canals were selected [18]. The inclusion criteria were as follows: no root canal treatment, internal or external resorption, calcification, immature root apices, caries/cracks/fractures on the root surface, and/or root canal curvature of less than 10 degrees.

A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal to confirm the patency. The working length was determined by subtracting 1 mm from the distance to the apical foramen. Root canals were prepared using ProTaper Universal rotary instruments (Dentsply, Maillefer) up to size F4 (size 40, 0.06 taper). Specimens were irrigated with 2 ml of 5% NaOCl (Werax; SDD, Izmir, Turkey) at each instrument change. A final irrigation was applied using 5 ml of 17% EDTA (Werax) for 1 min and 5 ml of 5% NaOCl for 1 min. The specimens were randomly distributed into four groups according to the sealer type ($n = 39$).

AH Plus Group

The specimens were randomly subdivided into three groups according to the final irrigation protocol ($n = 13$). In the CI group, the blunt-tip needle was placed into the root canal within 2 mm of the working length and 5 ml of NaOCl was utilized for 1 minute.

In the PIPS tip group, final irrigation was performed via the laser irradiation protocol, which was done by means of an Er:YAG laser with a wavelength of 2,940 nm (Fidelis AT, Fotona, Ljubljana, Slovenia). The laser activation was performed with a 14-mm-long and conical 300 μm diameter PIPS tip at 0.9 W, with 30 mJ/pulse and at 30 Hz for the very short pulse (VSP, 100 μs) mode [19,20]. The air and water on the laser system were switched off. Subsequently, 0.5 ml NaOCl was applied into the root canal and the tip was inserted into the coronal access opening of the pulp chamber only. When the irrigating solution in the coronal reservoir diminished, the remaining 4.5 ml NaOCl was gradually sent overall the root canal opening. The laser activation was proceeded during the placement of irrigant. The total activation time was 1 minute, and the total size of NaOCl was 5 ml.

In the PUI group, 0.5 ml of NaOCl was placed into the root canal via a blunt-tip needle; a stainless steel file #20/25 (IrriSafe tip, Satelec Acteon Group, Merignac, France) was then placed 2 mm short of the working length with an up-and-down movement, and the ultrasonic device (P5 Newtron XS, Satelec Acteon Group, Merignac, France) was activated for 1 minute at the power setting "blue" 11 in accordance with manufacturer instruction.

After the final irrigation procedures, the root canal was irrigated with 5 ml of distilled water and dried using paper points (Dentsply Maillefer). For fluorescence under confocal laser scanning microscopy, all the root canal sealers were mixed with 0.1% fluorescent rhodamine B isothiocyanate (Bereket Chemical Industry, Istanbul, Turkey). All the labeled root canal sealers were placed into the canal to 1 mm short of the working length using a size 30 lentulo spiral. A single gutta-percha cone (ProTaper Universal F4,

Dentsply Maillefer) was then slightly coated with labeled epoxy resin-based sealer, AH Plus (Dentsply DeTrey, Konstanz, Germany), and placed in the root canal to the WL. After the root filling, the coronal opening was filled with a temporary filling material (Cavit, 3M; ESPE, St. Paul, MN), and the specimens were stored at 100% humidity, 37°C for 1 week to completely set.

iRoot SP Group

The specimens were randomly sub-divided into same three groups according to the final irrigation protocol ($n = 13$) and all procedures were performed as in the AH Plus group. Differently from AH Plus group, iRoot SP sealer was used for the root canal obturation.

MTA Fillapex Group

Similarly, the specimens were randomly sub-divided into three groups according to the final irrigation protocol ($n = 13$), and all procedures were performed as in AH Plus group but with MTA Fillapex sealer.

GuttaFlow Bioseal Group

Likewise, the specimens were randomly sub-divided into three groups according to the final irrigation protocol ($n = 13$) and all procedures were performed as in AH Plus group, but with GuttaFlow Bioseal sealer.

Confocal Laser Scanning Microscope Analysis

After the root canal sealer had set, each specimen was sectioned perpendicular to its long axis using a precision saw (IsoMet 1000; Buehler, Lake Bluff, IL) at a slow speed under water cooling. Three slices were obtained from each tooth at depths of 2, 5, and 8 mm (apical, middle, and coronal) and approximately 1 ± 0.1 mm thickness. The sections were polished with silicon carbide abrasive paper. The samples were then mounted onto glass slides and examined with a Leica TCS-SPE confocal laser scanning microscope (Leica, Mannheim, Germany) at 10× with a wavelength of 560–600 nm. In case when the entire canal could not examined in one image, further partial images were taken and then assembled as a singly image using Photoshop (Adobe Systems, Inc., San Jose, CA). Digital images were imported into the ImageJ program (ImageJ software, NIH) to measure the total dentinal tubule penetration area. The dentinal tubule penetration area was measured as micrometers (μm) and converted to square millimeters (mm^2) for the statistical analysis.

Statistically Analysis

The data were analyzed using the three-way analysis of variance (ANOVA) and Tukey's *post hoc* tests to detect the effects of the independent variables (root canal sealers, final irrigation regimens, and root canal thirds) and their interactions on the dentinal tubule penetration into the root canal dentin ($P = 0.05$). All statistical analyses were made using software (SigmaStat for Windows Version 3.5; Systat Software, Inc., Erkrath, Germany) at a significance level of 0.05 and a confidence interval of 95%.

RESULTS

Three-way ANOVA for the root canal sealer, final irrigation technique, root canal third and the interaction terms according the dentinal tubule penetration area are presented in Table 1. The three-way ANOVA indicated that the dentinal tubule penetration area values were significantly affected by the root canal sealers ($P < 0.001$), by the final irrigation techniques ($P < 0.001$), and also by the root canal thirds ($P < 0.001$). Additionally, there were significant interactions between the root canal sealers and the final irrigation techniques ($P < 0.001$), between the root canal sealers and the root canal thirds ($P = 0.027$), and between the final irrigation techniques and the root canal thirds ($P < 0.001$). The statistical analysis also showed that there were significant interactions between all three parameters ($P = 0.001$).

The mean and standard deviations of the total dentinal tubule penetration area values (mm^2) according to the various root canal sealers, final irrigation techniques, and the root canal thirds are indicated Figure 1, and representative images from each group are shown in Figure 2. Regardless of the usage of final irrigation techniques, the three-way ANOVA indicated that iRoot

TABLE 1. Three-Way ANOVA for the Root Canal Sealer, Final Irrigation Technique, Root Canal Third and the Interaction Terms According the Dentinal Tubule Penetration Area

Source of variation	Type III sum of squares	df	Mean square	*F*	Sig.	Partial η^2
Sealer	1,579	3	0.526	13,548	0.000	0.086
Final irrigation	5,370	2	2,685	69,094	0.000	0.242
Root canal third	24,360	2	12,180	313,442	0.000	0.592
Sealer x final irrigation	0.970	6	0.162	4,161	0.000	0.055
Sealer x root canal third	0.561	6	0.093	2,406	0.027	0.032
Final irrigation x root canal third	2,594	4	0.649	16,690	0.000	0.134
Sealer x final irrigation x root canal third	1,306	12	0.109	2,801	0.001	0.072
Total	138,550	468				

Statistically significant difference at $P < 0.05$.

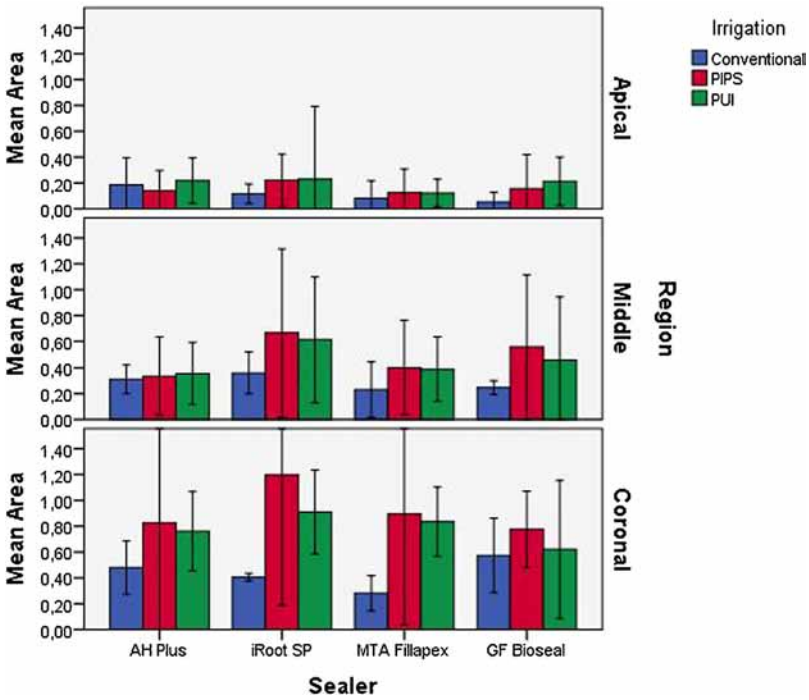

Fig. 1. Mean and standard deviations of total dentinal tubule penetration area values (mm^2) by root canal sealer, final irrigation technique, and root canal third.

SP root canal sealer exhibited significantly higher penetration area than the other sealers ($P < 0.001$), while there were no statistically significant differences between the AH Plus, MTA Fillapex, and GuttaFlow Bioseal sealer ($P > 0.05$). Regardless of the root canal sealer used, both PIPS and PUI had significantly higher penetration than CI ($P < 0.001$). There were no statistically significant differences between PIPS and PUI ($P = 0.092$). The coronal third had a greater dentinal tubule penetration area than the middle and apical thirds ($P < 0.001$), and the middle third had a greater dentinal tubule penetration area than the apical third ($P < 0.001$).

DISCUSSION

Penetration of the root canal sealer into the dentinal tubule can provide a mechanical interlocking between the sealer and root dentin [21]. On the other hand, varied physical and chemical properties of the sealer [22] and different irrigant delivery and/or agitation systems [23,24] can influence the depth of penetration. Therefore, the aim of this *in vitro* study was to assess the dentinal tubules penetration of four different sealers after the application of three different final irrigation techniques: CUI, PIPS, and PUI, using a laser scanning confocal microscope. According to the results of this study, iRoot SP root canal sealer exhibited a significantly greater penetration area than the AH Plus, MTA Fillapex, and Gutta Flow Bioseal, irrespective of the final irrigation procedure. Thus, the present study data rejected the first null hypothesis that there would be no difference among the sealer in terms of penetration into dentinal tubules. These results were in accordance with the view that the depth of dentinal tubule penetration of a sealer appears to be influenced by the chemical and physical characteristics of the materials that make up the sealer [7].

There is no published study of the dentinal tubule penetration effectiveness of the iRoot SP root canal sealer. The higher dentinal tubular penetration area of the iRoot SP root canal sealer can be attributed to its extremely small particle size (less than two microns) and high level of viscosity. These specifications may improve flow of the sealer into dentinal tubules, anatomic irregularities, and gutta-percha [25,26]. Moreover, iRoot SP exhibits minimal or no shrinkage during the setting phase because of the calcium silicate ingredient, which utilizes the moisture in dentinal tubules to initiate and complete the setting reaction [27]. In addition, iRoot SP root canal sealer exhibits 0.2% expansion during the setting period. These characteristics also support the spread of the sealer over the dentin walls of the root canal and filling of the lateral canals [28]. All these features may contribute to the higher dentinal tubule penetration observed here.

GuttaFlow Bioseal is quite a new sealer and, to the best of our knowledge, there is no study of this sealer, in the literature as yet. Moreover, there is no study either of the dentinal tubule penetration effectiveness of the GuttaFlow Bioseal predecessors, GuttaFlow and GuttaFlow 2. However, another silicone-based sealer, RoekoSeal, was showed to have a similar dentinal penetration as that of AH Plus sealer in a confocal laser scanning microscope study, as used here [29]. This study indicated that, further

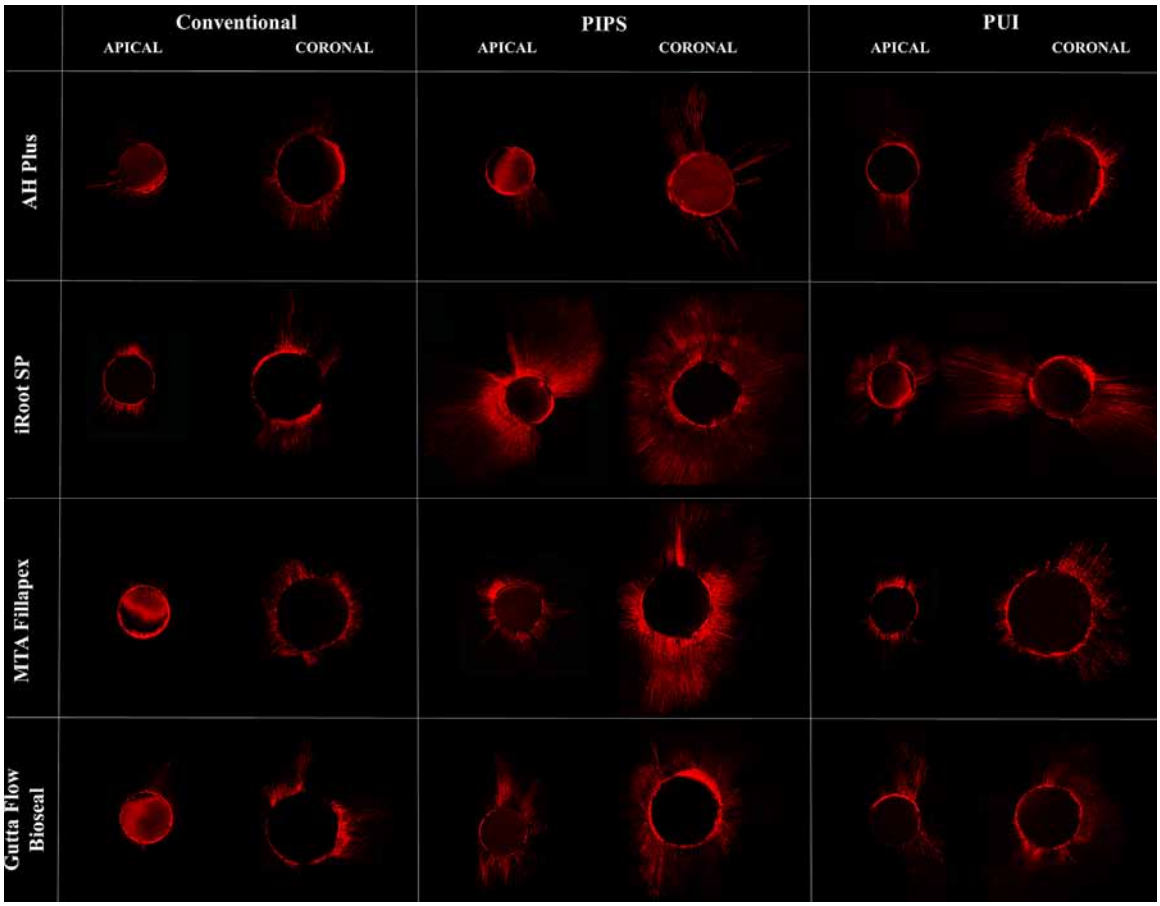

Fig. 2. Representative confocal laser scanning microscopic images from each group at coronal and apical thirds.

studies should investigate not only dentinal penetration but also physical and chemical properties in order to understand clinic applicability of this new bioactive sealer.

The dentinal tubule penetration of the AH Plus and MTA Fillapex root canal sealers has been evaluated with Amorosa-Silva et al. [30] reporting that their penetration into the dentinal tubules was statistically similar. The finding in the present study was in agreement with this study. Kuci et al. [31] evaluated the penetration into dentinal tubules of the MTA Fillapex and AH 26 root canal sealers. Differently from this study, the effects of the cold lateral compaction and warm vertical compaction techniques were investigated. This study found that greater dentinal tubule penetration was found when tMTA Fillapex was used with cold lateral compaction and AH 26 with warm vertical compaction. These results were associated with a greater flow of MTA Fillapex under compaction pressure and decreased viscosity of AH 26 at the higher temperature. In the present study, only a single gutta-percha cone was placed into the root canal and without any pressure. Our different results can be linked to this.

According to the results of the present study, the dentinal tubule penetration of the irrigation solution was better in the coronal thirds of the root canals than in the middle and apical thirds, and in the middle than apical thirds. Other investigations into dentinal tubule penetration have also reported decreasing penetration values from the coronal to apical parts [23,32,33]. The poorer dentinal tubule penetration in the apical thirds can be explained by the ineffective delivery of irrigant to this region of the canal, the smaller diameter and reduced number of dentinal tubules in this third, and its greater more tubular sclerosis [34,35].

According to the results of the present study, PIPS and PUI both obtain a higher dentinal penetration than CI. This finding is in conformance with an earlier study, which investigated various root canal irrigant agitation methods in respect of the penetration of endodontic irrigants into dentinal tubules. The results of that study showed that Nd:YAG laser activation with either NaOCl or EDTA was much better than NaOCl irrigation alone for sealer penetration into dentinal tubules and as effective as an EDTA final flush [23]. Also, another study demonstrated that ultrasonic agitation significantly improved the penetration of an endodontic irrigant when compared to sonic agitation [36].

The PIPS technique is based upon photo-acoustic and -mechanical action without needing to arrive to the root apex, which makes it dissimilar to other agitation techniques.With this technique, each impulse reacts with the water molecules, prompting expansion and serial shock waves that cause the creation of an effective streaming fluid [37]. Similarly, the PUI technique is based on the transmission of acoustic energy to an irrigant in the root canal space through ultrasonic waves and can cause acoustic streaming of the irrigant [38]. The higher dentinal tubule penetration ratio for these types of activation can thus be attributed to the acoustic energy and high-speed fluid motion. Before the root canal obturation, this suggests that the activation of the irrigant and creation of streaming with Er:YAG laser activation with PIPS or PUI has a positive effect on the dentinal penetration of the resin sealer to root dentin as compared to that achieved with CI. Thereby, the present study data rejected the second null hypothesis that there would be no difference for the sealer penetration after the application of different final irrigation techniques into dentinal tubules.

For the dentinal tubule penetration evaluation in the confocal laser scanning microscopy two parameters have been measured, the maximum depth of penetration and the percentage of sealer penetration,employing a similar method to that used by Gharib et al. [32]. Unique or a number of measurements were performed to calculate the deepest penetration and the outlined areas along the canal walls in which sealer penetrated into dentinal tubules, with distances divided by the canal circumference to calculate the percentage of sealer penetration. However, these methods have some limitations. Single/multiple measurements could have affect the overall depth and the thicker/looser penetration also changed the percentage. Therefore, in the present study, the ImageJ program was used to measure the total dentinal tubule penetration area. This program can calculate area and pixel value statistics of user-defined selections and intensity-thresholded objects.

Within the limitations of this study, the following conclusions can be made:

- iRoot SP root canal sealer exhibited a significantly greater penetration area than the AH Plus, MTA Fillapex, and GF Bioseal sealers, among which there were no statistically significant differences,
- Er:YAG laser activation with PIPS and PUI had a significantly greater penetration than the control group and there were no statistically significant differences between PIPS and PUI,
- iRoot SP root canal sealer usage after the final irrigation procedures with PIPS or PUI seems advantageous in terms of dentinal tubule penetration. Further studies should be conducted to confirm these findings.

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ORIGINAL ARTICLE

The effect of photon-initiated photoacoustic streaming, ultrasonically and sonically irrigation techniques on the push-out bond strength of a resin sealer to the root dentin

Merve Akcay · Hakan Arslan · Merve Mese · N. Nur Sahin

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Abstract
Objectives The present study investigated the effects of various irrigation activation techniques, including laser-activated irrigation using a laser with a novel tip design (photon-induced photoacoustic streaming, PIPS) on the bond strength of an epoxy resin-based sealer to root dentin.
Materials and methods Seventy-two single-rooted human mandibular premolars were prepared using the rotary system to size 40 and randomly divided into four groups ($n=18$) according to the final irrigation activation technique used as follows: conventional irrigation (CI), laser-activated irrigation with PIPS (LAI-PIPS), passive ultrasonic irrigation (PUI), and sonic irrigation (SI) with 5 mL of 17 % EDTA and 2.5 % NaOCl. The root canals were then obturated with gutta-percha and AH PlusJet sealer. A push-out test was used to measure the bond strength between the root canal dentin and the sealer. The data were analyzed using the two-way analysis of variance and least significant difference (LSD) post hoc tests ($P=0.05$).
Results The LAI-PIPS and PUI resulted in higher push-out values compared to CI and SI ($P<0.05$). There were no statistically significant differences between CI and SI ($P=0.978$) and between LAI-PIPS and PUI ($P=0.051$). There was a statistically significant interaction between the final irrigant activation techniques used and root canal thirds ($P<0.05$). A chi-square test revealed no significant differences in the failure mode within the groups ($P>0.05$).
Conclusions The use of LAI-PIPS and PUI can provide higher bond strength of resin sealer to root dentin compared to CI and SI techniques.
Clinical relevance The activation of the irrigant and the creation of the streaming have a positive effect on the bond strength of the resin sealer to root dentin.

Keywords Laser-activated irrigation · PIPS · Push-out bond strength · Resin sealer · Sonic irrigation · Ultrasonic irrigation

M. Akcay (✉) · M. Mese · N. N. Sahin
Department of Pediatric Dentistry, Faculty of Dentistry, Izmir Katip Çelebi University, İzmir, Turkey
e-mail: merve.akcay@ikc.edu.tr

H. Arslan
Department of Endodontics, Faculty of Dentistry, Ataturk University, Erzurum, Turkey

Introduction

The success of root canal treatment depends on an effective chemomechanical preparation and three-dimensional obturation of the root canal system [1, 2]. During chemomechanical preparation, endodontic instruments may not be able to access the whole root canal system, especially irregular and accessory canals [3]. After chemomechanical preparation, a smear layer consisting of microorganisms, their by-products and necrotic tissue form on the root canal walls [4]. The smear layer can inhibit the penetration of the root canal irrigation solutions and medicaments into dentinal tubules [5]. Furthermore, the smear layer adversely affects the penetration of the root canal sealers inside the dentinal tubules [6]. For these reasons, removal of the smear layer with intracanal medicaments is recommended to disinfect the entire root canal system and to enable the penetration of the root canal sealer into the dentinal tubules [7].

Different manual agitation techniques and machine-assisted agitation devices have been proposed to improve the efficacy of irrigation solutions including brushes, hand-activated files, or gutta-percha cones, sonic, and ultrasonic systems [8]. In recent years, researchers have focused on the laser devices for irrigant activation [9–13]. Laser-activated

irrigation produces explosive vapor bubbles with a secondary cavitation effect, providing effective removal of the debris and smear layer from the complex root canal systems. Photon-induced photoacoustic streaming (PIPS) is a novel laser agitation technique, which uses an erbium:yttrium-aluminum-garnet (Er:YAG) laser. The laser has a new design for use in endodontics, with both a radial and stripped tip of novel design at subablative levels. This technique uses low-energy levels and short microsecond pulse rates (50 μs) to generate peak power spikes. The profound photoacoustic shock wave allows for three-dimensional movement of the irrigation solutions. In contrast to other agitation techniques, the tip is only inserted into the canal orifice without moving into the root apex [9]. Adhesion of the root canal sealers to the root canal dentin by close contact is crucial to resist the disruption of the established seal via micromechanical retention or friction during intraoral tooth flexure [14, 15] or preparation of cores or postspaces along the coronal and middle thirds of canal walls [16]. Comparisons of the different agitation techniques are needed to understand the micromechanical forces exerted by these techniques and their effect on the adhesion of the root canal sealers. Thus far, no studies have investigated the push-out bond strength of the endodontic sealers to root canal dentin after using PIPS as the final irrigation technique. Therefore, the aim of the present study was to investigate the effect of various irrigation techniques including laser-activated irrigation with a novel tip design (LAI-PIPS), on the bond strength of an epoxy resin-based sealer (AH Plus Jet) to root canal dentin. The null hypothesis was that there would be no difference between the laser-activated irrigation and the other agitation techniques in terms of the push-out bond strength of the endodontic sealer.

Material and methods

Mandibular premolars were selected from a collection of teeth that had been extracted for reasons unrelated to this study. The specimens were immersed in 0.5 % Chloramine T solution (Merck, Darmstadt, Germany) for 48 h for disinfection. Then, they were stored in 4 °C distilled water until they were used. The soft tissue and calculus were removed mechanically from the root surfaces with a periodontal scaler. The teeth were verified radiographically as having a single root canal without calcification. The exclusion criteria consisted of a tooth having more than a single root canal and apical foramen, root canal treatment, internal/external resorption, immature root apices, caries/cracks/fractures on the root surface, and/or root canal curvature of more than 10°.

According to these criteria, 72 mandibular premolar teeth with similar root lengths from the cementoenamel junction to the root apex were selected. The specimens were sectioned using a diamond disk to obtain a standardized root length of 21 mm. They were not completely decoronated to protect the crown part as a reservoir for the irrigation solution. After preparing the endodontic access cavity, the size of the apical foramen was controlled by inserting a #15 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) to the working length. If the K-file extruded beyond the apical foramen, the tooth was excluded from the study. Then, a #10 stainless steel K-file was inserted into the canal until its tip was slightly visible at the apical foramen. Endodontic working length was set by deducting 1 mm from the initial length. ProTaper rotary instruments (Dentsply-Maillefer) were used to shape the root canals. The instrumentation sequence was as follows: Sx, S1, S2, F1, F2, F3, and F4 (size 40). The first three shaping files were used with a brushing motion, and the finishing files were used with a non-brushing motion until the working length was reached. The root canals were flushed between the instrument changes with 2 mL of 2.5 % NaOCl solution (ImidentMed, Konya, Turkey) using a size 27 gauge notched-tip needle (Ultradent, UT, USA). The specimens were then randomly divided into four groups according to the final irrigation procedures, as follows (n=18):

Conventional irrigation (CI) with an open-ended needle A total of 5 mL 17 % EDTA was administered with a size 27 gauge notched-tip needle for 30 s. Subsequent to the EDTA irrigation, 5 mL 2.5 % NaOCl was applied for 30 s.

Laser-activated irrigation with PIPS (LAI-PIPS) The root canals were irrigated using the laser irradiation protocol, which was performed with an Er:YAG laser (Fidelis AT; Fotona, Ljubljana, Slovenia) at a wavelength of 2940 nm using a PIPS tip. The 14-mm-long and 300-μm-diameter quartz tip was applied at 0.9 W, 30 Hz, and 30 mJ per pulse. The laser's water and air systems were turned off. One milliliter of 17 % EDTA was placed in the root canal, and the optical fiber was placed in the pulp chamber and then activated for 30 s. When the irrigating solution in the coronal reservoir decreased, the EDTA was refreshed. The total volume of 17 % EDTA used was 5 mL. Then, 1 mL of 2.5 % NaOCl was placed in the root canal, and the optical fiber was placed in the pulp chamber and then activated for 30 s. When the irrigating solution in the coronal reservoir decreased, the NaOCl was refreshed. The total volume of 2.5 % NaOCl used was 5 mL.

Passive ultrasonic irrigation (PUI) A total of 5 mL of 17 % EDTA and 5 mL of 2.5 % NaOCl was continuously agitated using an ultrasonic device (Anthos u-PZ6, Imola, Italy). As described above, 1 mL of 17 % EDTA was placed in the root canal, and an ultrasonic file (size 15: 0.02 taper) was placed in the canal 2 mm short of the working length without touching the walls, enabling it to vibrate freely,

and then activated at 25 % power for 30 s. When the irrigating solution in the coronal reservoir decreased, the EDTA was refreshed. A total of 5 mL of 2.5 % NaOCl was continuously activated in a similar manner for 30 s.

Sonic irrigation (SI) The same irrigation procedures described above were performed using the EndoActivator handpiece (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA). A total of 5 mL of 17 % EDTA (30 s) followed by 5 mL of 2.5 % NaOCl (30 s) was agitated using the EndoActivator handpiece, which was set at 10,000 cycles per minute with a red (25/04) tip, inserted 2 mm short of the working length.

In all the groups, the irrigation solution was transported with the Surgic XT Plus device (NSK, Kanuma, Japan) to fix the flow rate of the irrigating solution constantly and equally (0.16 mL/s). After the procedures, whole root canals were irrigated with 5 mL of distilled water and dried using paper points (Dentsply Maillefer). A single gutta-percha cone surface (F4, Dentsply Maillefer) was then slightly coated with a thin layer of the freshly mixed [17] epoxy resin-based sealer, AH Plus Jet (Dentsply DeTrey, Kontanz, Germany). The sealer was only covered around the circumference of the gutta-percha cone, and the excess sealer was slipped to the glass plate. Then, the single gutta-percha cone was placed in the root canal to the working length. Because the root canals were prepared using rotary instruments up to F4 files, all specimens were obturated using the single technique with matching taper F4 gutta-percha cones to obtain standard specimens for the push-out test [18]. Mesiodistal and buccolingual radiographs were taken to confirm the complete filling. After the root filling, the coronal opening was filled with a temporary filling material (Cavit; 3M ESPE, Seefeld, Germany) and the specimens were stored at 100 % humidity, 37 °C for 1 week to completely set.

Each specimen was sectioned perpendicular to its long axis using a precision saw (Isomet 1000; Buehler, Lake Bluff, IL, USA) at a slow speed under water cooling. Three slices were obtained from each tooth (n=54 for each group) at depths of 4, 7, and 10 mm (apical, middle, and coronal) and approximately 1±0.1 mm thickness. The thickness of each slice was confirmed with a digital caliper (Teknikel, Istanbul, Turkey). Both the apical and coronal aspects of the specimens were then microscopically examined to confirm a circular canal shape [19]. Both diameters of each hole were measured under a stereomicroscope (Zeiss Stemi 2000C; Carl Zeiss; Jena; Germany) at ×32 magnification to determine the diameters of the pluggers to be used for the push-out test. The push-out test was performed on each specimen with a universal test machine (AGS-X, Shimadzu Corporation, Tokyo, Japan) at a crosshead speed of 1 mm per minute by applying a continuous load to the apical side of each slice using 0.6-, 0.7-, and 0.8-mm-diameter cylindrical pluggers, matching the diameter of each canal third. The diameter of the pluggers was approximately at least 80 % of the diameter of the canal. The maximum load applied to the filling material before the failure was recorded in Newtons (N) and converted to megapascals (MPa) according to the following formula:

$$\text{push-out bond strength (MPa)} = \text{maximum load (N)}/\text{adhesion area of the root filling (A)}\ (\text{mm}^2).$$

The adhesion area of the root canal filling was calculated using the following equation:

$$A = (\pi r1_{+}\pi r2)XL,$$

where $L=\sqrt{(r1-r2)2+h2}$, where $r1$ is the smaller radius, $r2$ is the larger radius of the canal diameter (mm), h represents the thickness of the root section (mm), and π is the constant 3.14 [20].

After the test procedure, each specimen was visually examined under a stereomicroscope at ×32 magnification to evaluate the failure mode. Three types of the failure were classified: adhesive failure (between the sealer and root dentin), cohesive failure (within the sealer or root dentin), and mixed (a combination of cohesive and adhesive) [21].

The data were analyzed using the two-way analysis of variance (ANOVA) and least significant difference (LSD) post hoc tests to detect the effect of the independent variables (final irrigant activation techniques and root canal thirds) and their interactions on the push-out bond strength (P=0.05). The failure mode data were statistically analyzed using a chi-square test (P=0.05). All statistical analyses were performed using software (SigmaStat for Windows Version 3.5; Systat Software, Inc., Erkrath, Germany) at a significance level of 0.05 and a confidence interval of 95 %.

Results

Figure 1 shows representative scanning electron microscopic images of selected samples from middle third representing the different irrigant activation techniques at 1500 magnification. The two-way ANOVA indicated that the push-out bond strength values were significantly affected by both the final irrigant activation techniques (P<0.001) and the root canal thirds (P<0.001). However, there was no interaction between the final irrigant activation techniques used and the root canal thirds (P=0.675) (Table 1).

The mean and standard deviation of the push-out bond strength values (MPa) of the sealer to root canal dentin according to the different irrigation techniques and the root canal third are shown in Table 2 and Fig. 2. The LAI-PIPS and PUI techniques resulted in higher push-out values compared to CI and SI (P<0.05). There were no statistically significant differences

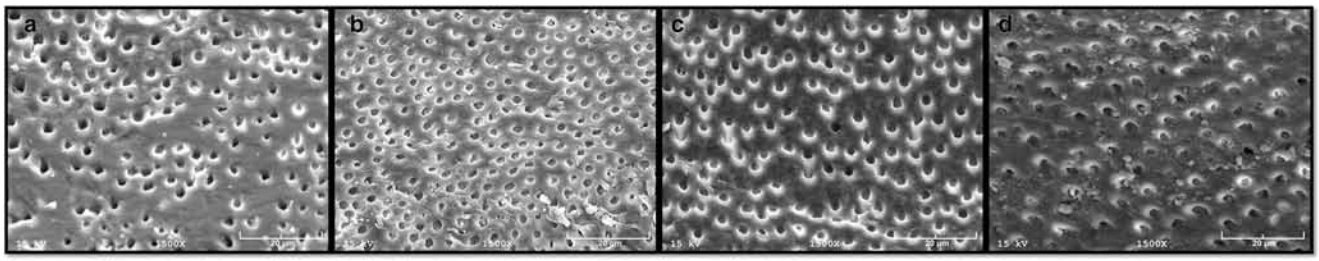

Fig. 1 **a** Effect of 17 % EDTA and 2.5 % NaOCl (conventional irrigation, CI) on middle third of the root canal wall. **b** Effect of Er:YAG laser irradiation using the PIPS tip (LAI-PIPS) on middle third of the root canal wall. **c** Effect of passive ultrasonic irrigation (PUI) on middle third of the root canal wall. **d** Effect of sonic irrigation (SI) on middle third of the root canal wall [scanning electron microscopy (SEM) ×1500]

between CI and SI (P=0.978) or between LAI-PIPS and PUI (P=0.051). There was a statistically significant interaction between the final irrigant activation techniques and the root canal thirds (P<0.05). The coronal third had higher bond strength values than the middle third and the apical third (P<0.001). The middle third had higher bond strength values than the apical third (P<0.001).

The chi-square test revealed no significant differences in the failure mode within the groups (P>0.05). Adhesive failure between the resin sealer and dentin was the most frequent type of failure mode in all the groups. Only one mixed failure was observed for the LAI-PIPS and SI groups and two mixed failure were observed for the PUI and CI groups.

Discussion

Adhesion of the sealers to the root canal dentin by close contact and penetration of the sealer tags into dentinal tubules is crucial for micromechanical retention or frictional resistance [15]. Numerous investigators have evaluated the adhesion of the resin-based sealer to root dentin after different irrigation regimens and found that the irrigation regimens affect the bond strength of the root filling negatively or positively [22, 23]. Comparisons of different agitation techniques are needed to understand the micromechanical forces exerted by these techniques and their effect on the adhesion of the root canal sealers. However, to the best of our knowledge, there are no data in the literature about the push-out bond strength of an epoxy resin-based sealer to root canal dentin after LAI-PIPS application as the final irrigation technique. Therefore, the aim of the present study was to investigate the effect of various irrigation techniques including LAI-PIPS on the bond strength of an epoxy resin-based sealer (AH Plus Jet) to root canal dentin.

In the present study, all controllable factors except the final irrigation protocol were standardized to the greatest extent possible. Specimens having similar root lengths were selected and sectioned from the same length. Then, specimens were instrumented using the same technique.

In the present study, the bond strength of an epoxy resin-based sealer was evaluated using a push-out test method. It has been reported that as the bond between the root canal sealer and root dentin increases, it is likely that the fracture resistance increases and the apical leakage reduces. Thus, the clinical longevity of the endodontic treatment can be improved [24]. Moreover, the root canal sealer should be able to remain in the same location under dislocating forces such as mechanical stresses caused by tooth function or operative procedures [25]. To evaluate the resistance to dislocating forces, the push-out test has been shown to be efficient, practical, and reliable [25–28]. Although this test method was not advisable for thermoplastic materials such as gutta-percha [29], Pane et al. [19] demonstrated that the push-out test could still be suitable for ranking the bonding of root filling materials. In addition, investigators indicated that a plugger size 70 to 90 % of the canal diameter did not affect the bond strength [19]. Therefore, different plugger size approximately at least 80 % of the diameter of the canal was used for this study. According to the results of this study, LAI-PIPS and PUI were superior to

Table 1 Two-way ANOVA for the final irrigation technique, root canal third, and the interaction terms according to the push-out bond strength data. The final irrigation technique and root canal third variables statistically affected the push-out bond strengths (P<0.005); however, the interaction between these variables did not affect the push-out bond strength values (P>0.005)

Source of variation	Sum of squares	df	Mean squares	F	P value*
Final irrigation technique	36.037	3	12.012	8.166	<0.001
Root canal third	249.073	2	124.537	84.660	<0.001
Final irrigation technique×root canal third	5.903	6	0.984	0.669	0.675
Total	591.104	215	2.749		

*P<0.05 (statistically significant difference)

Table 2 Mean (standard deviation) of the push-out bond strength values (MPa) of sealer to root canal dentin according to the different irrigation techniques and the root canal third

Root canal third	Final irrigation technique				
	Conventional irrigation (CI)	Laser-activated irrigation-PIPS (LAI-PIPS)	Passive ultrasonic irrigation (PUI)	Sonic irrigation (SI)	Total
Coronal	5.19 (0.81)^{A,a}	6.50 (2.28)^{A,b}	5.70 (0.61)^{A,a}	5.34 (1.98)^{A,a}	5.68 (1.64)^{A}
Middle	4.18 (0.97)^{B,a}	5.36 (0.95)^{B,b}	4.67 (1.29)^{B,ab}	4.13 (1.43)^{B,a}	4.59 (1.25)^{B}
Apical	2.82 (0.32)^{C,a}	3.28 (0.59)^{C,a}	3.40 (0.59)^{C,a}	2.75 (1.02)^{C,a}	3.06 (0.72)^{C}
Total	4.06 (1.22)^{a}	5.05 (1.97)^{b}	4.59 (1.29)^{b}	4.07 (1.84)^{a}	

Mean values represented with the same lowercase letters (row) are not significantly different according to LSD test ($P>0.05$). Mean values represented with the same uppercase letters (column) are not significantly different according to LSD test ($P>0.05$)

SI (EndoActivator) and CI with regard to the push-out bond strength of the resin sealer to the root dentin. Thus, the present study data rejected the null hypothesis that there would be no difference between the LAI and the other agitation techniques in terms of the push-out bond strength of the endodontic sealer.

Divito et al. [9] demonstrated that the tips in LAI-PIPS resulted in significantly better cleaning of the root canal walls in comparison with conventional irrigation procedures. Similarly, Lloyd et al. [30] reported that laser-activated irrigation using PIPS tips significantly eliminates more debris from the complex canal spaces compared to standard needle irrigation. A recent study also showed that LAI-PIPS was found to be more effective than conventional, sonic, and ultrasonic irrigation techniques in removing apically placed dentinal debris [31]. Correlatively, Ehsani et al. [24] demonstrated that the application of Er,Cr:YSGG did not adversely affect the push-out bond strength of RealSeal SE sealer to dentin and they also indicated that the use of this laser system could be suitable for removing the debris and smear layer from root canals. Varella et al. [32] showed that treatment of the root dentinal walls with a Er,Cr:YSGG laser enhanced cleaner surfaces and, subsequently, better adaptation of the filling material to the root canal walls when compared to a combination of EDTA and NaOCl. The results of the present study showed that LAI with PIPS tip has a positive effect in increased adhesion and performing higher mean bond strength values. This could be due to a number of reasons, the most likely is removing the debris and the smear layer on the root canal walls which may provide excellent adhesion and higher mean bond strength values that were observed in the LAI-PIPS group.

Previous studies have shown that the use of erbium lasers in the root canal may result in side effects. Matsuoka et al. [33] observed carbonization and cracks on the root canal walls when laser tips were used for the preparation of the root canal. Kimura et al. [34] monitored a temperature increase of up to 6 °C. Unlike other laser tip, the subablative parameters in the PIPS technique result in a photomechanical streaming effect, which occurs when the light energy is pulsed in a fluid rather than thermal effect [35, 36].

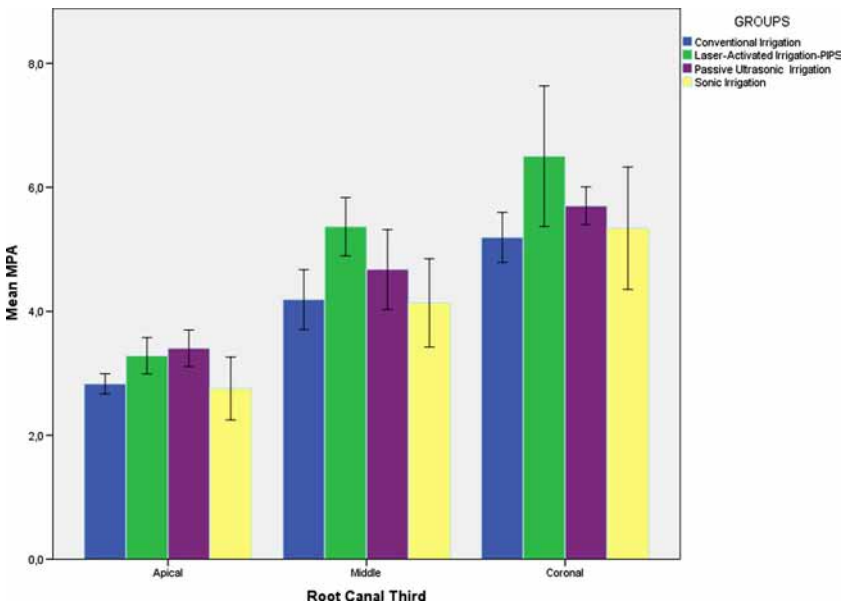

Fig. 2 Mean and standard deviation of the push-out bond strength values (MPa) of the sealer to root canal dentin according to the different irrigation techniques and root canal thirds

Er:YAG laser irradiation is highly absorbed by hydroxyapatite and water [37, 38]. With a low power, each impulse interacts with the water molecules, resulting in the expansion of vapor bubbles and the formation of a void in front of the laser light. Successive shock waves lead to the formation of a powerful stream of liquid [9, 39, 40]. The photomechanical-induced streaming effect occurs when only the tip of the laser is placed in the pulp chamber of the tooth. In a previous study, the researchers observed the high-speed motion of fluid when the tip of an Er:YAG was placed a distance of 5 mm from the apical stop of an artificial glass root canal model. They reported that the bubble caused by the laser activation of the irrigating solution increased in size and reached up to 1800 μm in 220 μs [41]. In the present study, although the tip of the laser was inserted only into the pulp chamber, it was superior to SI and CI when applied 2 mm short of the working length.

The traditional laser applications in the root canal necessitate conventional preparation for at least up to size 30 and the laser tip need to reach apical third of the root. However, the PIPS tip does not need to reach the root apex, and it is only placed into the coronal reservoir of the root canal. Therefore, this technique allows for minimally invasive preparation of the root canal [9, 35]. The effect may be explained by the increased NaOCl reaction kinetics with the laser activation [42].

According to the results of the present study, LAI with PIPS may be beneficial in obtaining high bond strength between resin sealer and root dentin. This finding is in agreement with a previous investigation that examined the effect of different root canal irrigant agitation protocols in the penetration of an endodontic irrigant into dentinal tubules. The results of that study showed that ultrasonic agitation was significantly more successful than sonic agitation [43]. Also, another study demonstrated that sonic activation did not significantly improve the penetration of the sealer when compared to CI [44]. Similarly, in the present study, SI technique resulted in similar values compared to CI.

As reported by Ahmad et al. [45], the PUI technique is based upon the transmission of the acoustic energy to an irrigant in the root canal space through ultrasonic waves and can cause acoustic streaming of the irrigant. The acoustic streaming effect of the irrigant in the ultrasonic has been shown to be more effective than syringe irrigation in removing artificially created dentine debris placed in simulated uninstrumented extensions and irregularities in root canals [46]. De Moor et al. [47] evaluated the efficacy of LAI with erbium lasers and PUI in removing artificially placed dentin debris in root canals. They showed that the application of the LAI technique for 20 s is as efficient as PUI for 3×20 s. In the present study, the acoustic energy with both PUI and LAI-PIPS resulted in similar bond strength, and the bond strength was greater than that achieved in the CI and SI groups. This suggests that the activation of the irrigant and the creation of the streaming have a positive effect on the bond strength of the resin sealer to root dentin.

Conclusions

Within the limitations of the present study, it can be concluded that the use of laser-activated irrigation with a novel tip design (PIPS) and PUI can provide higher bond strength of resin sealer to root dentin compared to CI and SI techniques.

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Conflict of interest The authors declare that they have no conflict of interest.

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Enhanced Removal of *Enterococcus faecalis* Biofilms in the Root Canal Using Sodium Hypochlorite Plus Photon-Induced Photoacoustic Streaming: An *In Vitro* Study

Mohammed Al Shahrani, BDS,[1] Enrico DiVito, DDS,[2,3] Christopher V. Hughes, DMD, PhD,[4,5] Dan Nathanson, DMD, MSD,[6] and George T.-J. Huang, DDS, MSD, DSc[1,7]

Abstract

Objective: The purpose of this study was to determine the effectiveness of laser-activated irrigation by photon-induced photoacoustic streaming (PIPS) using Er:YAG laser energy in decontaminating heavily colonized root canal systems *in vitro*. ***Materials and methods:*** Extracted single-rooted human teeth ($n = 60$) were mechanically and chemically prepared, sterilized, inoculated with *Enterococcus faecalis* for 3 weeks, and randomly assigned to four groups ($n = 15$): Group I (control, no decontamination), Group II (PIPS + 6% NaOCl), Group III (PIPS + saline), and Group IV (6% NaOCl). PIPS settings were all preset to 50 μsec pulse, 20 mJ, 15 Hz, for an average power of 0.3 W. After decontamination, the remaining live microbes from all specimens were collected and recovered via plate counting of the colony-forming units (CFUs). Randomized root canal surfaces were examined with scanning electron microscopy and confocal laser microscopy. Mean variance and Dunnett's t test (post-hoc test) comparisons were used to compare mean scores for the three groups with the control group. ***Results:*** The CFU analysis showed the following measurements (mean ± SE): Group I (control), 336.8 ± 1.8; Group II (PIPS + NaOCl), 0.27 ± 0.21; Group III (PIPS + saline), 225.0 ± 21; and Group IV (NaOCl), 46.9 ± 20.29. Group II had significantly lower CFUs than any other groups ($p < 0.05$). Both imaging analyses confirmed levels of remaining bacteria on examined root surfaces. ***Conclusions:*** The use of the PIPS system along with NaOCl showed the most efficient eradication of the bacterial biofilm. It appears that laser-activated irrigation (LAI) utilizing PIPS may enhance the disinfection of the root canal system.

Introduction

Effective cleaning and shaping of the root canal system to maximally eliminate microbes is a prerequisite for successful endodontic treatment.[1–3] One important aspect of successful treatment involves the irrigant selected as well as how it is delivered and agitated.[4] Various approaches to agitate the irrigant have been tested. Sonic and ultrasonic irrigation techniques appear to be more effective than syringe irrigation alone.[4–6] Laser-activated irrigation (LAI) utilizing laser energy has been found to enhance the irrigation efficacy of NaOCl.[7,8] This is because the Er:YAG's wavelength is absorbed more effectively by the water molecules within the irrigants, resulting in more aggressive irrigant agitation.[9–11]

A new LAI system device that has been recently introduced, photon-induced photoacoustic streaming (PIPS), uses a very low power source (subablative) to rapidly pulse laser light energy, which is absorbed by the molecules within the irrigant. This transfer of energy results in a series of rapid and powerful shockwaves, capable of forcefully propelling the irrigant throughout the entire root canal system.[12,13] The specially designed Er:YAG laser-based PIPS tip utilizes a

[1]Boston University, Department of Endodontics, Henry M. Goldman School of Dental Medicine, Boston, Massachusetts.
[2]Arizona Center for Laser Dentistry, Private Practice, Scottsdale, Arizona.
[3]Arizona School of Dentistry and Oral Health, Mesa, Arizona.
[4]Boston University, Department of Pediatric Dentistry, Henry M. Goldman School of Dental Medicine, Boston, Massachusetts.
[5]Department of Pediatric Dentistry, Rutgers School of Dental Medicine, Rutgers, The State University of New Jersey, Newark, New Jersey.
[6]Boston University, Department of Biomaterials and Prosthodontics, Henry M. Goldman School of Dental Medicine, Boston, Massachusetts.
[7]University of Tennessee Health Science Center, Department of Bioscience Research, College of Dentistry, Memphis, Tennessee.

tapered tip with 3 mm of polyamide sheathing stripped from its distal end to greatly improve the transfer of light energy into the irrigant. Previous studies indicate that PIPS appears to improve canal wall cleanliness with a greater number of open tubules than when these same irrigants were used without PIPS.[13] In comparison with an ultrasonic device, PIPS-activated irrigation was shown to remove more bacteria/film in the root canal space.[14] The purpose of this *in vitro* study was to evaluate the effectiveness of PIPS associated or not with 6% NaOCl in decontaminating root canal systems inoculated with heavily colonized *Enterococcus faecalis*.

Material and Methods

Sample collection

The procedures utilized in this study conformed to the protocols approved by the Institutional Review Board of the Arizona School of Dentistry and Oral Health (ASDOH) (IRB# 2009-26). Sixty-eight extracted teeth were collected from the clinic in the Department of Oral and Maxillofacial Surgery, and immediately placed in 10% formalin (Fisher Scientific Company LLC, Kalamazoo, MI) fixative solution. Only intact teeth with single canals were selected for this study.

Sample preparation

Each sample tooth was accessed, and patency was established and maintained using a size 15 K-file (Dentsply Maillefer, Tulsa, OK). The coronal third of each canal was enlarged using the crown-down technique, starting with Gates Glidden burs (sizes #4–2). A minimal preparation protocol was followed with the largest file used to working length being a #25/0.08 taper Twisted File (SybronEndo, Orange, CA). During instrumentation, RC Prep (Premium Products, Plymouth Meeting, PA) was used as a lubricant, followed by irrigation with NaOCl (6%) after each instrument use and recapitulation. At the completion of the canal preparation an aliquot of 1 mL 17% ethylenediaminetetraacetic acid (EDTA) was placed into the canal for 1 min to remove the smear layer, which was followed by needle irrigation with 6% NaOCl for 1 min. Teeth were then autoclaved in phosphate-buffered saline (PBS) (PBS, pH 7.2), at 120°C for 20 min. Eight teeth were randomly selected for a plate count technique (described subsequently) test and scanning electron microscopy (SEM) imaging to confirm the patency of dentinal tubules and the complete eradication of the preexisting microbial colonization or biofilm and smear layer.

E. faecalis *culture and inoculation*

Growth of *E. faecalis* (ATCC 4083) was maintained by weekly subculturing in trypticase soy agar plates. The agar plates contained BHI agar (Becton, Dickinson, and Co., Sparks, MD), yeast extract (Fisher Biotech, Fair Lawn, NJ), 5 g/mL hemin, 0.3 g/mL vitamin K, and 5% sheep blood (Becton, Dickinson, and Co.). Microorganisms grown on agar plates in a 37°C incubator for 72 h were inoculated into BHI broth and incubated overnight. Cells were scattered by vortexing and repeated passage to ensure a homogeneous population of scattered planktonic bacteria. Cell numbers were then measured by spectrophotometry (Spectronic 20 Genesys, Thermo Electron Scientific Instruments Corporation, Madison, WI) at 600 nm in 1 mL cuvettes (0.1 optical density unit equals $\sim 10^8$ cells/mL).[15]

Each tooth sample was transferred to a 2 mL sterile tube. One milliliter of BHI broth containing 10^8 *E. faecalis* grown in the exponential phase was delivered, via a syringe with a 30 gauge irrigation needle, into the prepared root canal system. After bacterial inoculation into the canal, the entire tooth specimen was submerged in the BHI broth. All sample tubes were kept in a warm chamber at 37°C for 3 weeks. The medium was changed daily with fresh BHI broth. This process was to establish *E. faecalis* biofilm. After the incubation period, the medium was aspirated from the tubes. All procedures were conducted under sterile conditions. The outcome key was to count the residual level of colony-forming unit (CFUs).

Cleaning and decontamination of the root canal system

The experiment included 60 teeth that were randomly divided into four independent groups ($n = 15$ per group) (Table 1). Group I was the control group, and did not undergo any irrigation treatment. Group II samples were activated with PIPS utilizing 6% NaOCl as the irrigant, whereas Group III samples were activated with PIPS utilizing saline as the irrigant. Group IV samples were irrigated with 21 mL 6% NaOCl, delivered via a 30 gauge needle syringe for a total of 90 sec, but no PIPS. The 6% NaOCl was chosen for its effectiveness in disinfecting *E. faecalis* biofilms.[16]

Each tooth in all four groups underwent the following procedures: The surface of each tooth sample was wiped with a clean gauze pad soaked with NaOCl, after which the tooth was mounted onto a sterile plastic holder. The apex was sealed with two layers of nail varnish. The PIPS groups (II and III) were exposed to laser irradiation by an Er:YAG

Table 1. Treatment Protocol

	Intervention				
	6% NaOCl		*Saline*		
Group	*Volume (mL)*	*Time (sec)*	*Volume (mL)*	*Time (sec)*	*Volume/time*
Group I-control	None	None	None	None	None
Group II-(PIPS + NaOCl)	21	90	7	60	28/150
Group III-(PIPS + saline)	None	None	28	150	28/150
Group IV-passive NaOCl irrigation	21	90	7	60	28/150

PIPS, photon-induced photoacoustic streaming.

laser (Fotona LightWalker DT Ljubljana, Slovenia) with a wavelength of 2940 nm in 30 sec exposure intervals. The laser was set to 50 μsec pulse duration at a 15 Hz pulse rate and 20 mJ of energy, thereby delivering a total of 0.3 W of power. A newly designed PIPS quartz tip was used (600 μm diameter, 9 mm long). The tip was tapered, and had 3 mm of the polyamide sheath stripped back from its end (Fig. 1A). PIPS utilizes a unique tapered and stripped tip that increases the available surface interface for photons of light escaping. Setting for PIPS was established to be below the threshold of dentin ablation ($\leq$20 mJ), thereby avoiding thermal damage as seen with other laser techniques. Also, PIPS utilizes extremely low microsecond pulse durations (50 μsec) generating greater peak powers than longer pulse durations. This creates powerful pressure and shockwaves that travel three dimensionally throughout the fluid-filled root canal systems without the need to place the tip near the morphologically delicate apical third.[17] Both the air and water spray feature of the laser unit was set to ''off.'' The tip was then placed in the coronal pulpal chamber of the access opening only (Fig. 1B), remaining stationary, and was not advanced apically into the root canal during laser activation. The canal system was passively filled with 6% NaOCl via 30 gauge needle syringe. A laser activation using PIPS tip protocol was followed. Thirty seconds on, then 30 sec off, and this cycle was performed three times (i.e., total of 90 sec of activation). The off or ''resting'' phase in-between laser activation allowed for greater release of the more active forms of NaOCl as described by the literature.[7] The amount of NaOCl solution used during each 30 sec exposure measured out to be 7 mL per cycle, hence the total NaOCl irrigation volume used was 21 mL ($3\times7=21$). The canal was then syringe irrigated with sterile saline for 60 sec.

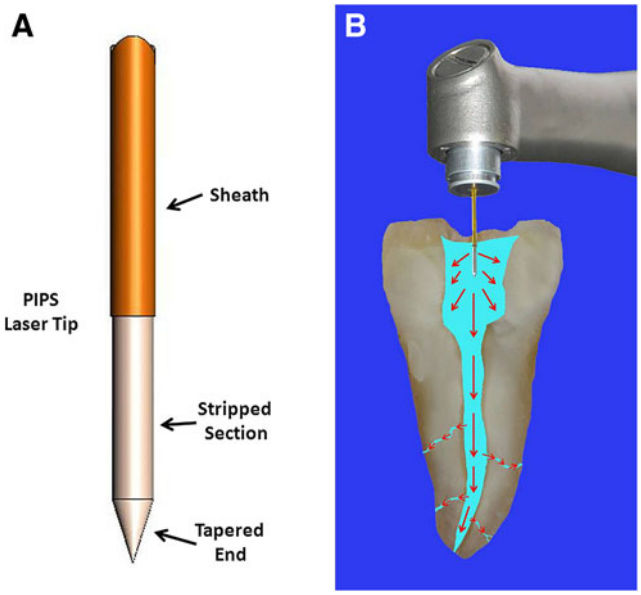

FIG. 1. A close-up view of the photon-induced photoacoustic streaming (PIPS) tip and its composition, with stripped sheath that helps to propagate the shockwaves in the root canal system **(A)**. Illustration shows how the PIPS is placed in the coronal aspect of access only, not in the canal, and how it delivers the shock waves **(B)**.

Microbial counts

After root canal decontamination, the root canal was filled with 50 μL sterile BHI broth. Paper points (Course sized, Dentsply Maillefer) were immediately placed into the root canal space to absorb the broth until the canal dried. The paper points were then placed into a microfuge tube containing 500 μL BHI broth, and the tube was vortexed to release the microbes into the broth medium. The vortexed broth was then inoculated onto agar plates containing sheep blood. The formation of bacterial colonies (CFUs) after 24 h was observed, and the CFUs were counted.

SEM

Five teeth were randomly selected from each group. A diamond disc was used to cut a groove along the long axis of the tooth without reaching the root canal system. A chisel was then used to split the tooth open into two pieces. These procedures were performed in a clean area. to avoid contaminating the samples. Both tooth fragments were placed in a sterile 2 mL tube, and one of them was fixed with 3.0% formaldehyde plus 1.5% glutaraldehyde in 0.1M Na cacodylate plus 5 mM Ca^{2+}, 2.5% sucrose, pH 7.4 for SEM. The other one was processed without fixation for confocal laser microscopy (CLM).

The fixed sample was dehydrated with 10% ethanol and sequentially transferred to higher percentages of ethanol until reaching 100% ethanol. The ethanol content was then replaced by hexamethyldisilazane (HMDS) through a graded series of ethanol HMDS mixtures as follows: (1) 25% HMDS in ethanol, (2) 50% HMDS, and (3) 75% HMDS followed by three exchanges in 100% HMDS. The last bath of HMDS was reduced in volume until the liquid just covered the sample. This bath was allowed to evaporate for at least 8 h, leaving the sample completely devoid of any moisture. Subsequently, samples were mounted onto a stub and gold sputter coated for SEM analysis under 15 kW.[18] The root canal surface of each specimen was examined randomly under two magnifications, 750$\times$ and 1500$\times$, respectively.

CLM

The other halves of the split root samples were used to perform the CLM. The tooth specimens were split into two pieces, using the method described, and placed in 2 mL tubes. Then the root surface of one half was exposed to the reagents of a LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, Inc., Eugene, OR) for 15 min, according to the manufacturer instructions. Negative control tooth specimen only received canal enlargement, using the same method as for experimental tooth samples, and was not infected. The specimen tubes were covered with aluminum foil to prevent the sample from light exposure, and kept in a refrigerator set at 4°. CLM analysis allowed distinguishing viable from nonviable bacteria on root canal walls and in dentin tubules. To detect the presence of green biofilms (living) or red biofilms (dead), we used a Zeiss LSM 510 confocal microscope (Carl Zeiss, Germany) with 40$\times$ objective. Green fluorescence was detected using a 30 mW argon 488 nm laser, set at an output of 8% acousto-optic tunable filter (AOTF) and red with 1.5 mW HeNe 543 nm

laser, set to output at 50% AOTF. This resulted in 2.4 and 0.75 mW, respectively, of illumination power of the samples.

Data analysis

The ANOVA model was used to compare the CFU means with an overall $\alpha \leq 0.05$. Dunnett's t test (post-hoc test) comparison was used to compare the mean score for the three techniques with the control group.

Results

Effective elimination of E. faecalis *biofilm demonstrated by microbial analysis*

The recovered bacteria presented in Table 2 shows that Group II had essentially no viable *E. faecalis* recoverable from the decontaminated canals via the sample collection process. Out of 15 samples, only 2 had recovered colonies (#3, 3 colonies and #15, 1 colony). The traditional NaOCl irrigation group (Group IV) was the next most effective approach to decontaminating the canal, with 10 out of 15 samples having recovered *E. faecalis*, each ranging between 2 and 296 colonies. The ANOVA procedure suggests that the four different techniques yield significantly different means of recovered live *E. faecalis* CFU counts, as the F test is highly significant ($F = 121.514$, p value < 0.002). The observed power for the analysis is 1.0. The model adequacy measure R^2 suggests that 86.7% variability in the recovered live *E. faecalis* CFU counts can be explained by the one way ANOVA model. All three techniques have significant difference among mean scores from the control group, as suggested by Dunnett's t test (post-hoc test) in Table 3. The maximum difference may be seen in Group II (PIPS + NaOCl).

SEM analysis revealing decontaminated canal walls after PIPS and NaOCl treatment

It was shown by other reports that 10 days after inoculation of *E. faecalis*, a biofilm was formed with microbial penetration into dentinal tubules, and a thick biofilm was established on dentin surface after 2–3 weeks of inoculation.[19–21] In our present studies in the control group (Group I), multilayers of bacterial colonization resembling a mature biofilm formation was observed on the root canal wall surface (Fig. 2A, B). In contrast, the PIPS + NaOCl group (Group II) showed a complete depletion of any bacteria or colonies in the samples (Fig. 2C, D). Three weeks of *E. faecalis* colonization from a saturated loading dose of bacteria were removed by the PIPS + NaOCl cleaning process. In Group III, saline was activated by PIPS, and the effect of PIPS in removing *E. faecalis* colonies was clearly visible, yet not as significant as when it was accompanied with NaOCl (Fig. 2E, F). For the NaOCl only group (Group IV), there was still significant colonization observed, as shown in Fig. 2G–I. This demonstrates that NaOCl is more effective when laser activated with PIPS.

CLM analysis showing dead bacteria after PIPS and NaOCl treatment

The split-opened tooth samples were examined as shown in Fig. 3A. The negative control tooth sample that was sterile, and not exposed to bacteria, showed no autofluorescence background (Fig. 3B). The control (Group I) showed much green (living) fluorescence (Fig. 3C). Conversely, Group II (PIPS + NaOCl) showed little green and mostly red (dead) fluorescence (Fig. 3D). As anticipated, Group III (PIPS + saline) showed much green fluorescence, indicating less effectiveness when compared with use of NaOCl with PIPS (Fig. 3E). Finally, Group IV (NaOCl and conventional needle irrigation alone) showed red fluorescence limited to the superficial layer only, rather than the deeper penetration seen when NaOCl was activated with PIPS, resulting in the presence of live bacteria in the dentinal tubules (Fig. 3F).

Discussion

This *in vitro* study model tested laser-activated irrigation using an Er:YAG laser and PIPS technique in conjunction with both NaOCl and saline. Results showed that NaOCl activated by PIPS was the most effective method for removing *E. faecalis* biofilm in the root canal system when compared with the other irrigation techniques tested. This method both mechanically and chemically debrides and decontaminates the root canal system using Er:YAG laser energy at subablative power levels with a short 50 μsec pulse duration at 15 Hz and 0.3 W of power. The heavy biofilms in the root canal system established by *E. faecalis* were effectively eliminated when using PIPS in conjunction with NaOCl. This finding could be attributed to the known bactericidal effects of NaOCl enhanced by the photomechanical effect seen when light energy is pulsed in liquid.[22] The possible reasons for differences in the efficacy of lasers in endodontic therapy could be the result of the different parameters used in various methods, including the delivery technique, tip design, the time of application within the canal, presence of an aqueous solution that would affect the absorption of the laser beam and power of the laser, and, finally, the density of energy delivered.[23]

Most of the previous literature cited utilizes the thermal effect of lasers to disinfect the canal. Lasers used in a thermal capacity have inherent disadvantages. Conversely, PIPS utilizes a photoacoustic, subablative technique and does not require heat to create the shockwave.[12,24,25] Instead, it is a photoacoustic event and because the energies required are so low, ≤ 20 mJ, the levels of heat transfer are

TABLE 2. RECOVERED LIVE *ENTEROCOCCUS FAECALIS* AFTER THE TREATMENT

	Recovered live E. faecalis *CFU counts*			
	Group I (Control)	*Group II (PIPS + NaOCl)*	*Group III (PIPS + saline)*	*Group IV (NaOCl only)*
Mean ± SE	336.8 ± 1.8	0.27 ± 0.21	225.0 ± 21.2	46.9 ± 20.29

CFU, colony-forming units.

TABLE 3. DEPENDENT VARIABLE: RECOVERED LIVE *ENTEROCOCCUS FAECALIS* CFU COUNTS (DUNNETT'S *T* TEST)

Treatment groups (I)	*Control group (J)*	*Mean difference (I-J)*	*Standard error*	*Significance*	*95% confidence interval upper limit*
PIPS + NaOCl	Control	−336.5333	0.76354	3.784E-29	−292.7849
NaOCl Irrigation	Control	−289.8667	0.76354	1.022E-09	−246.1182
PIPS + NaCl	Control	−81.8000	20.76354	0.00175	−38.0515

CFU, colony-forming units; I, J, randomly chosen letters to represent the experimental or control groups.

referred to as ''subablative.'' There is an negligible increase in temperature in the root canal space up to only 1.5°C.[12]

PIPS protocol also utilizes alternating 30 sec cycles of activation and 30 sec of ''resting'' from laser activation. Activation has been shown to be a strong modulator of the reaction rate of NaOCl, whereas during the rest interval, the consumption of available chlorine increases significantly. This effect seems to be more pronounced after irrigant activation by laser.[7]

One of the major etiologies of endodontic failure is the persistence of a bacterial biofilm following root canal therapy.[26] Investigators have examined different methods in order to disinfect the root canal systems. These methods have included various techniques and protocols, including machine-assisted irrigation.[27] Lasers have been utilized to eliminate the bacterial biofilms from the root canal system with varying degrees of success. *E. faecalis* is a well-studied microorganism in the endodontic literature both because of its virulence and because it is the microorganism most often isolated in failed root canal treatments.[28] This makes the results of this study noteworthy. Our study shows that following decontamination and mechanical conventional use of NaOCl, the activation of NaOCl with PIPS for 90 sec along

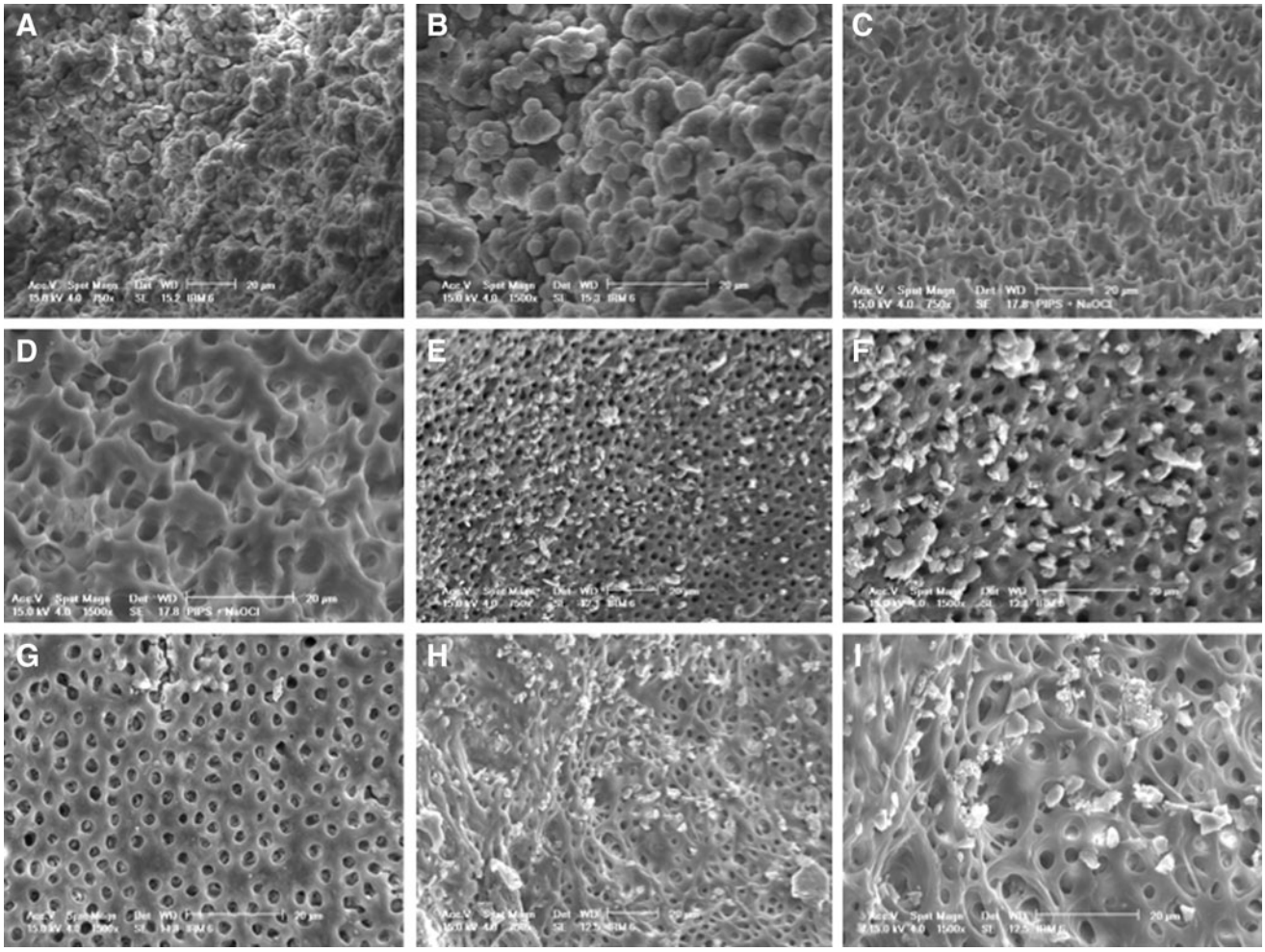

FIG. 2. Scanning electron microscope analysis of root canal surface. (**A** and **B**) Group I shows *E. faecalis* colonies attached to the root canal surface. (**C** and **D**) Group II (PIPS + NaOCl) shows a clean root canal surface. (**E** and **F**) Group III (PIPS + saline) shows colonies attached to the root canal surface. (**G–I**) Group IV (irrigation with NaOCl) shows some colonies and the other image shows no colonies.

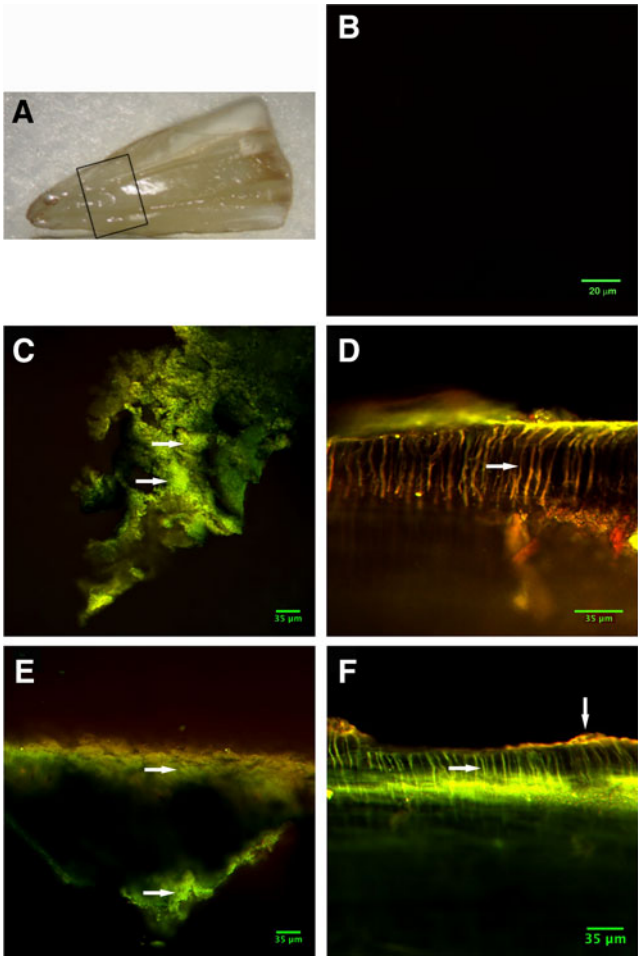

FIG. 3. Confocal scanning laser microscopy analysis of live/dead bacteria on root canal surface. **(A)** Example of a split-opened tooth sample showing the exposed root canal surface. The box at the mid-root area of the root canal system indicates where the imaging analysis was performed. **(B)** Negative control sterile tooth samples showing no detectable auto-fluorescence background. **(C–F)** Experimental samples with live bacterial biofilms are shown in green fluorescence and the dead bacterial biofilm in red fluorescence. **(C)** Group I, a control sample with no treatment, the green fluorescence (arrows) indicate live bacteria. **(D)** A representative sample from Group II (PIPS + NaOCl) showing red fluorescence (arrow) in dentinal tubules indicative of dead bacteria. **(E)** Group III, (PIPS + saline), the green indicates still live bacteria (marked with arrows) with some red dead bacteria. **(F)** Group IV, NaOCl with no PIPS, shows the red fluorescence (upper vertical arrow) on the superficial layer with green florescence deeper in dentin tubules (lower horizontal arrow) where NaOCl was unable to penetrate without laser activation.

with 90 sec of resting, was sufficient to achieve near-zero growth of *E. faecalis* within the canal system under our experimental settings. This demonstrates that PIPS does have a positive (mechanical) cleaning effect. PIPS is made more effective when used in combination with a known cleaning irrigant such as NaOCl. Most likely, the acoustic streaming and forceful shockwaves created by the tapered and stripped PIPS tip design create a more effective disruption and eradication of biofilm via its photomechanical effects as opposed to the thermal effects of laser energy as described in past literature.[25] From the chemical perspective, we assume that the greater ability for disinfection is caused by the photoacoustic effect of PIPS, which actively liberates the antimicrobial hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) from NaOCl.[7] It should be noted that in the clinical setting, particularly in repeat treatment cases, *E. faecalis* may live in a starvation phase. They have been found to be more resistant to NaOCl and intracanal medicaments.[29,30] Further analysis using the bacteria in the starvation phase is needed to validate the efficiency of PIPS using NaOCl as the irrigant. Another concern is the concentration of NaOCl chosen for the present study. One recent report demonstrated that 6% NaOCl was most effective against 3-week-old *E. faecalis* biofilm.[16] However, the risk of extruding the irrigant out of the apex should be considered. Whether 6% NaOCl is the most optimal concentration for the PIPS disinfection protocol should also be tested.

Our results agree with those of previous studies that have confirmed the efficacy of PIPS in the eradication of bacterial biofilm.[14,22] We consider that the very low energy levels (20 mJ) and the high peak power (400 W) produced by the 50 μsec pulse of this Er:YAG laser generate photoacoustic shockwaves that allows streaming of irrigants three dimensionally inside the root canal system without the need to place the tip inside the canals. PIPS can have additional advantages over other systems. Effective canal cleaning resulted even when canal preparation with endodontic instruments was kept to a minimum (in this study #25/08 taper), thus allowing the canal to remain mostly in its natural state. Time savings can result when only minimal instrumentation is required and when all canals can be irrigated at the same time. Even more importantly, minimal instrumentation greatly reduces the chance of iatrogenic events occurring, such as file breakage, ledging, perforation, and root fracture. Because PIPS is a photoacoustic event and not a thermal event, as is the case with most other laser techniques, there is no risk of thermal damage to the tooth structure or periodontium.

Conclusions

Laser-activated irrigation using PIPS protocol and NaOCl significantly enhanced the antimicrobial effect by eliminating bacterial biofilm *in vitro*. This study suggests that PIPS is a promising adjunctive method to conventional root canal therapy.

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Author Disclosure Statement

Drs. Mohammed Al Shahrani, Christopher V. Hughes, Dan Nathanson, and George T.-J. Huang have no competing financial interests. Dr. DiVito is a partner with Medical

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Address correspondence to:
George T.-J. Huang
Department of Bioscience Research
College of Dentistry
University of Tennessee Health Science Center
875 Union Avenue
Memphis, TN 38163

E-mail: gtjhuang@uthsc.edu

Efficacy of Needle Irrigation, EndoActivator, and Photon-initiated Photoacoustic Streaming Technique on Removal of Double and Triple Antibiotic Pastes

Hakan Arslan, DDS, PhD, Merve Akcay, DDS, PhD,† Ismail Davut Capar, DDS, PhD,‡ Hüseyin Ertas, DDS, PhD,‡ Evren Ok, DDS, PhD,§ and Banu Uysal, DDS‡*

Abstract

Introduction: Photon-induced photoacoustic streaming (PIPS) is a novel technique used for the removal of material on root canal walls, such as bacteria and the smear layer. This study evaluated the efficacy of needle irrigation, the EndoActivator System (Dentsply Tulsa Dental Specialties, Tulsa, OK), and PIPS on the removal of antibiotic pastes from an artificial groove created in a root canal. **Methods:** Root canal preparation was performed up to size #40 on 84 extracted single-rooted teeth using ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland). The specimens were then split longitudinally, and 2 standardized grooves were prepared in the coronal and apical part of each segment. Double (DAP) and triple antibiotic pastes (TAP) were placed in the grooves for 4 weeks, and the root halves were reassembled. Needle irrigation, the EndoActivator System, and PIPS were used for the removal of DAP and TAP. The root segments were disassembled, and the amount of remaining antibiotic pastes was evaluated under a stereomicroscope at 20× magnification using a 4-grade scoring system. The data were evaluated statistically using Mann-Whitney *U* tests with a 95% confidence level ($P = .05$). **Results:** PIPS removed significantly more antibiotic pastes than the EndoActivator and needle irrigation ($P < .001$). The EndoActivator was superior to needle irrigation in removing antibiotic pastes ($P < .001$). There were no statistically significant differences between DAP and TAP and between coronal and apical thirds in their removing from artificially created grooves ($P > .05$). **Conclusions:** PIPS was more effective in removing both DAP and TAP from artificial grooves in root canals than the EndoActivator System and needle irrigation. The EndoActivator was also more effective than needle irrigation. It is difficult to completely remove antibiotic pastes from root canals. *(J Endod 2014;40:1439–1442)*

Key Words

Double antibiotic paste, EndoActivator, endodontics, photon-induced photoacoustic streaming, regenerative, triple antibiotic paste

Antibiotic pastes have been used for root canal treatment and especially for revascularization treatment (1, 2). Triple antibiotic paste (TAP) has been found to have antimicrobial properties and to be biocompatible (3–6). It consists of ciprofloxacin, metronidazole, and minocycline and was developed by Hoshino et al (4). Because case reports have shown that minocycline causes visible crown discoloration (7, 8), minocycline was eliminated in a double antibiotic paste (DAP) that consists of only ciprofloxacin and metronidazole (9, 10).

Ruparel et al (11) showed that both DAP and TAP had detrimental effects on human stem cells in the apical papilla. Thus, these pastes should be removed completely from root canals to inhibit their detrimental effects on stem cells. Likewise, antibiotic pastes should be removed because it may be detrimental to sealer setting, sealer penetration, or other properties of sealers (7, 12).

Photon-induced photoacoustic streaming (PIPS), a light energy phenomenon, has been proposed recently. This technique differs from other agitation techniques by the placement of only the tip into the coronal portion (13). In this technique, an erbium:yttrium-aluminum-garnet laser is used with both a radial and stripped tip of novel design at subablative power settings (0.3 W). This technique uses low- energy levels and short microsecond pulse rates (50 μs) to generate peak power spikes. The profound photoacoustic shock wave it induces facilitates 3-dimensional movement of the irrigation solutions (13). Therefore, this technique results in significantly better debridement of root canal than conventional irrigation (14). Peeters and Suardita (15) also used a plain fiber tip to activate the irrigating solution in the pulp chamber and showed that the use of a laser with a plain fiber tip can produce cavitation in the irrigant and has potential as an improved alternative method for the removal of the smear layer. In another study, it has been reported that the plain fiber tip in the pulp chamber can drive the irrigation solution to the end of the canal without harming the apical tissues (16).

Antibiotic pastes were previously removed using various irrigating solutions such as sodium hypochlorite (NaOCl), EDTA, and sterile saline (1, 17–19). In a recent study by Arslan et al (20), it has been shown that it was difficult to remove TAP from grooves within root canals using irrigating solutions without ultrasonic agitation. Using 2.5% NaOCl and passive ultrasonic irrigation with 1% NaOCl improved the removal of TAP. The present study evaluated the effect of needle irrigation, the EndoActivator System (Dentsply Tulsa Dental Specialties, Tulsa, OK), and PIPS on the removal of DAP and

From the *Department of Endodontics, Faculty of Dentistry, Ataturk University, Erzurum; Departments of †Pedodontics and ‡Endodontics, Faculty of Dentistry, Katip Çelebi University, İzmir, Turkey; and §Department of Endodontics, Faculty of Dentistry, Şifa University, İzmir, Turkey.

Address requests for reprints to Dr Hakan Arslan, Department of Endodontics, Faculty of Dentistry, Ataturk University, Erzurum, 25640, Turkey. E-mail address: hakan.arslan@ikc.edu.tr

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TAP from artificial grooves in root canals. The null hypotheses were that the removal of antibiotic pastes was not affected by the (1) irrigation technique, (2) root canal third, or (3) type of antibiotic paste.

Materials and Methods

The study included 84 single-rooted, noncarious, human teeth with similar sizes and completed apices. Soft tissues and calculus were removed mechanically from the root surfaces with a periodontal scaler. Single-root canals were confirmed with radiographs, and the teeth were then stored in distilled water until use. Teeth were decoronated with a diamond bur under water coolant to obtain a standardized root length of 14 mm. The root canals were shaped with ProTaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to an F4 (D0 = .40 mm). During preparation, the root canals were irrigated with 2 mL 1% NaOCl solution after each instrument. The specimens were fixed in Eppendorf vials (Eppendorf-Elkay, Shrewsbury, MA) with a silicone material (Optosil; Heraeus Kulzer, Hanau, Germany). After removal from the impression material, all the roots were split longitudinally into 2 halves after separation with a diamond disk. A longitudinal groove 3-mm long, 0.2-mm wide, and 0.5-mm deep was then cut in the root canal wall of one half of each tooth at a distance of 2–5 mm from the apex in the apical third, and another longitudinal groove was created in the other half at a distance of 9–12 mm from the apex in the coronal third. A toothbrush was used to remove debris from the root halves and grooves, and a final flush was applied using 5 mL 17% EDTA for 60 seconds and 5 mL 1% NaOCl for 60 seconds. The root canals were then dried with paper points (Dentsply Maillefer).

Application of Antibiotic Pastes

DAP. Equal portions of metronidazole (Eczacibasi, Istanbul, Turkey) and ciprofloxacin (Biofarma, Istanbul, Turkey) were mixed with distilled water (powder/liquid ratio of 3:1).

TAP. Equal portions of metronidazole (Eczacibasi), ciprofloxacin (Biofarma), and minocycline (Ratiopharm, Ulm, Germany) were mixed with distilled water (powder/liquid ratio of 3:1). The apical and coronal grooves were filled with DAP in 42 specimens, and the grooves in the remaining 42 teeth were filled with TAP. The root halves were reassembled, and a size 40 gutta-percha point was placed into the root canal. All gaps along the tooth and the apices were sealed with wax to prevent the overflow of the irrigating solution and to create a closed-end channel for obtaining a vapor lock effect (21, 22). Specimens were then remounted in Eppendorf vials. Access to the root canals was temporarily sealed with a cotton pellet and hydraulic temporary restorative material (MD-Temp; Meta Biomed Co Ltd, Cheongju, Korea), and the specimens were then kept at 37°C with 100% humidity for 4 weeks. The specimens were divided randomly into 4 groups (n = 14) and irrigated as follows:

1. Needle irrigation–DAP: 6 mL 1% NaOCl via a size 27-G blunt-tip needle (Ultradent, South Jordan, UT) was used for 60 seconds. The needle was inserted into the root canal within 2 mm of the working length without binding. The flow rate of the irrigating solution was 0.1 mL/s.
2. Needle irrigation–TAP: TAP was removed from the artificially created grooves using the needle irrigation as stated previously.
3. EndoActivator–DAP: DAP was removed from the artificially created grooves using the EndoActivator handpiece. A total of 6 mL 1% NaOCl was agitated for 60 seconds using the EndoActivator handpiece set at 10,000 cycles/min with a red tip (25/04) inserted 2 mm short of the working length.
4. EndoActivator–TAP: TAP was removed from the artificially created grooves using the EndoActivator handpiece as stated earlier.

PIPS–DAP. In this group, DAP was removed using the laser irradiation protocol, which was performed by an erbium:yttrium-aluminum-garnet laser with a wavelength of 2,940 nm (Fidelis AT; Fotona, Ljubljana, Slovenia); a 14-mm-long, 300-μm quartz tip was tapered and had 3 mm of the polyamide sheath stripped back from its end. The tip was applied with 0.3 W, 15 Hz, and 20 mJ per pulse. The water and air on the laser system were turned off. Then, 0.5 mL 1% NaOCl was placed into the root canal, and the optical fiber was placed into the coronal part of the root canal. When the irrigating solution in the coronal reservoir decreased, 0.5 mL 1% NaOCl was refreshed. The optical tip was activated for 30 seconds in each application. The total activation time was 60 seconds, and the total volume of 1% NaOCl was 6 mL.

PIPS–TAP. TAP was removed from the artificially created grooves using PIPS as stated earlier. The root canals were dried with paper points, and the roots were disassembled to evaluate the removal of the antibiotic pastes. Digital images at 20× magnification were obtained using a stereomicroscope (Olympus BX43; Olympus Co, Tokyo, Japan) attached to a digital camera (Olympus SC100; Olympus Soft Imaging Solution GmbH, Munster, Germany) and were transferred to the computer. The digital images were coded to avoid identifying the specimens. Two calibrated observers were blinded to the technique used to remove the antibiotic pastes. Reference photographs were selected for each score. Then, calibration of the observers was performed on the photographs of 50 root halves. After scoring, the results were also discussed using reference photographs. Finally, the main evaluation was performed by the observers. The amount of antibiotic pastes remaining in the grooves was scored using the following scoring system described by van der Sluis et al (23): (0) groove was empty, (1) antibiotic paste was present in less than half of the groove, (2) antibiotic paste covered more than half of the groove, and (3) the groove was completely filled with antibiotic paste (Fig. 1*A–H*).

Photographs were evaluated by the observers 1 week later, and the kappa test was used to analyze interobserver agreement. The differences in the scores of antibiotic pastes among the different groups were analyzed with the Kruskal-Wallis and Mann-Whitney U tests. Testing was performed at the 95% confidence level (P = .05). All statistical analyses were performed using IBM SPSS Statistics 20 software (IBM SPSS Inc, Chicago, IL).

Results

Reliability between the observers was good (kappa value = 0.908), and the difference between the matched scores never exceeded 1 unit. Intraindividual reproducibility was 99% (166/168) for the first observer and 98% (164/168) for the second observer.

The Kruskal-Wallis test revealed significant differences between the groups for coronal and apical thirds (P < .001). The Mann-Whitney U test for paired comparisons showed that PIPS was superior in removing antibiotic pastes regardless of their composition compared with the EndoActivator and needle irrigation groups (P < .001) (Figs. 2 and 3). The EndoActivator System was superior to needle irrigation in removing antibiotic pastes (P < .001).

Although the mean rank of the values observed in the apical thirds was superior to those observed in the coronal thirds, there was no statistically significant difference between them (P > .05). Also, there were no statistically significant differences between the 2 antibiotic pastes in their removal from the artificially created grooves (P > .05).

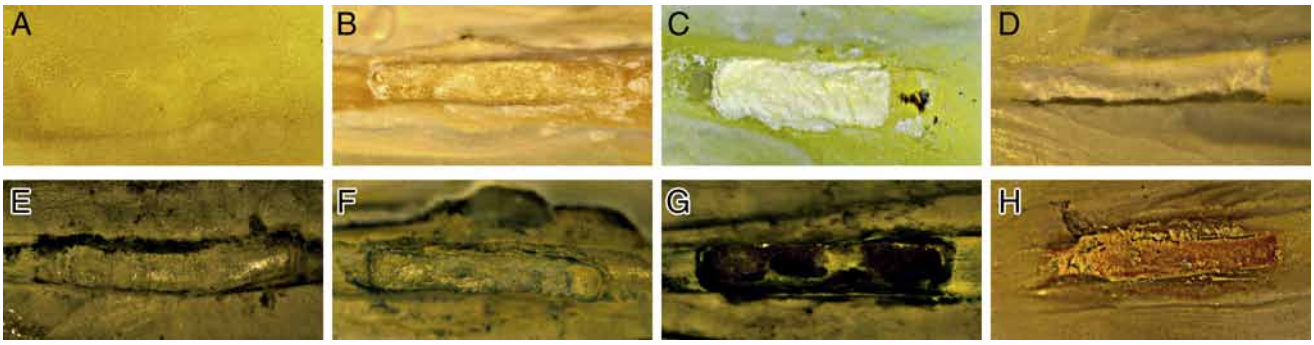

Figure 1. Representative images of scores for DAP ([*A*] score 0, [*B*] score 1, [*C*] score 2, and [*D*] score 3) and TAP ([*E*] score 0, [*F*] score 1, [*G*] score 2, and [*H*] score 3).

Discussion

There has been no study comparing the effectiveness of needle irrigation, the EndoActivator System, and PIPS in removing intracanal medicaments from root canal walls. Based on our results, PIPS was superior to the EndoActivator System and needle irrigation in removing both antibiotic pastes. Thus, the first null hypothesis was rejected. The PIPS technique is based on photoacoustic and photomechanical phenomena, which make it different from other agitation/activation techniques. These phenomena result from the use of subablative energy of 20 mJ at 15 Hz. At low power (0.3 W), each impulse interacts with the water molecules, creating expansion and successive shock waves that lead to the formation of a powerful streaming fluid (14). The second and third null hypotheses have to be accepted. The removal of antibiotic pastes was not affected by the root canal third and its type.

The elimination of bacteria and their byproducts from the root canal system is 1 of the goals of root canal therapy. Thus, the combination of the instrumentation and various irrigation solutions and medicaments was suggested (24–26). Calcium hydroxide has been established as the most frequently used medicament because of its antimicrobial efficacy against most bacterial species identified in endodontic infections (27). Because infections of the root canal system are considered to be polymicrobial, consisting of both aerobic and anaerobic bacteria species, different antibiotic combinations have also been used (2, 28, 29). In a previous report by Er et al (2), the root canal of a mandibular premolar with a large periapical lesion was initially filled with calcium hydroxide paste. However, because of the enlargement of the periradicular lesion despite medicament placement, they changed the treatment protocol, and the root canals were filled with TAP. The TAP was removed after 3 months, and the periapical lesion showed complete healing after 12 months. Taneja and Kumari (29) tried to treat a tooth with a large periapical lesion; however, the treatment protocol was changed to the use of TAP as an intracanal medicament because the symptoms did not subside. They concluded that TAP can be used clinically in the treatment of teeth with large periradicular lesions. In a case of retreatment of a resected tooth by Kusgoz et al (28), it has been shown that TAP can be used clinically in the treatment of an unsuccessfully resected tooth associated with a large periapical lesion. Recently, the use of antibiotic pastes in revascularization cases is popular. After the disinfection procedure with antibiotic pastes, the treatment strategy includes antibiotic paste removal followed by the placement of mineral trioxide aggregate (17, 18). Likewise, medicaments should be removed to avoid an effect on sealer penetration and tooth discoloration sealer setting and other properties (7, 12). Also, it has been shown that antibiotic pastes had a detrimental effect on human stem cells in the apical papilla (11). Thus, these pastes should be removed completely from root canals. However, it is difficult to remove completely antibiotic pastes from the root canal using conventional methods (20). Therefore, in the present study, PIPS and the EndoActivator System were used to remove antibiotic pastes from the root canals in comparison with needle irrigation. The needle irrigation resulted in the worst scores, which was harmonious with the findings of the previous report (20).

Previously, antibiotic pastes have been left in the root canal for up to 3 months in root canal treatment and revascularization treatment (1, 2, 30, 31). In the present study, antibiotic pastes were left for 4 weeks in the grooves to simulate clinical conditions. A 6-mL standard volume of 1% NaOCl was used in all groups. Irrigation time was set at 60 seconds, similar to the activation procedure. The design of this study was based on studies described by Lee et al (32), van der Sluis et al (23, 33, 34), and Rödig et al (35). The standardized size and location of the grooves are advantages of the *in vitro* model. Thus, the design provides researchers a standardized evaluation with high intraobserver reproducibility, good interobserver agreement, and discrimination between mechanical removal of the medicament, and the influence of the irrigant alone is better. In the present study, before the main evaluation, reference photographs were selected for each score to calibrate the observers. Fifty photographs were scored by observers. After the discussion on the photographs, the main evaluation was performed by the same calibrated observers. However, the complexity of a natural root canal system cannot be simulated by standardized grooves (35). Moreover, this experimental design does not address paste that diffused into the dentinal tubules (a potential reservoir for the medicament).

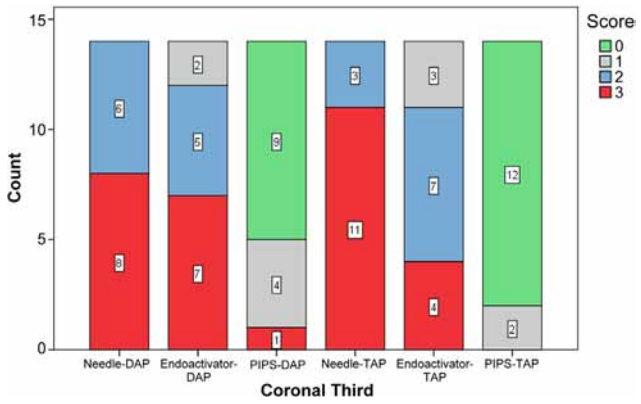

Figure 2. The distribution of scores for removing antibiotic pastes in the coronal third.

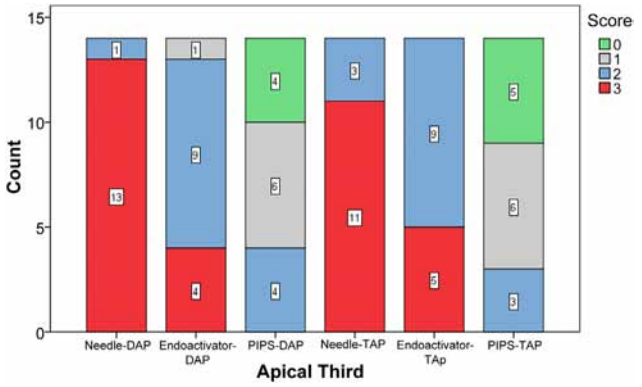

Figure 3. The distribution of scores for removing antibiotic pastes in the apical third.

The EndoActivator System has previously been compared with manual and ultrasonic irrigation (36–38). Kanter et al (36) showed that the EndoActivator was significantly superior to ultrasonic irrigation in removing debris. In addition, the EndoActivator was found to be more effective than ultrasonic irrigation in removing the smear layer (38). However, in another study, the EndoActivator was found to be similar to ultrasonic irrigation in removing the smear layer (37). In the present study, the EndoActivator was found to be superior to needle irrigation in the removal of antibiotic pastes from artificially created grooves.

Conclusions

Under the conditions of this study, it was difficult to completely remove antibiotic pastes from root canals. PIPS was more effective in removing both DAP and TAP from artificial grooves in root canals than the EndoActivator System and needle irrigation. The EndoActivator was also more effective than needle irrigation.

Acknowledgments

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

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An *in vitro* comparison of irrigation using photon-initiated photoacoustic streaming, ultrasonic, sonic and needle techniques in removing calcium hydroxide

H. Arslan[1], M. Akcay[2], I. D. Capar[3], G. Saygili[3], T. Gok[3] & H. Ertas[3]

[1]Department of Endodontics, Faculty of Dentistry, Ataturk University, Erzurum; [2]Department of Pedodontics, Faculty of Dentistry, Izmir Katip Celebi University, İzmir; and [3]Department of Endodontics, Faculty of Dentistry, Izmir Katip Celebi University, İzmir, Turkey

Abstract

Arslan H, Akcay M, Capar ID, Saygili G, Gok T, Ertas H. An *in vitro* comparison of irrigation using photon-initiated photoacoustic streaming, ultrasonic, sonic and needle techniques in removing calcium hydroxide. *International Endodontic Journal*, **48**, 246–251, 2015.

Aim To evaluate the effect of various techniques including photon-initiated photoacoustic streaming (PIPS), ultrasonic, sonic and needle irrigation on the removal of calcium hydroxide [$Ca(OH)_2$] from artificial grooves created in root canals.

Methods The root canals of 48 extracted single-rooted teeth with straight canals were prepared using ProTaper rotary instruments up to size 40. After the specimens had been split longitudinally, a standardized groove was prepared in the apical part of one segment that was filled with $Ca(OH)_2$ powder mixed with distilled water. Each tooth was reassembled and the apices closed with wax. The specimens were irrigated for 60 s with one of the following techniques: needle irrigation using 17% EDTA, PIPS with 17% EDTA, ultrasonic irrigation using 17% EDTA and sonic irrigation (EndoActivator) using 17% EDTA. The root segments were then disassembled, and the amount of remaining $Ca(OH)_2$ evaluated under a stereomicroscope at 25× magnification. A pixel count of $Ca(OH)_2$ remaining on the artificially created grooves was recorded as a percentage of the overall groove surface. The data were evaluated statistically using one-way analysis of variance and the least significant difference post hoc tests at 95% confidence level ($P = 0.05$).

Results Photon-initiated photoacoustic streaming was superior in removing $Ca(OH)_2$ as compared to needle irrigation ($P < 0.001$), sonic irrigation ($P < 0.001$) and ultrasonic irrigation ($P = 0.046$).

Conclusion Photon-initiated photoacoustic streaming provided complete removal of $Ca(OH)_2$ from artificial grooves in straight root canals. Ultrasonic irrigation enhanced the $Ca(OH)_2$ removal capacity of irrigating solution but did not provide complete removal from artificial grooves.

Keywords: calcium hydroxide, endodontics, photoacoustic streaming, PIPS, sonic, ultrasonic.

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Introduction

Calcium hydroxide [$Ca(OH)_2$] is used in root canal treatment and has good antimicrobial properties against the majority of endodontically relevant pathogens (Athanassiadis *et al.* 2007). Research has shown that remnants of $Ca(OH)_2$ on dentine walls can affect

Correspondence: Dr. Hakan Arslan, Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum 25240, Turkey (Tel.: +90 442 231 2270; Fax: +90 442 231 2270; e-mail: dt_hakan82@hotmail.com).

the penetration of sealers into the dentinal tubules and increase apical leakage (Kim & Kim 2002). Therefore, complete removal of $Ca(OH)_2$ placed inside the root canal prior to root filling is recommended. However, several studies have demonstrated that it is difficult to completely remove $Ca(OH)_2$ from root canals using irrigating solutions alone (Lambrianidis *et al.* 1999, Rödig *et al.* 2010, Arslan *et al.* 2012, Capar *et al.* 2013).

Sonic, ultrasonic and laser activation of irrigating solutions has been widely used to improve their chemical and mechanical effectiveness (van der Sluis *et al.* 2007a, De Moor *et al.* 2009, Jiang *et al.* 2010, Macedo *et al.* 2010, Arslan *et al.* 2013a,b). A novel laser agitation technique, photon-initiated photoacoustic streaming (PIPS), has been proposed. This technique differs from other agitation techniques as only the tip of the device is placed into the orifice. In this technique, an erbium : yttrium-aluminium-garnet (Er : YAG) laser is used with a radial and stripped tip of novel design at subablative power settings. There is limited information about the effect of PIPS on the removal of $Ca(OH)_2$ in the literature. The current study evaluated the effect of various techniques (PIPS, ultrasonic, sonic and needle) on the removal of $Ca(OH)_2$ from an artificial groove created in a root canal. The null hypothesis was that there is no difference between the techniques.

Materials and methods

Forty-eight single-canal, noncarious human mandibular premolar teeth with straight roots were selected from a collection of teeth that had been extracted for orthodontic reasons or periodontal diseases with mature apices and stored in distilled water until use. The specimens were decoronated to obtain a standardized root length of 14 mm using a diamond disc. The working length was determined by subtracting 1 mm from the length at which the tip of a size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) extruded apically. ProTaper rotary instruments (Dentsply Maillefer) were used for root canal shaping procedures. The instrumentation sequence was as follows: Sx, S1, S2, F1, F2, F3 and F4 (size 40, 0.06 taper). The first three shaping files were used with a brushing motion, and the finishing files were used with a nonbrushing action until the working length was reached. The root canals were flushed with 1 mL of 1% NaOCl solution between each instrument change.

After instrumentation, the specimens were fixed in modified Eppendorf vials (Eppendorf-Elkay, Shrewsbury, MA, USA) with a silicone material (Optosil; Heraeus Kulzer, Hanau, Germany). After removal from the impression material, all the roots were grooved longitudinally on the buccal and lingual surfaces with a diamond disc under copious water irrigation, avoiding penetration into the root canal. The roots were then split into two halves with a small chisel. A longitudinal groove of approximately 3 mm long, 0.5 mm wide and 0.2 mm deep was then cut in the root canal wall of one half of each tooth at a distance of 2–5 mm from the apex with a scaler adapted on an ultrasonic device (Anthos u-PZ6, Imola, Italy) to simulate an uninstrumented canal extension in the apical region. A toothbrush was used to remove debris from the root halves and grooves. A final flush was applied using 5 mL of 17% EDTA and 5 mL of 2.5% NaOCl, each for 1 min. The root canals were then dried with paper points.

Powder of $Ca(OH)_2$ (Kalsin; Spot Dis Deposu A.Ş., Izmir, Turkey) was mixed with distilled water, and the grooves were filled with $Ca(OH)_2$. The root halves were reassembled, and the specimens remounted in the Eppendorf vials. Access to the root canals was temporarily sealed with a cotton pellet and Cavit (Espe, Seefeld, Germany), and the specimens were then kept at 37 °C with 100% humidity for 1 week. The specimens were divided randomly into four groups ($n = 12$) defined by irrigation technique (needle, PIPS, ultrasonic and sonic) and irrigated as follows.

Needle irrigation with EDTA

Five millilitres of 17% EDTA (Werax; Spot Dis Deposu A.Ş., Izmir, Turkey) via a size 27 gauge blunt-tip needle (Ultradent; South Jordon, Utah, USA) was used for 60 s. The needle was placed at a distance of 1 mm from working length, and it was moved backwards and forwards. The average pressure was 69.6 kPa, and the flow rate was 0.083 mL s^{-1}.

Photon-initiated photoacoustic streaming

The laser irradiation protocol was performed by an Er : YAG laser with a wavelength of 2940 nm (Fidelis AT; Fotona, Ljubljana, Slovenia). A 14-mm long, 300-μm diameter quartz tip was applied with 0.9 W, 30 Hz and 30 mJ per pulse with the laser system water and air turned off. One millilitre of 17% EDTA

was placed into the root canal, and the optical fibre was placed into the coronal part of the root canal and activated for 20 s. When the irrigating solution in the coronal reservoir decreased, the EDTA was refreshed. Three applications were performed for a total activation time of 60 s and with a total volume of 17% EDTA of 5 mL.

Ultrasonic irrigation

A total of 5 mL of 17% EDTA was agitated using an ultrasonic device (Anthos u-PZ6; Imola, Italy). As with the PIPS technique, 1 mL 17% EDTA was placed into the root canal, and then an ultrasonic file (size 20, 0.02 taper) was placed into the canal 1 mm short of the working length and without touching the walls, enabling it to vibrate freely. The file was activated for 20 s. When the irrigating solution in the coronal reservoir decreased, the EDTA was refreshed. Again, three applications were performed for a total agitation time of 60 s with a total volume of 17% EDTA of 5 mL.

Sonic irrigation

A total of 5 mL of 17% EDTA was agitated for 60 s using the EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) handpiece set at 10 000 cycles per min, with a red (25/04) tip inserted 2 mm short of the working length.

Following each irrigation procedure, the root canals were given a final irrigation with 5 mL distilled water and dried with paper points. Then, the roots were disassembled to evaluate the removal of the $Ca(OH)_2$. Digital images at 25× magnification were obtained using a stereomicroscope (Zeiss Stemi 2000C; Carl Zeiss, Jena, Germany) attached to a digital camera and were transferred to the computer.

A pixel count of $Ca(OH)_2$ remaining on the artificially created grooves was recorded as a percentage of the overall groove surface (Fig. 1). Data were subjected to statistical analysis using one-way analysis of variance and the least significant difference post hoc tests at 95% confidence level ($P < 0.05$). The statistical analyses were performed using IBM® SPSS® Statistics 20 software (IBM SPSS Inc., Chicago, IL, USA).

Results

The mean percentage of the remaining $Ca(OH)_2$ was 75% for the needle irrigation, 0% for the PIPS, 24% for the ultrasonic irrigation and 54% for the sonic irrigation (Fig. 2). PIPS was superior in removing $Ca(OH)_2$ as compared to the needle irrigation ($P < 0.001$), sonic irrigation ($P < 0.001$) and ultrasonic irrigation ($P = 0.046$) (Fig. 3). Ultrasonic irrigation was superior to needle irrigation ($P < 0.001$) and sonic irrigation ($P = 0.017$). There was no statistically significant difference between needle and sonic irrigation ($P = 0.084$).

Discussion

One of the goals of root canal treatment is elimination of bacteria and their by-products from the root canal system. $Ca(OH)_2$ has been established as the most frequently used medicament because of its antimicrobial efficacy against most bacterial species identified in endodontic infections (Byström *et al.* 1985, Kawashima *et al.* 2009). Intracanal medicaments should be removed completely from root canals to avoid negative effects on sealer penetration. However, it is difficult to remove completely $Ca(OH)_2$ from the root canal using conventional methods. Therefore, in the present study, sonic, ultrasonic and laser (PIPS) activation was used to remove $Ca(OH)_2$ from an artificial groove created in straight root canals in comparison with needle irrigation. The main finding of this study was that in the PIPS group all of the specimens were scored as achieving a complete removal of medication. The PIPS was statistically superior to the needle, sonic and ultrasonic irrigation techniques in removing $Ca(OH)_2$. Therefore, the null hypothesis that there is no difference between various techniques is

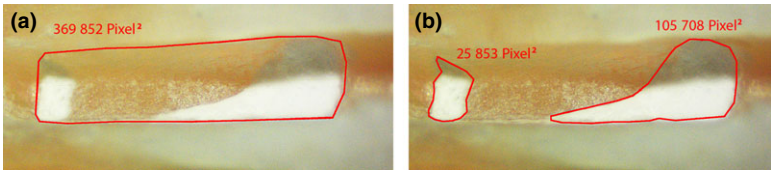

Figure 1 Demonstration of the pixel count of $Ca(OH)_2$ remaining on the artificially created grooves; (a) overall groove surface and (b) the remaining $Ca(OH)_2$.

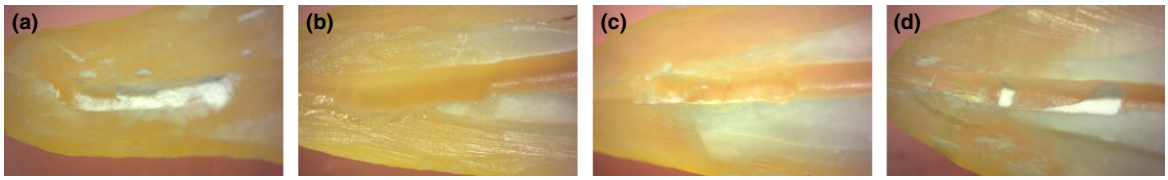

Figure 2 Representative images for the groups; (a) needle irrigation, (b) Photon-initiated photoacoustic streaming (PIPS)-*note the complete removal*, (c) ultrasonic irrigation and (d) sonic irrigation.

rejected. In the present study, the techniques were compared in straight root canals and further studies should be conducted to evaluate the effectiveness of the techniques in curved root canals.

The PIPS technique is based on photoacoustic and photomechanical phenomena, which make it different from other agitation techniques. PIPS tips have been used at subablative levels with specific models and settings and with a radial and stripped tip of novel design. This technique uses low energy levels and short microsecond pulse rates (50 μs) to generate peak power spikes. In this technique, each impulse interacts with the water molecules, creating expansion and successive shock waves that lead to the formation of a powerful streaming fluid and facilitates three-dimensional movement of the irrigation solutions (DiVito *et al.* 2012). The use of erbium lasers may result in side effects in the root canal such as carbonization and cracks in the root canal walls or temperature increasing (Kimura *et al.* 2002, Matsuoka *et al.* 2005). However, the subablative parameters in the PIPS technique result in a photomechanical effect, which occurs when the light energy is pulsed in a fluid, rather than thermal effect (Peters *et al.* 2011, DiVito *et al.* 2012).

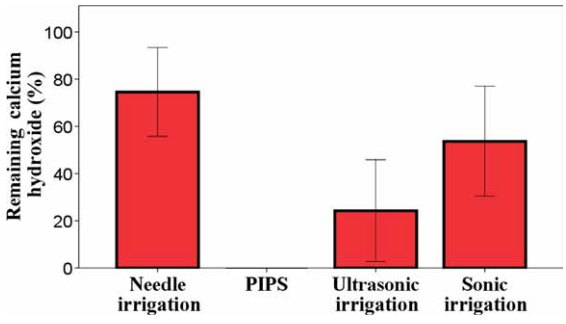

Figure 3 Graphical demonstration of the percentage of the $Ca(OH)_2$ according to the groups. Photon-initiated photoacoustic streaming (PIPS) was statistically superior to the other groups ($P < 0.05$). Ultrasonic irrigations was also superior to the sonic and needle irrigation ($P < 0.05$).

Bubbles, the formation of an empty space in a liquid, are the basis of cavitation. Er : YAG laser irradiation is highly absorbed by hydroxyapatite and water (Paghdiwala 1991, Armengol *et al.* 1999). When Er : YAG laser irradiation is absorbed by water, the energy causes evaporation (Brugnera *et al.* 2003, Kivanc *et al.* 2008). The vapour bubble starts to expand and form a void in front of the laser light. Matsumoto *et al.* (2011) demonstrated that the bubble increased in size and reached up to 1800 μm in 220 μs when a 300-μm laser tip was used, as in the present study. They observed that when the laser tip was inserted 2 mm and 5 mm short of the bottom of an artificial glass root canal model, the second cavitation bubbles were clearly observed at the bottom of the root canal model. Therefore, they suggested that it is not always necessary to insert the laser tip up to the canal terminus because the cavitation bubbles also assist in cleaning the apical region. In the present study, this finding was confirmed. The PIPS optic tip was inserted only the coronal part of the straight root canals, and the apically placed $Ca(OH)_2$ was effectively removed by this technique.

Despite the traditional laser applications, the PIPS tip does not need to reach the root apex, and it is placed into the coronal reservoir only of the root canal. Therefore, this technique allows for minimally invasive preparation of the root canal (DiVito *et al.* 2012). The affect may be explained by the increased liquid reaction kinetics with the laser activation (de Groot *et al.* 2009, Macedo *et al.* 2010). Laser-activated irrigation using PIPS tips has been shown to be effective in significantly better cleaning of the root canal walls in comparison with conventional irrigation procedures (DiVito *et al.* 2012). In a recent study, it was demonstrated that PIPS tips eliminated organic debris from canal isthmi at a significantly greater level compared with standard needle irrigation (Lloyd *et al.* 2014). Also, in a similar experimental set-up with the present study, PIPS was found to be more effective than sonic and ultrasonic techniques in removing apically placed debris (Arslan *et al.* 2014).

In the present experiment, the PIPS tip had a positive effect in removing $Ca(OH)_2$ from an artificial groove created in the apical third of the straight root canals, and this result is compatible with those of aforementioned studies.

Activation of the irrigant in the ultrasonic system has been shown to be more effective than syringe irrigation in removing $Ca(OH)_2$ from the root canal walls (Maalouf *et al.* 2013, Yucel *et al.* 2013). As mentioned above, in the present study, ultrasonic irrigation was superior to needle irrigation. However, PIPS and ultrasonic irrigation techniques are based upon the transmission of acoustic energy to an irrigant in the root canal space (Ahmad *et al.* 1987, DiVito *et al.* 2012), and the PIPS tip was more effective than the ultrasonic system in terms of removing $Ca(OH)_2$ from an artificial groove created in the apical third of the straight root canals in this study.

Jiang *et al.* (2010) reported that no cavitation seemed to take place either on the sonic tip itself or on the wall of the glass model of the root canal. They explained this by the velocity of the sonic tip, which was below the threshold needed for cavitation. Recently, Macedo *et al.* (2014) confirmed this result. In the present study, the cavitation effects of the techniques were not directly evaluated. However, sonic irrigation was found to be similar to needle irrigation in removing $Ca(OH)_2$ from artificially created grooves and had no perfect score for any sample. The ineffectiveness of sonic irrigation could result from its inability to create cavitation.

Rödig *et al.* (2010) compared the efficacy of different irrigating solutions in the removal of $Ca(OH)_2$ from root canals. According to the their results, chelating agents such as citric acid and EDTA showed the best results, and the addition of NaOCl to the chelators did not result in significant improvement of $Ca(OH)_2$ removal. Thus, in the present study, the control group included the single use of a chelating agent (EDTA) followed by irrigation with distilled water. EDTA irrigation with needle was not able to completely remove the $Ca(OH)_2$ from artificially created grooves. This finding was in accordance with those of Rödig *et al.* (2010).

The design of this study was based on studies described by Lee *et al.* (2004), van der Sluis *et al.* (2005a, b, 2007b) and Rödig *et al.* (2010). The standardized size and location of the grooves are advantages of the *in vitro* model. In addition, in the present study, a pixel count of $Ca(OH)_2$ remaining on the artificially created grooves was recorded as a percentage of the overall groove surface. The design provides researchers with a standardized evaluation that has high reproducibility, and discrimination between mechanical removal of the medicament and influence of the irrigant alone is enhanced. However, the complexity of a natural root canal system cannot be simulated by standardized grooves (Rödig *et al.* 2010). Moreover, this experimental design does not address medicament that diffused into the dentinal tubules.

Conclusion

Photon-initiated photoacoustic streaming provided complete removal of $Ca(OH)_2$ from artificial grooves in straight root canals. This technique could be beneficial in endodontics for activating irrigating solutions. Ultrasonic irrigation enhanced the $Ca(OH)_2$ removal capacity of irrigating solution but did not provide complete removal. Sonic irrigation was inefficient and similar to needle irrigation. Further studies should be conducted to determine apical extrusion during activation procedures.

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ORIGINAL ARTICLE

Effect of PIPS technique at different power settings on irrigating solution extrusion

Hakan Arslan · Merve Akcay · Hüseyin Ertas · Ismail Davut Capar · Gökhan Saygili · Merve Meşe

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Abstract The aim of this study was to determine the effect of photon-induced photoacoustic streaming (PIPS) technique at different power settings on extrusion of irrigating solution. Root canal preparation was performed up to a #30 file on 64 extracted single-rooted mandibular premolar teeth, which were then divided into four groups. Each group was irrigated with one of the following irrigation methods: (a) irrigation with conventional irrigation open-ended needles, (b) continuous ultrasonic irrigation, (c) 0.3 W PIPS, or (d) 0.9 W PIPS. Apical extrusion of irrigating solution was evaluated using a modified model. The net weight of the extruded irrigating solution was measured for each group, and the resulting data were analysed statistically using Kruskal-Wallis at a 95 % confidence level ($P<0.05$). Although the 0.9 W PIPS group resulted in the largest quantity of irrigation solution, the ultrasonic group was the smallest. However, the difference between these groups was not statistically significant at the 95 % level of confidence ($P>0.05$). PIPS at both 0.3 W and 0.9 W resulted similar solution extrusion to the conventional irrigation or ultrasonic irrigation.

Keywords Activation · Agitation · Apical extrusion · Endodontics · Photoacoustic streaming · PIPS · Ultrasonic

H. Arslan (✉) · H. Ertas · I. D. Capar
Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum 25640, Turkey
e-mail: dt_hakan82@hotmail.com

M. Akcay · M. Meşe
Department of Pedodontics, Faculty of Dentistry, Katip Çelebi University, İzmir, Turkey

G. Saygili
Department of Endodontics, Faculty of Dentistry, Katip Çelebi University, İzmir, Turkey

Introduction

Irrigation plays an important role in root canal treatment, and some irrigating solutions can show inefficiency in removing material from the root canal walls [1, 2]. Different irrigation agitation methods with sonic and ultrasonic [3] and laser devices [4–6] have been proposed. A novel laser agitation technique, photon-induced photoacoustic streaming (PIPS), has been proposed. This technique differs from other techniques that the tip is placed in the coronal portion only [7]. In this technique, the erbium:yttrium-aluminum-garnet (Er:YAG) laser is used with a radial and stripped tip of novel design at subablative power settings. Nevertheless, this technique does not have any additional benefits of reducing the number of bacteria in the root canal [8–10]. Divito et al. [11] demonstrated that it could have significantly better results in the removal of smear layer.

Undesired irrigating solution extrusion has been described in previous case reports, resulting in complications such as facial swelling and pain [12, 13]. Kleier et al. [14] surveyed Diplomates of the American Board of Endodontics and reported that 42 % of the respondents had experienced at least one complication, and 38 % experienced more than one complication. Consequently, irrigating solution extrusion with different setups has been increasingly investigated by researches [15–18].

Laser activation of irrigating solutions using erbium lasers can create pressure waves. George and Walsh [19] reported that caution should be used when using such lasers in combination of irrigants such as sodium hypochlorite and hydrogen peroxide. The aim of this study was to determine the effect of the PIPS technique at different power settings on extrusion of irrigating solution. The null hypothesis was that there would be no difference between the PIPS, conventional, and ultrasonic irrigation techniques in terms of the amount of irrigating solution extrusion.

Materials and methods

Mandibular premolars were selected from among teeth that had been extracted for reasons unrelated to the current study. Specimens were immersed in a 0.5 % Chloramine-T solution (Merck, Germany) for 48 h for disinfection. Then, the teeth were stored in distilled water at 4 °C. Soft tissues and calculus were removed mechanically from the root surfaces using a periodontal scaler. The teeth were verified radiographically for having a single root canal without calcification. The teeth with more than a single root canal and apical foramen, root canal treatment, internal/external resorption, immature root apices, caries/cracks/fractures on the root surface, and/or root canal curvature more than 10° were excluded from the study. After the access cavity prepartion, a #10 stainless steel K-file (G-Star, Shenzhen, China) was inserted into the canal until its tip was slightly visible at the apical foramen. Endodontic working lengths were set by deducting 1 mm from the initial length. The size of the apical foramen was controlled by inserting a #15 K-file through the root canal. If the K-file extruded beyond the apical foramen, the tooth was excluded from the study.

According to these criteria, 64 mandibular premolar teeth were selected. Root canal shaping procedures were performed using ProTaper rotary instruments (Dentsply, Ballaigues, Switzerland) up to F3 (#30). Root canals were irrigated with 2 mL of the 1 % NaOCl (ImidentMed, Konya, Turkey) using a 27-gauge notched-tip needle (Ultradent, UT, USA) between instrument changes. The root canals were irrigated with a final rinse of 5 mL distilled water and dried with paper points.

Quantification of the extruded irrigating solution

To quantify the extruded irrigating solution in this study, a modification of the method used by Altundasar et al. [20], Myers et al. [21], and Huang et al. [22] was done. A cube-shaped sponge of 0.119±0.02 g was used to simulate resistance of periapical tissues. Each sponge was placed into an ampule. The total weigh was measured as "sponge weight+ ampule and its cap weights." An ampule with its sponge and a cap were weighed using a microbalance (TW423L; Shimadzu, Tokyo, Japan). Another cap was drilled, and an Eppendorf tube and a needle (to equalize the internal and external pressures) were placed in the drilled hole. The gap in the hole, the Eppendorf tube, and the needle were carefully filled with adhesive (Pattex Super Glue; Türk Henkel, Inc., Istanbul, Turkey) to prevent irrigating solution extrusion through the hole.

The tip and cap of the Eppendorf tube were cut, and the sponge was placed inside the tube (Fig. 1a). The cap of the Eppendorf tube was drilled, the tooth was placed in the cap, and the gap between the tooth and cap was filled with adhesive. The tooth was pushed in the sponge, and the cap was seated in the Eppendorf tube (Fig. 1b). The tooth was isolated with a rubber dam ligated with thread. An aspirator was used to suction overflowed irrigating solution from the tooth crown (Fig. 1c).

Sixty-four specimens, which were randomly divided into four groups (n=16), were irrigated using one of the four techniques. In all groups, the flow rate of the irrigating solution was constant and equal to 0.16 mL/s.

Group 1: conventional irrigation with open-ended needle

Five milliliters of physiologic saline with a 27-gauge notched-tip needle (Ultradent, UT, USA) was used for 30 s. The needle was attached to the Surgic XT Plus device (NSK, Kanuma, Japan), inserted in the canal within 2 mm from the working length without binding, and then inserted with an up-and-down motion.

Group 2: continuous ultrasonic irrigation

An open-ended notched-tip needle (Ultradent) was inserted in the canal within 2 mm from the working length, and the irrigation started with 5 mL of physiologic saline. The irrigating needle was placed in the access cavity, and an ultrasonic file (size 15:0.02 taper) was attached to an ultrasonic device (Anthos u-PZ6, Imola, Italy), which was inserted in the root canal within 2 mm from the apical foramen and activated at 25 % power. The continuous ultrasonic irrigation took 30 s.

Group 3: PIPS at 0.3 W

An Er:YAG laser with a wavelength of 2,940 nm (Fidelis AT; Fotona, Ljubljana, Slovenia) was used. A 14-mm long, 300-μm quartz laser tip was applied with 0.3 W, 15 Hz, and 20 mJ per pulse. The pulse duration was 50 μs. The water and air on the laser system were turned off. The open-ended notched-tip needle (Ultradent) was inserted in the canal within 2 mm from the working length, and the irrigation started. The irrigating needle was placed in the access cavity and the optical fiber was inserted in the access cavity. The laser irradiation took 30 s.

Group 4: PIPS at 0.9 W

The same PIPS technique, which has been mentioned in the previous group, was done again but at a different power setting: 0.9 W, 30 Hz, and 30 mJ per pulse. The laser irradiation took 30 s.

After the experimental procedures, the sponge was pushed in the ampule, and the ampule, sponge, and its cap were weighed. The net weight of the extruded irrigating solution was determined by subtracting the initial (pre-irrigation) weight from the last (post-irrigation) (grams).

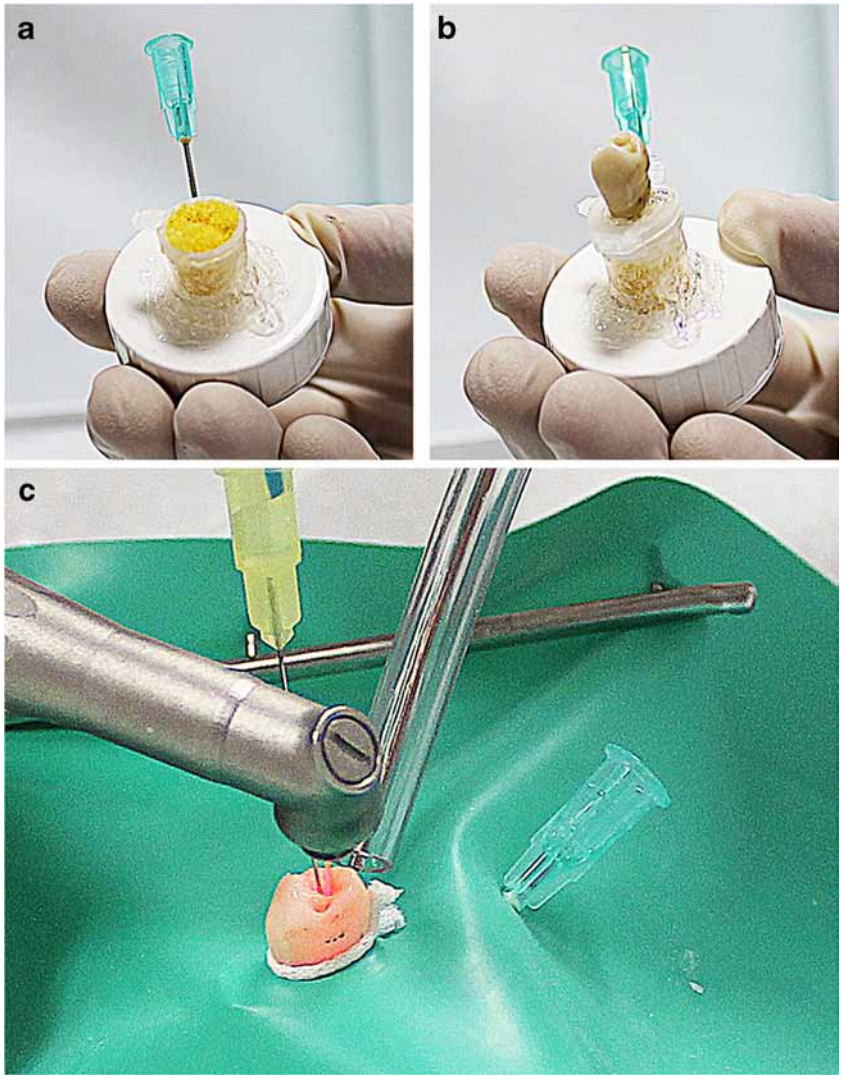

Fig. 1 Experimental procedures in the study. **a** The sponge was placed in the Eppendorf tube to simulate resistance of the periapical tissue, **b** the tooth was pushed in the sponge and the cap was seated in the Eppendorf tube, and **c** the tooth was isolated using a rubber dam

Data were analysed using a Kruskal-Wallis test. The testing was performed at a 95 % confidence level (P=0.05). All statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL, USA).

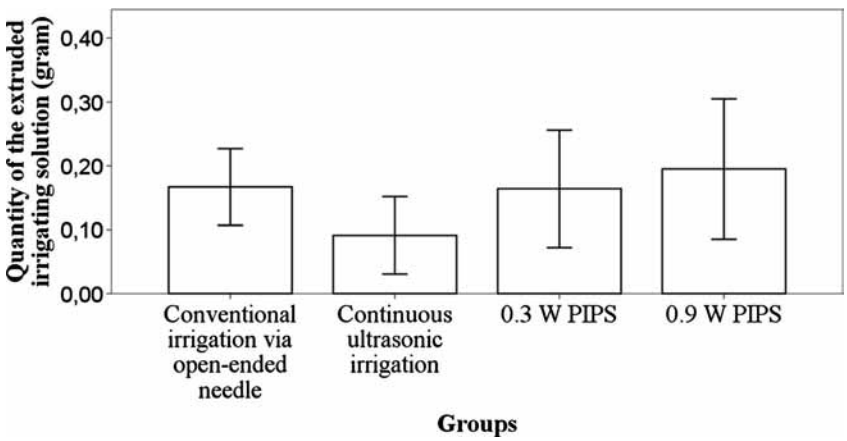

Fig. 2 The quantity of extruded irrigating solution according to the groups. There were no statistically significant differences between the groups (P>0.05). The *error bars* represent confidence intervals

Results

Figure 2 shows the quantity of apically extruded irrigating solution for each group. Mean and standard deviations of the extruded irrigating solution amount was 0.16±0.11 g for conventional irrigation, 0.09±0.11 g for continuous ultrasonic irrigation, 0.16±0.17 g for 0.3 W PIPS, and 0.19±0.20 g for 0.9 W PIPS groups. The largest quantity of extruded irrigating solution occurred in the 0.9 W PIPS group, and the smallest was in the ultrasonic irrigation group. However, according to the Kruskal-Wallis test there were not statistically significant between the groups at a confidence level of 95 % ($P>0.05$) (effect size=1.1286).

Discussion

The apical extrusion of irrigating solution in root canal treatment can lead to complications, such as damage of periapical tissues, pain, and burning sensation [12, 23, 24]. Thus, irrigation techniques need to be evaluated to determine the one, which results in less extrusion. In vitro models try to simulate clinical situations. In previous studies, in vitro models included the root apex suspended in air or water [22, 25]. Altundasar et al. [20] used a flower-arranged foam to simulate the resistance of the periapical tissues and to collect any extruded irrigating solution. This study presented a modified model based on previous studies [20–22]. In this modified model, a sponge simulates the periapical tissue resistance [20], and the extruded irrigating solution is collected in an ampule [21, 22].

In a recent study, Boutsioukis et al. [25] evaluated the effect of ultrasonic, sonic, and manual dynamic agitation on irrigating solution extrusion. They found that there was not statistically significant difference between the ultrasonic agitation and the control group. Desai and Himel [17] evaluated the safety of various intracanal irrigation systems, and they found no statistically significant difference between conventional (manual syringe and Max-I-probe needle) and ultrasonic irrigation. These findings are in harmony with our results. In this study, the ultrasonic irrigation did not result in more irrigating solution extrusion when compared to the control group.

In the present study, the PIPS and ultrasonic techniques were compared with the conventional irrigation technique. The ultrasonic and PIPS techniques require prior filling of the root canals with irrigating solution, followed by continuous solution activation through the optical or ultrasonic tip. When such activation occurred, less irrigating solution extrusion was observed compared to the conventional technique. Moreover, in the conventional technique the needle gets closer to the apex than in the PIPS and ultrasonic groups. These factors could affect the results of studies evaluating the extrusion of irrigating solution [25].

Our results show that PIPS at both 0.3 and 0.9 W did not result in more solution extrusion than conventional irrigation and ultrasonic irrigation. Therefore, the null hypothesis that there is no difference between the PIPS, conventional, and ultrasonic irrigation techniques in terms of irrigating solution extrusion can be accepted.

Extrusion of irrigating solution can be affected by many factors, some of which were controlled in this study. In the present study, specimens with an apical diameter more than #15 K-file were excluded from the study. The root canals were instrumented up to the size 30 using the ProTaper Universal rotary files. The volume and flow rate of the irrigating solution had equal values in all groups. To standardize the irrigation flow rate and the pressure, a Surgic XT Plus device was used. A standard needle type was used. Precautions were taken to avoid extrusion of the irrigating solution in the ampule through the gaps in the modified model. Finally, sponges with the standardized weight were used to simulate resistance of periapical tissues.

Conclusion

Within the limitations of this study, all techniques led to extrusion of irrigating solution beyond the apical foramen. Nevertheless, PIPS at both 0.3 and 0.9 W resulted similar extrusion to conventional irrigation and ultrasonic irrigation. The modified model used here can be beneficial for further evaluation of apical extrusion.

Conflict of interest There are no conflicts of interest.

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Bond strength of self-adhesive resin cement to root dentin. Comparison of photon-initiated photoacoustic streaming technique with needle and ultrasonic irrigation

Hakan Arslan, Merve Akcay, Gokhan Saygili, Ahmet Keskı, İbrahim Talha MeŞe, Adem Gok & Mehmet Dalli

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ORIGINAL ARTICLE

Bond strength of self-adhesive resin cement to root dentin. Comparison of photon-initiated photoacoustic streaming technique with needle and ultrasonic irrigation

HAKAN ARSLAN[1], MERVE AKCAY[2], GOKHAN SAYGILI[3], AHMET KESKI[4], İBRAHIM TALHA MEŞE[4], ADEM GOK[5] & MEHMET DALLI[5]

[1]*Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum, Turkey,* [2]*Department of Pedodontics,* [3]*Department of Endodontics,* [4]*Department of Prosthodontics, and* [5]*Department of Restorative Dentistry, Faculty of Dentistry, Izmir Katip Çelebi University, İzmir, Turkey*

Abstract

Objective. The aim of this study was to investigate the effects of photon-initiated photoacoustic streaming (PIPS) with various irrigating solutions on the bond strength of a self-adhesive resin cement to root dentin. ***Materials and methods.*** Seventy-two mandibular premolar roots were divided into six groups after post space preparation and treated with a needle irrigation with distilled water and NaOCl, ultrasonic irrigation with NaOCl, PIPS with NaOCl, PIPS with EDTA and PIPS with distilled water at 0.3 W, 15 Hz and 20 mJ per pulse for 60 s. Fiber posts were cemented with a newly marketed, self-adhesive resin cement. The data obtained from the push-out tests were analyzed using analysis of variance (ANOVA) and LSD post-hoc tests ($p = 0.05$). ***Results.*** PIPS with distilled water resulted in higher push-out values than those of needle (with both distilled water and NaOCl) and ultrasonic irrigation ($p < 0.05$). ***Conclusions.*** The use of PIPS may provide higher bond strength of self-adhesive resin cement to root dentin than needle and ultrasonic irrigation techniques.

Key Words: *bond strength, fiber post, photoacoustic streaming, self-adhesive resin cement, ultrasonic*

Introduction

Fiber reinforced composite (FRC) posts have been widely used in restoring endodontically treated teeth to retain coronal restoration. FRC posts have a modulus of elasticity close to that of dentin [1,2]. They also have a good esthetic appearance, with no risk of gingival discoloration of the root surfaces due to corrosive products [3]. The longevity of the restorations depends on many factors, such as the effectiveness and durability of the bonding between the post, dentin, and the adhesive resin cement [4]. Effective bonding can contribute to reduced stress being generated on the root canal walls, thereby strengthening the remaining tooth structure and decreasing the risk of fracture [5,6].

The efficacies of various irrigating solutions were investigated as pre-treatment agents before the FRC post placement [3,7]. Recently, a novel laser agitation technique, photon-induced photoacoustic streaming (PIPS), has been proposed. This technique differs from other agitation techniques insofar as it involves the placement of only the tip into the coronal portion [8]. In this technique, an erbium:yttrium-aluminum-garnet (Er:YAG) laser is used with a radial and stripped tip of novel design at sub-ablative power settings. DiVito et al. [9] demonstrated that it results in a significantly better cleaning of the root canal walls.

Various strategies have been proposed to lute FRC posts to root dentin. Conventional dual-cured resin cements require the prior application of adhesive systems. Self-adhesive cements do not require any surface treatment of the root canals. They are easier to handle and have clinically effective bond strength [10]. The chemical reaction between the phosphate methacrylates in the self-adhesive cements and hydroxyapatite in dentin provides bonding [11].

The aim of this study was to evaluate the effect of PIPS with various irrigating solutions on the bond

Correspondence: Assistant Professor Dr Hakan Arslan, Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum, 25620, Turkey. Tel: +90 442 325 4040 2352. Fax: +90 442 325 2535. E-mail: dt_hakan82@hotmail.com

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strength to root dentin of a self-adhesive resin cement as compared to needle and ultrasonic irrigation. The null hypothesis of this study was that there were no significant differences among the techniques in terms of adhesion between the self-adhesive resin cement and root dentin.

Materials and methods

Human mandibular premolar teeth were selected from a collection of teeth that had been recently extracted for reasons unrelated to this study and stored in distilled water until use. Soft tissue and calculus were removed mechanically from the root surfaces with a periodontal scaler. Teeth with root canal treatment, restoration, immature root apices and/or coronal defects were excluded from the study. The teeth were verified radiographically as having a single root canal without calcification. According to these criteria, 72 mandibular premolar teeth were selected.

Each tooth was decoronated using a diamond disc (Diamond disc superflex 910S/220; North Bel, Italy) operated perpendicularly to its longitudinal axis to obtain a standardized root length of 15 mm. A size #15stainless steel K-file (Dentsply-Maillefer, Ballaigues, Switzerland) was moved down in the canal until the file was just visible. The working length was set by deducting 1 mm from this. The root canals were shaped with ProTaper rotary instruments (Dentsply-Maillefer) up to a size #40 (F4) at working length. The root canals were irrigated with 2 mL of 5% NaOCl (ImidentMedEndosolve-HP, Konya, Turkey) between instrument changes. The apexes of the specimens were closed with boxing wax and the root canals were irrigated first for 120 s with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) as a final flush and then for 120 s with 5 mL of 5% NaOCl, followed by a final rinse with 5 mL of distilled water to remove the smear layer.

The specimens were dried using paper points, with a single gutta-percha cone (F4; Dentsply-Maillefer) slightly coated with AH Plus (Dentsply DeTrey, Kontanz, Germany) and placed into the root canal to the working length. Temporary filling material (Cavit G; 3M ESPE, Seefeld, Germany) was used to seal the coronal orifice. After storage at 100% humidity for 1 week at 37°C; a portion of the root canal filling material was removed with gates-glidden drills. A 10-mm deep post space was made using a 1.4-mm size drill (Cytec Carbon; Hahnenkratt GmbH, Königsbach-Stein, Germany). The specimens were randomly divided into six groups (n = 12), as follows:

- *Needle irrigation with distilled water*: 5 mL of distilled water via a size 27 gauge blunt-tip needle (Ultradent, UT) was used for 60 s in this group.
- *Needle irrigation with NaOCl*: 5 mL of 5% NaOCl was used for 60 s in this group.
- *Ultrasonic irrigation with NaOCl*: 5 mL of 5% NaOCl was continuously activated via an ultrasonic device (Anthos u-PZ6, Imola, Italy). An ultrasonic file (size 15: 0.02 taper) was placed into the post space without touching the walls, enabling it to vibrate freely. The ultrasonic file was activated at 25% power for a total of 60 s.
- *PIPS with NaOCl*: 5 mL of 5% NaOCl was continuously activated via an Er:YAG laser with a wavelength of 2940 nm (Fidelis AT; Fotona, Ljubljana, Slovenia). A 14-mm long, 400-micron quartz tip was inserted into the post space as coronally as possible and applied at 0.3 W, 15 Hz and 20 mJ per pulse for 60 s. The water and air in the laser system were turned off.
- *PIPS with EDTA*: 5 mL of 17% EDTA was activated as stated above.
- *PIPS with distilled water*: 5 mL of distilled water was activated as stated above.

The flow rate of the irrigating solutions in all groups was 0.08 mL/s. After the procedures, the post space was irrigated with 5 mL distilled water and dried using paper points. Self-adhesive resin cement (RelyX U200; 3M ESPE, Seefeld, Germany) was applied directly into the post space. Size #2 carbon fiber posts (1.4 mm diameter) (Cytec Carbon; Hahnenkratt GmbH, Königsbach-Stein, Germany) were inserted into the post space. Excess cement was removed and light-cured for 40 s. The coronal opening was sealed using composite resin and the specimens were stored at 100% humidity, 37°C for 24 h to completely set. Each specimen was sectioned perpendicular to its long axis using a diamond disc (Arbor; Extec, Enfield, CT) and a precision saw (Isomet 1000; Buehler, Lake Bluff, IL) at a low speed with water cooling. Two slices of a 1 ± 0.1-mm thickness were obtained from each tooth (n = 24 for each group). The definitive thicknesses of the slices were recorded after measurement using a digital caliper. The diameter of each post was measured under a stereomicroscope (Zeiss Stemi 2000C; Carl Zeiss, Jena, Germany) at 32× magnification.

A push-out test was performed on each specimen with a universal test machine (AGS-X; Shimadzu Corporation, Tokyo, Japan) at a crosshead speed of 0.5 mm per minute. The maximum load at failure was recorded in Newtons (N) and converted to MPa by dividing the load by A, the bonded area. The bonded area was calculated using the formula $A = 2\pi rh$, where h represents the thickness of the section (mm) and r the radius of the post segment (mm) [12–14].

After the test procedure, each specimen was visually examined under a stereomicroscope (Zeiss Stemi 2000C; Carl Zeiss) at 32× magnification to evaluate

Table I. Bond strength ± standard deviation and failure modes in the groups.

Group	*n*	Bond strength ± SD	Failure modes		
			Adhesive	Cohesive	Mixed
Needle irrigation with distilled water	24	4.81 ± 2.16^{bc}	24 (100)	0 (0 %)	0 (0%)
Needle irrigation with NaOCl	24	3.50 ± 2.86^{a}	23 (95.8%)	0 (0 %)	1 (4.2%)
Ultrasonic irrigation with NaOCl	24	3.93 ± 2.19^{ac}	23 (95.8%)	0 (0 %)	1 (4.2%)
PIPS with NaOCl	24	5.20 ± 1.44^{b}	20 (83.3%)	3 (12.5%)	1 (4.2)
PIPS with EDTA	24	5.00 ± 2.43^{bc}	18 (75%)	5 (20.8%)	1 (4.2%)
PIPS with distilled water	24	6.48 ± 1.20^{d}	14 (58.3%)	8 (33.3 %)	2 (8.3%)

*Different letters shows the statistically significant differences.

failure mode. Three types of failure were classified: adhesive failure (between the cement and root dentin); cohesive fracture (within the dentin, cement layer or post); and mixed (a combination of the two; cohesive and adhesive). Data were analyzed using one-way analysis of variance (ANOVA) and LSD post-hoc tests (p = 0.05). The failure mode data were analyzed using the chi-square test (p = 0.05). All statistical analyses were performed using IBM® SPSS® Statistics 20 software (IBM SPSS Inc., Chicago, IL).

Results

Mean bond strength values, standard deviations and distribution of failure modes after the push-out test are shown inS Table I. PIPS with distilled water resulted in higher push-out values than those of needle (with both distilled water and NaOCl) and ultrasonic irrigation ($p < 0.05$) Figure 1.

For the needle groups, the NaOCl reduced the bond strength compared to distilled water. In the PIPS technique the use of distilled water resulted in higher push-out values than the use of NaOCl or EDTA ($p < 0.05$). There was no significant difference between the use of NaOCl and EDTA on the bond strength to root dentin of self-adhesive resin cement bond strength (p = 0.743). There was no statistically significant difference between needle and ultrasonic irrigation with NaOCl regarding the bond strength of self-adhesive resin cement bond strength to root dentin ($p > 0.05$). Adhesive failure between cement and dentin was the most frequent type of failure mode in all groups (Table I).

Discussion

The results from the present study did not support the null hypothesis, the PIPS technique was found to be superior to needle and ultrasonic irrigation. The higher mean bond strength values observed in the PIPS group may have been due to the fact that this technique is able to remove materials like the debris-smear layer on root canal walls that could negatively affect good bonding [9]. Er:YAG laser irradiation is highly absorbed by hydroxyapatite and water [15,16]. When Er:YAG laser irradiation is absorbed by water, the vapor bubble starts to expand and form a void in

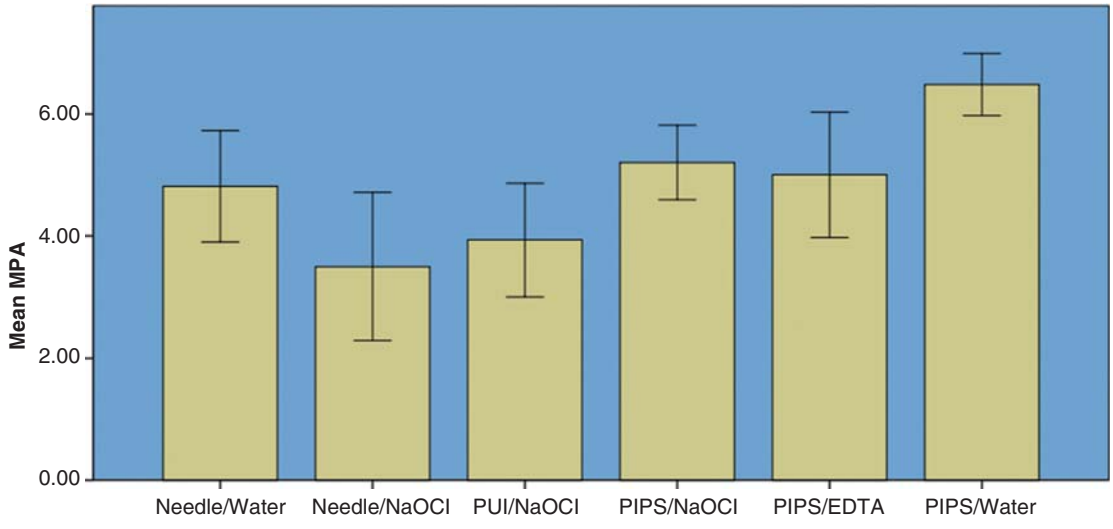

Figure 1. Mean bond strength of self-adhesive resin cement to root dentin after needle irrigation, ultrasonic irrigation, and PIPS.

front of the laser light. This causes shock waves and streaming of the liquid [17,18]. In a previous study, the bubble was shown to increase in size and reached up to 1800 μm in 220 μs [19]. It was observed that, when the laser tip was inserted to 2 mm and 5 mm short of the bottom of an artificial (glass) root canal model, the second cavitation bubbled at the bottom. Thus, it is not always necessary to insert the laser tip up to the apex, because the cavitation bubbles also assist in cleaning the apical region. The present study has confirmed this finding in real human teeth. Although the ultrasonic file was inserted into the apical part of the post space, PIPS was found to be the most effective technique. This suggests that PIPS may be beneficial in obtaining high bond strength between self-adhesive resin cement and root dentin.

Meire et al. [20] investigated the absorption of different endodontic irrigating solutions including water, NaOCl, and EDTA at wavelengths between 300–3000 nm. They found that water and NaOCl showed a markedly high absorption (> 40) at 2.940 nm (Er:YAG laser), while EDTA showed less absorption (= 33.5). Thus, in the present study, PIPS technique described for Er:YAG laser (2.940 nm) was used with different irrigating solutions and the effectiveness of the PIPS technique on the bond strength to the root canal dentin of a self-adhesive resin cement was evaluated for different irrigating solutions. Similar to the findings of Meire et al. [20], the use of distilled water in the PIPS technique was found to be superior to that of NaOCl and EDTA.

In the present study, the recently introduced RelyX U200 was used as self-adhesive resin cement. RelyX Unicem, ancestor of the RelyX U200, chemically interacts with calcium from hydroxyapatite. This interaction is the result of the chelation of the calcium ions by acid groups [11]. NaOCl and EDTA are widely used in the root canals to remove smear layer [21,22]. NaOCl removes the organic components and EDTA demineralized inorganic components. In the present study, NaOCl was found to be reducing the bond strength of self-adhesive resin cement to root dentin. Renovato et al. [14] used RelyX Unicem as a self-adhesive resin cement, which was the previous version of RelyX U200. They investigated the effect of irrigating solutions on the retention of glass-fiber posts luted with self-adhesive resin cement. According to the results of these investigators, EDTA and NaOCl irrigating solutions reduced the bond strength. This finding is harmonious with our findings.

Conclusion

Within the limitation of the present study, the use of the PIPS technique with distilled water is shown to provide a higher bond strength to root dentin of self-adhesive resin cement as compared to needle and ultrasonic irrigation techniques. Needle and ultrasonic irrigation resulted in similar push-out values. Moreover, the use of distilled water in PIPS technique is shown to be advantageous in increasing the bond strength to the root canal dentin of self-adhesive resin cement tested in this study when compared to the use of NaOCl and EDTA.

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***Declaration of interest*:** The authors deny any financial affiliations related to this study or its sponsors.

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ORIGINAL ARTICLE

Bleaching effect of activation of hydrogen peroxide using photon-initiated photoacoustic streaming technique

Hakan Arslan · Merve Akcay · Bilal Yasa · Huseyin Hatirli · Gökhan Saygili

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Abstract
Objectives This study aims to investigate the bleaching effectiveness of photon-initiated photoacoustic streaming (PIPS) using 35 % hydrogen peroxide on discolored teeth as compared with different devital bleaching techniques.
Materials and methods Fifty extracted human mandibular incisors were collected and artificially stained using sheep's blood. The teeth were then randomly divided into five groups according to the different bleaching procedures to be tested: walking bleach with sodium perborate and with 35 % hydrogen peroxide gel, both for 1 week; PIPS using 35 % hydrogen peroxide liquid for 30 min; and just 35 % hydrogen peroxide, as a liquid and as a gel (again, for 30 min). Spectrophotometric measurements were obtained on the buccal surfaces of the crowns, at the beginning, just after the bleaching procedures had been performed, and the following first, third, and seventh days. The ΔE values were calculated, and the data were analyzed with a two-way analysis of variance ($P=0.05$).
Results There were statistically significant differences between the PIPS technique using 35 % hydrogen peroxide liquid and the 35 % hydrogen peroxide liquid and gel without PIPS immediately after the procedures ($P<0.05$). On Days 1, 3, and 7, the PIPS technique further bleached specimens more than all of the other techniques ($P<0.05$).
Conclusions The PIPS technique using 35 % hydrogen peroxide was found to be more effective than all of the conventional techniques.
Clinical relevance Within limitations of this study, PIPS technique using hydrogen peroxide was superior to the conventional techniques. Further studies should be conducted to determine if the PIPS technique results in any complications, particularly cervical resorption.

Keywords Bleaching · Discoloration · Er/YAG · Hydrogen peroxide · Internal bleaching · Laser · Photon-initiated photoacoustic streaming · Sodium perborate

H. Arslan
Department of Endodontics, Faculty of Dentistry, Ataturk University, Erzurum, Turkey

M. Akcay
Department of Pedodontics, Faculty of Dentistry, Izmir Katip Celebi University, Izmir, Turkey

B. Yasa (✉) · H. Hatirli
Department of Restorative Dentistry, Faculty of Dentistry, Izmir Katip Celebi University, Izmir, Turkey
e-mail: bilalyasa@hotmail.com

G. Saygili
Department of Endodontics, Faculty of Dentistry, Izmir Katip Celebi University, Izmir, Turkey

Introduction

Systemic and local factors can cause intrinsic changes to the tooth surface [1]. The main intrinsic changes related to the endodontic process may result in a serious esthetic complaint. Internal bleaching is widely used to resolve this, as it is a minimally invasive, simple, and cost-effective intervention for discolored nonvital teeth [2].

Discolored nonvital teeth can be bleached with chemicals, such as sodium perborate, hydrogen peroxide, and carbamide peroxide. Hydrogen peroxide seems to be the optimum choice, as it produces free radicals, such as hydroperoxyl and hydroxyl. Moreover, it can penetrate to the enamel and dentin and release oxygen that breaks the double bonds of the organic and inorganic compounds [1, 3].

Bleaching agents can be applied in the pulp chamber followed by a heat source to catalyze and accelerate the breaking reaction. Photoxidation, the process for activating bleaching by light, causes the decomposition of the bleaching agents, thus releasing free radicals [4]. This mechanism may

be induced with halogen lamp appliances or lasers [3]. In order to accelerate the bleaching process, different laser devices have been used as light sources subsequent to the bleaching agent application [5, 6]. Laser devices also have been applied (internal bleaching) prior to the bleaching agent application, in order to increase the permeability of tubules and remove the smear layer (as a conditioner) [7]. However, laser energy has not yet been used at the same time as a bleaching agent for the purpose of agitating it.

A novel laser agitation technique, photon-induced photoacoustic streaming (PIPS), has been proposed. In this technique, an erbium/yttrium–aluminum–garnet (Er/YAG) laser with a radial and stripped tip of novel design is used at subablative power settings. This technique differs from other agitation techniques in the placement of only the tip into the coronal portion [8].

Cardoso et al. [9] evaluated the effect of the ultrasonic activation of bleaching agents on internal bleaching. To our knowledge, however, no studies have investigated the bleaching effect of laser agitation of bleaching agents on discolored teeth. Therefore, the aim of the present study was to investigate the bleaching effect of PIPS using 35 % hydrogen peroxide on discolored teeth as compared with walking bleaching techniques (sodium perborate and 35 % hydrogen peroxide gel),and 35 % hydrogen peroxide liquid and gel applications (without PIPS). The null hypothesis was that there is no difference between the PIPS using 35 % hydrogen peroxide and the other internal bleaching techniques.

Materials and methods

Specimen selection

Mandibular incisors were selected from a collection of teeth that had recently been extracted for reasons unrelated to this study. They were stored in distilled water until use. Fifty were selected from among those with similar mesiodistal (3.3±0.3) and buccolingual width (5.6±0.4) to obtain standard access cavity dimensions. Teeth with root canal treatment, restoration, immature root apices, and/or coronal defects were excluded from the study. The soft tissue and calculus were removed mechanically from the root surfaces with an ultrasonic scaler (Anthos u-PZ6; Imola, Italy), and then polished with pumice.

Preparation of specimens

A standard oval coronal access was performed, and the thickness of the buccal wall was gradually decreased with a size 6 number carbide bur and standardized at 2.6±0.3 by using an electronic digital caliper. Root canals were enlarged up to F3 using ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland). Coronal flaring was performed using size 4–5 Gates Glidden burs (Mani, Mani Inc., Takanezawa, Japan) to ease the placement of cement as a cervical plug. Specimens were irrigated to open dentinal tubules with combination of 5 mL of 17 % EDTA (Werax; Spot Dis Deposu A.S., Izmir, Turkey) and 5 mL of 5 % NaOCl, each for 60 s. Final rinse was performed using 5 mL of distilled water.

Artificial staining

The specimens were artificially stained as described by previous studies [9, 10], with a modified procedure based on that employed by Freccia and Peters [11]. First, the specimens were immersed in Eppendorf tubes containing sheep blood and centrifuged at 3,400 rpm for 20 min at 37 °C (Micro 220R; Hettich, Germany). After the plasma (supernatant) and precipitate were yielded, the plasma was removed. The Eppendorf tubes were then centrifuged for a further 20 min, followed by a centrifugation twice a day for a further 2 days. After each centrifugation, the teeth were irrigated with distilled water, reinserted into the Eppendorf tubes, and stored at 37 °C in 100 % humidity.

On the fourth day, the teeth were removed from the Eppendorf tubes and placed in clean ones. A total of 0.5 mL of distilled water was added to the original Eppendorf tubes (i.e., including blood), which were then centrifuged for a further 20 min to pioneer the hemolysis of erythrocytes. This resulted in a membranous precipitate and hemolysate, and the supernatant layer was removed. The teeth were then transferred back to these Eppendorf tubes, and the tubes were centrifuged again, for 20 min on three consecutive days. After 6 days, the blood was changed, and all the aforementioned procedures were repeated, over a further 6 days.

Baseline color measurement

After the artificial staining procedure, the teeth were cleaned in running water and dried with air spray, after which 2 mm thick glass-ionomer cement (Ketac Molar; 3M ESPE, Seefeld, Germany) was placed 1 mm apical to the cementoenamel junction (CEJ). A standardized circular strip with an internal diameter of 4 mm (external diameter=8 mm) was bonded to the buccal surface of the crown coronal to the CEJ to ensure that color measurement was performed on the same region at every turn with a vertical angle (Fig. 1) [12]. A baseline color measurement was performed using a noncontact-type spectrophotometer (Spectro Shade™ Micro; MHT, Milan, Italy) on the buccal surfaces of the crowns. The specimens were distributed equally according to the baseline color measurements across the five groups to be tested. Statistical analysis by one-way ANOVA confirmed no significant differences among the groups in terms of their baseline color (P=0.682).

Fig. 1 Representative image of the analysis of crown color using a standardized circular strip

Conventional walking bleaching with sodium perborate The walking bleach paste was prepared by mixing tetrahydrate sodium perborate powder (Sultan Healthcare, Hackensack, NJ, USA) and distilled water, in a ratio of 2 g of powder to 1 mL of liquid, to a consistency of wet sand. With a plastic instrument, the pulp chamber was packed with the paste and tiny cotton pellet was placed over the paste. After the access cavity had been closed with temporary filling material (META Biomed Co. Ltd., Cheongju, Korea), the bleaching agent was left for 1 week and the specimens were stored in distilled water at 37 °C in 100 % humidity.

Conventional walking bleaching with 35 % hydrogen peroxide gel The pulp chamber was filled with 35 % hydrogen peroxide gel (Opalescence®Endo; Ultradent Products Inc., South Jordan, UT, USA) via its syringe, and tiny cotton pellet was placed over the gel. After the access cavity had been closed with temporary filling material, the bleaching gel was left for 1 week and the specimens were stored in distilled water at 37 °C in 100 % humidity.

PIPS using 35 % hydrogen peroxide liquid A band was agglutinated above the endodontic access cavity to hinder irrigant extrusion during the activation procedure. It was holed using a size 40 spreader to allow needle and fiber tip insertion. Then, 0.5 mL of 35 % hydrogen peroxide liquid (Merck, Darmstadt, Germany) was inserted into the access cavity and activated by laser for 1 min. When the irrigating solution in the coronal reservoir decreased, the 35 % hydrogen peroxide liquid was refreshed, resulting in a total of 3 mL. After, the activated 35 % hydrogen peroxide liquid was left in the access cavity without any activation for 10 min of total time. These procedures were repeated for three times, totaling for 30 min, including laser activation time of 3 min. Laser activation was performed with an Er/YAG laser at a wavelength of 2,940 nm (Fidelis AT, Fotona, Ljubljana, Slovenia). A 14-mm-long and 300-μm-diameter quartz tip was applied with 0.9 W, 30 Hz, and 30 mJ/pulse. The water and air on the laser system were turned off, and the optical fiber was placed into the endodontic access cavity.

At the end of the 30 min, the bleaching agent was removed using an air water jet. After the access cavity of the tooth had been filled with cotton pellet, and sealed with temporary filling material, the specimens were stored for 1 week at 37 °C in 100 % humidity.

Thirty-five percent hydrogen peroxide liquid without PIPS A band was agglutinated above the endodontic access cavity, as stated. A total of 3 mL of 35 % hydrogen peroxide liquid (Merck) was inserted into the access cavity during 1 min and left for 10 min of total time. This procedure was repeated for three times, totaling 30 min. Application of temporary filling material and storage were the same as the group of PIPS using 35 % hydrogen peroxide liquid.

Thirty-five percent hydrogen peroxide gel without PIPS Thirty-five percent hydrogen peroxide gel (Opalescence®Endo; Ultradent Products Inc., South Jordan, UT, USA) was placed into the access cavity via its syringe and stirred with bonding brush during 1 min and left for 10 min of total time. Again, this procedure was repeated for three times, totaling 30 min. Application of temporary filling material and storage were the same as the group of PIPS using 35 % hydrogen peroxide liquid.

Tooth color assessment

Color measurements were recorded just after the bleaching procedures, except for both conventional walking bleaching groups, had been performed and on Days 1, 3, and 7. For standardization, the temporary filling material was not removed from the teeth in all of the groups. The color of each specimen was assessed by the CIE-Lab system in *L*a*b** mode using a spectrophotometer (Spectro Shade™ Micro) on the buccal surface of the crown by means of circular strip that was stack at the baseline color measurement. The spectrophotometer was calibrated at the beginning of the test procedure according to the manufacturer's recommendations.

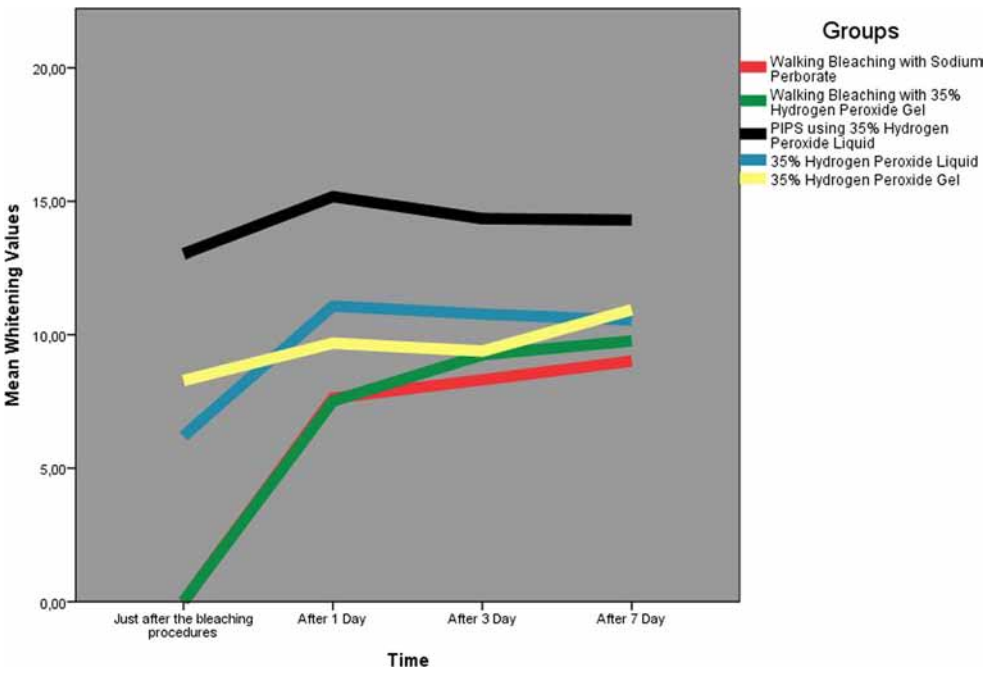

Fig. 2 Mean whitening values of the groups at the different times

The color measurements were performed thrice at each time point on a white background, and the mean of these measurements was calculated. The total color difference or distance between two colors (ΔE) was calculated using the formula below, where L^* represents the value of lightness/darkness, a^* represents the measurement along the red–green axis, and b^* represents the measurement along the yellow–blue axis:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

Statistical analysis

All statistical analyses were performed using software (SigmaStat for Windows Version 3.5; Systat Software Inc., Erkrath, Germany) at a significance level of 0.05 and confidence interval of 95 %. The data were subjected to statistical analysis using a two-way analysis of variance (ANOVA) considering two factors (bleaching procedures and time intervals). Tukey post hoc test was used for multiple comparisons.

Results

The data of two-way ANOVA revealed that the bleaching of the discolored teeth was significantly affected by the bleaching procedures ($P<0.001$) but not the factor of time interval ($P>0.05$). In addition, there was no significant interaction between the bleaching procedure and the time interval ($P>0.05$).

Figure 2 and Table 1 show the mean whitening values of the five groups at the four time intervals.

Immediately after the bleaching procedures, regardless of walking bleach groups the bleaching effect of the PIPS technique using 35 % hydrogen peroxide liquid was found to be superior to that of 35 % hydrogen peroxide liquid ($P<0.001$) and gel ($P=0.022$) applied for 30 min.

On Days 1, 3, and 7, the PIPS technique bleached specimens more than the walking bleaching techniques and the (non-PIPS) 35 % hydrogen peroxide gel and liquid ($P<0.05$). There were no statistically significant differences between the other groups ($P>0.05$) (Fig. 3).

Discussion

Activation of irrigating solutions with laser tips has recently become popular in endodontics [13–15]. The mechanism of laser-activated irrigation is based on the formation of bubbles [16]. When the energy of erbium lasers is absorbed by water, it causes evaporation [17, 18]. The vapor bubble starts to expand and form a void in front of the laser light. Assuming that this activation process may enhance the efficacy of the irrigating solution, the aim of the present study was to investigate the bleaching effect of PIPS using 35 % hydrogen peroxide on discolored teeth as compared with other internal bleaching techniques.

The main finding of this study was that the PIPS technique using 35 % hydrogen peroxide bleached specimens after 7 days more than did the walking bleaching techniques. In

Table 1 The mean (standard deviation) ΔE values of the groups at the time intervals

Groups	Time interval			
	Just after the bleaching	After 1 day	After 3 days	After 7 days
Walking bleaching with sodium perborate	N/a	7.62 (2.8) a	8.31 (3.43) a	9.01 (3.62) a
Walking bleaching with 35 % hydrogen peroxide	N/a	7.51 (2.68) a	9.25 (2.43) a	9.77 (2.61) a
PIPS using 35 % hydrogen peroxide liquid	13.03 (4.24) a	15.18 (4.23) b	14.35 (3.97) b	14.3 (3.47) b
Without PIPS 35 % hydrogen peroxide liquid	6.2 (3.83) b	11.08 (4.11) a	10.78 (3.99) a	10.55 (3.43) a
Without PIPS 35 % hydrogen peroxide gel	8.28 (3.36) b	9.68 (4.02) a	9.38 (3.7) a	10.94 (4.07) a

Different letters in the same column indicate statistically significant difference

N/a not applicable

addition, there were no statistically significant differences between walking bleaching techniques applied for 1 week and 35 % hydrogen peroxide liquid or gel applied for 30 min. Therefore, the null hypothesis that there is no difference between the PIPS technique using 35 % hydrogen peroxide and the other internal bleaching techniques is rejected.

In the PIPS technique, an Er/YAG laser is used with a radial and stripped tip of novel design at subablative power settings. Er/YAG laser irradiation is highly absorbed by hydroxyapatite and water [19, 20]. In the PIPS technique at low power, each impulse interacts with the water molecules, creating expansion and successive shock waves that lead to the formation of a powerful streaming fluid [21]. An interesting finding in the present study was that even immediately after the bleaching procedures had been performed, the bleaching effect of the PIPS technique using 35 % hydrogen peroxide liquid was superior to that of 35 % hydrogen peroxide liquid and gel applied for 30 min (i.e., without PIPS). This finding indicates that PIPS enhances the dentinal permeability of hydrogen peroxide in the short-term. However, as there is limited data on this in the literature, our finding should be confirmed in further studies.

In the present study, the specimens were stained using sheep's blood to simulate the clinical tooth discoloration. The specimens and sheep blood were placed in an Eppendorf tube and centrifuged to separate the blood in order to remove the plasma and yield the precipitate containing hemoglobin. This method was used as described by previous studies [9–11].

Lim et al. [2] artificially stained teeth using whole blood and bleached them using different bleaching agents. These authors found 35 % hydrogen peroxide

Fig. 3 Representative images of the specimens before and after the bleaching procedures, by group

to be more effective than sodium perborate for internal bleaching. In the present study, all of the hydrogen peroxide groups apart from the PIPS technique were not superior to the sodium perborate in terms of bleaching discolored teeth.

In in vitro bleaching efficacy studies, human maxillary incisors, human premolars, or bovine teeth were used [2, 7, 9, 10, 12]. In the present study, mandibular incisors were used to evaluate the bleaching effectiveness of PIPS using 35 % hydrogen peroxide. When compared with maxillary incisors, mandibular incisors are easy to obtain, and also, their diameters were suitable and easy to use for immersing in Eppendorf tubes for centrifugation. Previously, bleaching efficacy was evaluated using contact-type spectrophotometers whose measurement tips are suitable for wide teeth like maxillary incisors, premolars, or bovine teeth [7, 9]. However, the contact-type spectrophotometer is not suitable for mandibular incisors, which are smaller than the teeth aforementioned. In the present study, a noncontact-type spectrophotometer was used. The image was taken on the surface of tooth, and color was evaluated on this image (Fig. 1) [12]. The noncontact-type spectrophotometer could be beneficial for evaluating color changes in relatively smaller teeth.

It is well established that hydrogen peroxide is caustic, burning tissue on contact [1]. In the present study, therefore, a band was agglutinated above the endodontic access cavity to hinder hydrogen peroxide extrusion during the activation procedure. The PIPS technique in activating hydrogen peroxide was experimentally evaluated in the present study. Clinical usage cannot be suggested according to the present study, however, as further studies should be conducted to investigate further hindrance of hydrogen peroxide extrusion during the activation procedure.

Conclusions

Within the limitations of the in vitro study, the PIPS technique was more effective than both walking bleach techniques as well as both hydrogen peroxide groups. Bleaching materials have adverse effects, however, both are localized and systemic, such as cervical external resorption [22–24], reduction in microhardness of hard dental tissues [25], and toxicity [26]. Thus, further studies should be conducted to determine if the PIPS technique results in any complications, including cervical resorption.

Conflict of interest The authors declare that there are no conflicts of interests in writing this article.

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Effect of photon-initiated photoacoustic streaming on removal of apically placed dentinal debris

H. Arslan[1], I. D. Capar[2], G. Saygili[2], T. Gok[2] & M. Akcay[3]

[1]Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum; [2]Department of Endodontics, Faculty of Dentistry, Izmir Katip Celebi University, İzmir; and [3]Department of Pedodontics, Faculty of Dentistry, Izmir Katip Celebi University, İzmir, Turkey

Abstract

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Aim To compare the efficacy of photon-induced photoacoustic streaming (PIPS) technique with conventional, sonic and ultrasonic irrigation on the removal of apically placed dentinal debris from an artificial groove created in a root canal.

Methodology Root canal preparation was performed up to size 40 on 48 extracted single-rooted teeth using ProTaper rotary instruments. The specimens were then split longitudinally, and a standardized groove was prepared in the apical part of each segment. Each groove was filled with dentinal debris mixed with 5% NaOCl. Each tooth was reassembled and irrigated as follows: (i) conventional irrigation with 1% NaOCl, (ii) sonic, (iii) ultrasonic irrigation, and (iv) PIPS. The root segments were disassembled, and the amount of remaining dentinal debris was evaluated under a stereomicroscope at 20× magnification, using a four-grade scoring system. The data were evaluated statistically using Kruskal–Wallis and Mann–Whitney *U*-tests with a 95% confidence level ($P = 0.05$).

Results Photon-induced photoacoustic streaming removed significantly more dentinal debris than conventional irrigation ($P < 0.001$), sonic irrigation ($P < 0.001$) or ultrasonic irrigation ($P = 0.005$). There was no significant difference between sonic and ultrasonic irrigation ($P = 0.377$).

Conclusions Photon-induced photoacoustic streaming was more effective than conventional, sonic and ultrasonic irrigation in the removal of apically placed dentinal debris.

Keywords: EndoActivator, endodontics, photoacoustic streaming, PIPS, sonic, ultrasonic.

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Introduction

The goal of biomechanical preparation is to clean, shape and disinfect the root canal system. However, chemomechanical preparation leaves untouched zones, debris, the smear layer and microorganisms and their by-products, which can result in persistent inflammation (Vertucci 1984, Wu & Wesselink 2001b, Wu *et al.* 2001a). That is why irrigation plays an essential role in root canal treatment. However, because irrigating solutions can be ineffective in removing material from the root canal walls (Torabinejad *et al.* 2003, Mancini *et al.* 2009), improved irrigation agitation methods such as sonic and ultrasonic devices have been proposed (Guerisoli *et al.* 2002). Recently, agitation of irrigants using lasers has gained popularity (De Moor *et al.* 2009, de Groot *et al.* 2009, Moon *et al.* 2012).

A novel laser agitation technique, photon-induced photoacoustic streaming (PIPS), has been proposed. This technique differs from other agitation techniques in that only the tip is placed into the canal orifice

Correspondence: Hakan Arslan, Department of Endodontics, Faculty of Dentistry, Atatürk University, Erzurum 25240, Turkey (Tel.: +90.442.231.1900; fax: +90.442.236.0945; e-mail: dt_hakan82@hotmail.com).

(DiVito & Lloyd 2012a). In this technique, an erbium/yttrium–aluminium–garnet (Er/YAG) laser is used with a radial and stripped tip of novel design at subablative power settings (0.3 W). Although it has been shown that this technique has no additional benefit for the reduction in bacteria from the root canals (Peters *et al.* 2011, Pedulla *et al.* 2012, Zhu *et al.* 2013), DiVito *et al.* (2012b) demonstrated that it results in significantly better removal of the smear layer. Peeters & Suardita (2011) used a plain fibre tip to activate the irrigating solution in the pulp chamber and demonstrated that the use of a laser with a plain fibre tip can produce cavitation in the irrigant and has potential as an improved alternative method for the removal of the smear layer. In another study, it was reported that the plain fibre tip in the pulp chamber can drive the irrigation solution to the end of the canal without harming the apical tissues (Peeters & Mooduto 2013).

Previously, it has been shown that large amounts of debris remain in root canal irregularities after the use of conventional syringe irrigation (Goodman *et al.* 1985, Wu & Wesselink 2001b). If these untouched zones with debris remaining after conventional techniques are not well cleaned, it is not possible to provide direct contact for the medicaments with bacteria or to fill the root canal completely (Siqueira & Lopes 1999). There is limited information on PIPS and its ability to remove dentinal debris from root canals. Thus, the aim of this study was to compare the efficacy of PIPS in removing dentinal debris from an artificial groove created in the apical third of the root canals. The null hypothesis was that there is no difference between PIPS and the other irrigation techniques.

Materials and methods

A total of 48 single-rooted, noncarious, freshly extracted, maxillary human anterior teeth with fully formed apices were used. Soft tissues and calculus were mechanically removed from the root surfaces with a periodontal scaler. The teeth were verified radiographically as having a single root canal without calcification. The teeth were then stored in distilled water at room temperature until use. Specimens were decoronated with a diamond disc under water coolant to obtain a standardized root length of 13 mm. Root canal shaping procedures were performed with ProTaper rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to an F4 (size 40, .06 taper) master apical file size. Root canals were irrigated with 2 mL 1% NaOCl (ImidentMed, Konya, Turkey) between instrument changes. All the specimens were grooved longitudinally on the buccal and lingual surfaces with a diamond disc under copious water irrigation, avoiding penetration into the root canal. The roots were then split into two halves with a small chisel.

Next, a standardized longitudinal groove (3 mm in length, 0.5 mm in width and 0.2 mm in depth) was cut into the root canal wall of one half of each tooth at a distance of 2–5 mm from the apex to simulate an uninstrumented canal extension in the apical region. A toothbrush was used to remove debris from the root halves and grooves. A final flush was applied using 5 mL of 17% EDTA for 1 min and 5 mL of 2.5% NaOCl for 1 min. The root canals were then dried with paper points.

Dentinal debris application

To obtain dentine powder, a number of teeth were split longitudinally and dentinal debris was obtained using round burs. The debris was mixed with 5% NaOCl 5 min before use. The standardized grooves were filled with dentinal debris using a spreader. The root halves were reassembled, and all gaps along the tooth and the apices were sealed with wax to prevent the overflow of the irrigating solution and to create a closed-end channel so as to obtain a vapour lock effect (Alfredo *et al.* 2009, Pedulla *et al.* 2012). The specimens were divided randomly into four groups ($n = 12$) and irrigated as follows:

Conventional irrigation: 6 mL of 1% NaOCl via a size 27 gauge blunt-tip needle (Ultradent, South Jordan, UT, USA) was used for 1 min. The needle was inserted into the root canal within 1 mm of the working length without binding. The flow rate of the irrigating solution was 0.1 mL s^{-1}.

Sonic irrigation: 0.5 mL of 1% NaOCl was flushed into the root canal using a needle; a red (size 25, .04 taper) sonic tip was then inserted 2 mm short of the working length, and the sonic handpiece (EndoActivator; Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) was activated for 1 min at 10 000 cycles min^{-1} (Klyn *et al.* 2010). During the activation procedure, irrigation was gently continued through the root canal opening using 5.5 mL of irrigating solution.

Ultrasonic irrigation: 0.5 mL of 1% NaOCl was placed into the canal as in the sonic group; a smooth ultrasonic file (size 15, .02 taper) was then inserted

1 mm short of the working length (Lee *et al.* 2004, van der Sluis *et al.* 2005, Rodig *et al.* 2010), and the ultrasonic device (Anthos u-PZ6, Imola, Italy) was activated for 1 min at 25% power. During activation, irrigation was gently continued through the root canal opening using 5.5 mL of irrigating solution.

Pips: dentinal debris was removed using the laser irradiation protocol, which was performed by an Er/YAG laser with an emission wavelength of 2940 nm (Fidelis AT, Fotona, Ljubljana, Slovenia). A 14-mm-long and conical, cylindrical (tapered) 300-μm fibre tip was applied at 0.3 W, 15 Hz and 20 mJ per pulse. The water and air on the laser system were turned off. Then, 0.5 mL 1% NaOCl was placed into the root canal, and the optical fibre was placed approximately 1 mm below the root canal orifice. When the irrigating solution in the coronal reservoir decreased, the supplemental NaOCl was applied through the root canal opening. The laser activation was continued during the placement of irrigant. The total activation time was 1 min, and the total volume of 1% NaOCl was 6 mL.

For all groups, the total volume of 1% NaOCl was 6 mL and the exposure time to 1% NaOCl was 1 min. The root canals were dried with paper points, and the roots were disassembled to evaluate the removal of the dentinal debris. Digital images at 20× magnification were obtained using a stereomicroscope (Olympus BX43; Olympus Co., Tokyo, Japan) attached to a digital camera and were transferred to the computer. The digital images were coded to avoid identifying the specimens. Two calibrated observers were blinded to the technique used to remove dentinal debris. The amount of dentinal debris remaining in the grooves was scored using the following scoring system, described by van der Sluis *et al.* (2007):

0: Groove was empty;

1: Dentinal debris was present in less than half of the groove;

2: Dentinal debris covered more than half of the groove;

3: The groove was completely filled with dentinal debris;

Photographs were evaluated by the observers 1 week later, and the Kappa test was used to analyse interexaminer agreement. The differences in the dentinal debris scores among the different groups were analysed with Kruskal–Wallis and Mann–Whitney *U*-tests. Testing was performed at the 95% confidence level ($P = 0.05$). All statistical analyses were performed using IBM® SPSS® Statistics 20 software (IBM SPSS Inc., Chicago, IL, USA).

Results

The scores for the dentinal debris remaining in the grooves for all groups are shown in Fig. 1. The Kruskal–Wallis test revealed significant differences between

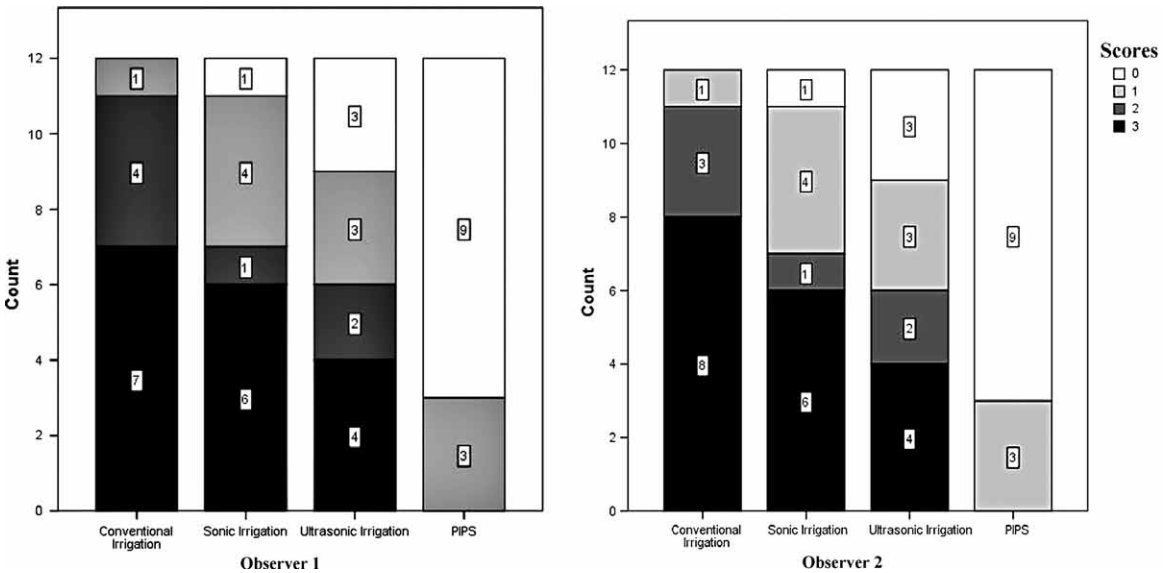

Figure 1 Distribution of scores for removal of apically placed dentinal debris after agitation with different protocols according to Observers 1 and 2.

the groups ($P < 0.001$). The Mann–Whitney *U*-test revealed that PIPS removed more dentinal debris than conventional irrigation ($P < 0.001$), sonic irrigation ($P < 0.001$) and ultrasonic irrigation ($P = 0.005$). In the PIPS group, the majority (75%) of specimens were assessed to be totally free from debris. The percentages of complete removal of dentinal debris (Score 0) for conventional, sonic and ultrasonic irrigation techniques were 0%, 8.3% and 25%, respectively. Conventional irrigation had the most remaining debris, although there were no significant differences between conventional irrigation and sonic ($P = 0.309$) and ultrasonic irrigation ($P = 0.061$). There was also no significant difference between sonic and ultrasonic irrigation ($P = 0.377$). Intraindividual reproducibility was 98% (47/48) for each examiner. The reliability between the examiners was good (κ value = 0.971), and the difference between the matched scores never exceeded one unit.

Discussion

This study compared the removal of apically placed dentinal debris with conventional, sonic and ultrasonic irrigation to that obtained using PIPS. PIPS removed more debris compared with the other agitation techniques. Therefore, the null hypothesis that there is no difference between PIPS and the other irrigation techniques can be rejected.

DiVito *et al.* (2012b) demonstrated that laser-activated irrigation using PIPS tips resulted in a significantly better cleaning of the root canal walls in comparison with the conventional irrigation procedures. In a recent study, Lloyd *et al.* (2013) also showed that laser-activated irrigation using PIPS tips eliminated organic debris from canal isthmus at a significantly greater level compared with standard needle irrigation. The results of the present study revealed that laser-activated irrigation with PIPS tip had a positive effect in removing dentinal debris from an artificial groove created in the apical third of the root canals, and this result is harmonious with those of aforementioned studies.

Bubbles, the formation of an empty space in a liquid, are the basis of cavitation. Er/YAG laser irradiation is highly absorbed by hydroxyapatite and water (Paghdiwala 1991, Armengol *et al.* 1999). When Er:YAG laser irradiation is absorbed by water, the energy causes evaporation (Brugnera *et al.* 2003, Kivanc *et al.* 2008). The vapour bubble starts to expand and form a void in front of the laser light. Matsumoto *et al.* (2011) demonstrated that the bubble increased in size and reached up to 1800 μm in 220 microseconds when a 300 μm laser tip was used, as in the present study. They stated that when the laser tip was inserted 2 and 5 mm short of the bottom of an artificial glass root canal model, the second cavitation bubbles were clearly observed at the bottom of the artificial root canal. Therefore, they suggested that it is not always necessary to insert the laser tip up to the terminus of the canal, because the cavitation bubbles also assist in cleaning the apical region. In the present study, this finding has been confirmed. The PIPS optic tip was inserted only in the coronal part of the root canals, and the apically placed dentinal debris was effectively removed.

Photon-induced photoacoustic streaming tips have been used at subablative levels with specific models and settings and with a radial and stripped tip of novel design. This technique uses low energy levels and short microsecond pulse rates (50 μs) to generate peak power spikes. The profound photoacoustic shock wave it induces facilitates three-dimensional movement of the irrigation solutions (DiVito & Lloyd 2012a). Previous studies have shown that the use of erbium lasers in the root canal may result in side effects. Matsuoka *et al.* (2005) observed carbonization and cracks on the root canal walls when the laser tips were used for root canal preparation. Kimura *et al.* (2002) monitored a temperature increase of up to 6 °C. The subablative parameters in the PIPS technique result in a photomechanical effect, which occurs when the light energy is pulsed in a fluid, rather than thermal effect (Peters *et al.* 2011, DiVito *et al.* 2012b).

The traditional laser applications necessitate conventional preparation for at least up to size 30 and the laser tip need to reach apical third of the root. However, the PIPS tip does not need to reach the canal terminus, and it is placed into the coronal reservoir only of the root canal. Therefore, this technique allows for minimally invasive preparation of the root canal (DiVito & Lloyd 2012a, DiVito *et al.* 2012b). The effect may be explained by the increased NaOCl reaction kinetics with laser activation (de Groot *et al.* 2009, Macedo *et al.* 2010).

Both PIPS and ultrasonic irrigation techniques are based upon the transmission of acoustic energy to an irrigant in the root canal space (Ahmad *et al.* 1987, DiVito *et al.* 2012b). The acoustic streaming effect of the irrigant in the ultrasonic has been shown to be more effective than syringe irrigation in

removing artificially created dentine debris placed in simulated uninstrumented extensions and irregularities in root canals (Lee *et al.* 2004). In a recent study, De Moor *et al.* (2010) evaluated the efficacy of laser-activated irrigation with erbium lasers and passive ultrasonic irrigation in terms of removing artificially placed dentine debris in root canals. They showed that the application of the laser-activated irrigation technique for 20 s was as efficient as passive ultrasonic irrigation for 3 × 20 s. Similarly, de Groot *et al.* (2009) revealed that laser-activated irrigation was significantly more effective in removing dentine debris from the apical part of the root canal than passive ultrasonic irrigation when the irrigant was activated for 20 s. In the present study, laser-activated irrigation with PIPS tip removed more dentinal debris than ultrasonic irrigation. This result can be explained by the high amounts of energy being transferred to the irrigant with laser activation compared with passive ultrasonic irrigation (de Groot *et al.* 2009).

Conclusion

Photon-induced photoacoustic streaming technique was significantly more effective than both sonic and ultrasonic irrigation in removing apically placed dentinal debris.

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ORIGINAL ARTICLE

Effectiveness of the erbium:YAG laser and new design radial and stripped tips in removing the smear layer after root canal instrumentation

E. DiVito · O. A. Peters · G. Olivi

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Abstract The aim of this study was to analyze in vitro the debriding ability of an Er:YAG laser system (2,940 nm) equipped with a newly designed radial and stripped tip of 400 μm diameter by scanning electron microscopy (SEM). A total of 80 single-rooted extracted human teeth were endodontically prepared with rotary instrumentation and standardized chemical irrigation using 5.25% sodium hypochlorite. At the end of mechanical instrumentation, four different final protocols were used. Group 1 was irrigated for 2 min with saline water as a control group. Groups 2, 3 and 4 were irradiated with an Er:YAG laser at 25 mJ and 15 Hz with a pulse duration of 50 μs and laser spray off using the tip in the coronal opening of the wet root canal. Different solutions and irradiation times were used: *group 2* 20 s, laser irradiation in sterile distilled water, wet canal; *group 3* 20 s, laser irradiation in 17% EDTA, wet canal; and *group 4* 40 s, laser irradiation in 17% EDTA, wet canal. Debridement of and smear layer removal from the apical third of root canals were evaluated by SEM. The study showed that standardized instrumentation, followed by a final Er:YAG laser irradiation in wet canals with EDTA irrigation resulted in more cleaning of the root canal walls and a higher quantity of open tubules in comparison with the traditional irrigation method.

Keywords Erbium:YAG · Laser · Smear layer · EDTA

E. DiVito
Private Practice, Arizona Center for Laser Dentistry and faculty, Arizona School of Dentistry and Oral Health,
Scottsdale, AZ, USA

O. A. Peters
Department of Endodontics, University of the Pacific Arthur A, Dugoni School of Dentistry,
San Francisco, CA, USA

G. Olivi
Department of Science and Technology of Biophysics, Medicine and Odontostomatology, University of Genoa,
Genoa, Italy

G. Olivi (✉)
Piazza F. Cucchi, 3,
00152, Rome, Italy
e-mail: olivi.g@tiscali.it

Introduction

The ability to successfully treat and remove the smear layer and bacteria continues to be a challenge in nonsurgical endodontic treatment of the root canal system. The shaping and cleaning of root canals is a key step during root canal treatment and unless all remnants of debris are removed, subsequent stages of obturation may also be jeopardized [1, 2]. Clinically, endodontic procedures use both mechanical instrumentation and chemical irrigants in the attempt to three dimensionally debride, clean and decontaminate the endodontic system [3, 4]. Some of these irrigation techniques include manual irrigation with needles and canulas, and the use of machine-assisted agitation systems and sonic and ultrasonic energy sources [5]. All file systems generate a smear layer and leave debris in the root canal. Irrigation with 5.25% sodium hypochlorite alone is unable to remove debris and the smear layer [6]. Other irrigants such as 2% chlorhexidine gluconate, 17% ethylene diamine tetraacetic acid (EDTA) and 10% citric acid have been used to help remove debris, but many studies have demonstrated the limited ability to effectively reach all internal faces of seemingly complicated root canal architecture [1, 2, 4, 6–8]. Although a recent study

[9] has shown excellent ability of a new file system operated with a continuous irrigation device to remove debris and the smear layer also from the apical third of the root canal, the literature shows that when compared to the coronal and middle thirds of relatively clean canals, the apical third of the root canal always presents a problem in regard to the ability to achieve the same cleanliness [5, 6, 10–12]. This fact may be of significance during root canal treatment because the presence of a smear layer and debris may prevent sealer adaptation to the canal walls and impede penetration of irrigants into dentinal tubules and accessory canals. Accordingly, some alternative, more effective method to debride, clean and penetrate the dentinal walls should be explored.

The effectiveness of lasers in dentistry continues to be an area of discussion. Although the use of lasers for nonsurgical endodontic treatment of the root canal system has been reported since the early 1970s [13, 14], acceptance has been slow. A common feature of dissatisfaction has been the thermal damage associated with the application of laser photonic energy [15–19]. Laser treatment can be a valuable tool for the removal of the dentinal smear layer, as a debridement device during endodontic treatment. The Er:YAG laser (wavelength 2,940 nm) is approved by the FDA for cleaning, shaping and enlarging the root canal [20]. Previous studies have tested the ability and the effects of this laser on root canal walls and have indicated that the Er:YAG laser is a suitable instrument for removal of the smear layer in root canals [21–26]. Furthermore, George et al. in an investigation of the ability of both the Er:YAG and Er,Cr:YSGG lasers equipped with conical shaped radially firing tips and plain tips to remove the smear layer from the apical third of the root canal showed a laser activation of EDTA and a better performance of conical fibers compared to plain fibers for improving the action of EDTAC in dissolving smear layer [27].

The aim of this in vitro study was to evaluate by scanning electron microscopy (SEM) the ability and effectiveness of the Er:YAG laser in removing the smear layer and debriding the root canal. A newly designed tip with a tapered radial firing end and 3 mm of the polyamide sheath removed was used. Using specific pulse rates, a short microsecond pulse duration and low energy during application, the thermal morphological effects described in the literature were minimized [21–29].

Materials and methods

Sample preparation

In this study, 80 recently extracted single-rooted human teeth were used. They were stored in physiological saline solution until use.

Root canal treatment

The access cavity to the canal orifice was first prepared with a tapered diamond bur creating a glide path for insertion of the first instrument (size #10 K file). The teeth were then minimally prepared using nickel/titanium rotary instruments in a sequential crown down method to a size 20/.06 (Profile GT; Dentsply Tulsa Dental, Tulsa, OK). The canals were irrigated during preparation with sodium hypochlorite. After reaching the final instrumentation size of 20/.06, an additional two 30-s cycles of irrigation with saline only were applied. The samples were then ready to be treated with the various laser protocols described.

Laser parameters

An Er:YAG laser with a wavelength of 2,940 nm (Fidelis; Fotona, Ljubljana, Slovenia) was used to irradiate the root canals after traditional instrumentation. A newly designed 12-mm long 400-μm quartz tip was used. The tip, as received directly from the manufacturer, was tapered and had 3 mm of the polyamide sheath stripped back from its end (Fig. 1a). The laser operating parameters used for all of the treatment groups (using the free-running emission mode) were as follows: 20 mJ per pulse, 15 Hz, and 50 μs pulse duration. The coaxial water spray feature of the handpiece was set to 'off'. The tip was placed into the coronal access opening of chamber only, and was kept stationary and not advanced into the orifice of the canal (Fig. 1b).

Laser irradiation and irrigation methods

After the mechanical preparation, the teeth were randomly divided into four groups (20 teeth each) and treated according to the following protocol:

Group 1	Saline water irrigation for 2 min as control group
Group 2	Laser irradiation, 20-s cycle in sterile distilled water
Group 3	Laser irradiation, 20-s cycle in 17% EDTA
Group 4	Laser irradiation, 40-s cycle in 17% EDTA

During the laser irradiation cycles, the root canals were continuously irrigated with 2 ml of fluid to maintain hydration and levels using a hand syringe with a 25 gauge needle positioned above the laser tip in the coronal aspect of the access opening, accordingly to the above protocol.

Temperature measurements

To identify possible thermal side effects, the temperature changes on the external root surface of three teeth in each laser group (for a total of nine teeth) were measured.

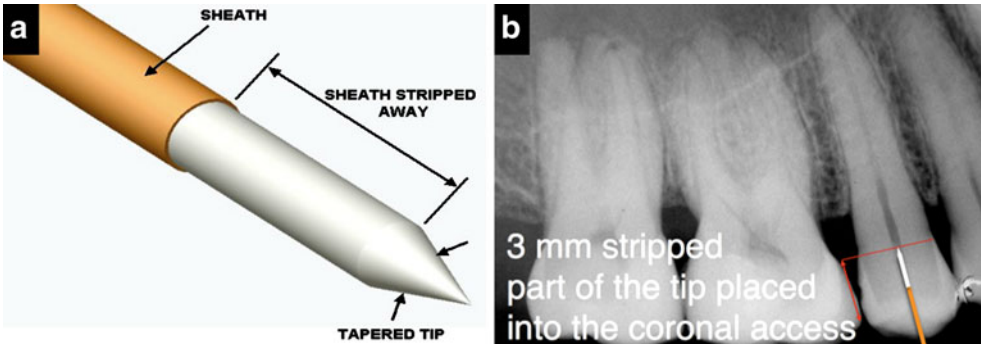

Fig. 1 **a** Diagram of the radial and stripped tip used in this laser study (courtesy of Medical Dental Advanced Technology Group). **b** Radiograph showing positioning of radial and stripped tip placed into the coronal access opening of the fluid filled chamber only and not advanced into the orifice of the canal

A modified thermocouple measurement sensor of 1.5 mm diameter (K-Type NiCr-Ni immersion sensor; TEL-Atomic, Jackson, MI) was placed on the root surface and attached with a silicon-based heat-conductive compound (340 heat sink compound; Dow Corning, Midland, MI) 5 mm from the apex. The temperature changes were monitored continuously throughout all the irradiation procedure periods (20 s for groups 2 and 3, and 40 s for group 4) starting from a room temperature of 21°C and recorded using a digital thermometer (digital quick response pocket thermometer; TEL-Atomic). The average value and the standard deviation of the three measurements per laser group were calculated. The temperatures were digitally displayed on the thermometer and subject to sensor errors of ±0.2°C.

Scanning electron microscopy

A F4000 field emission scanning electron microscope (Hitachi, Tokyo, Japan) was used. The prepared samples were sectioned longitudinally, dried, sputter-coated and only the apical third of the root canal (5 mm) examined. More than 150 photographs were taken at various magnifications ranging from ×300 to ×10,200 by the same operator and were evaluated by two additional blinded observers.

Quantitative evaluation

The smear layer was defined as the film retained on the dentin surfaces after application of the nickel/titanium rotary instruments. A scoring method for smear layer removal suggested by Hülsmann et al. was applied [10]. The three observers evaluated the amount of remaining smear layer. SEM images at magnifications in the range ×1,000 to ×2,000 were used for this quantitative assessment. A mean smear layer score was calculated for each specimen. The overall agreement of the observers was very good as indicated by a Fleiss' kappa of 0.82. A scoring index of 1 through 5 was used as described below:

Score 1	No smear layer; dentinal tubules open
Score 2	Small amount of smear layer; many dentinal tubules open
Score 3	Homogeneous smear layer covering the root canal walls; only a few dentinal tubules open
Score 4	Complete root canal wall covered by a homogeneous smear layer; no dentinal tubules open
Score 5	Heavy, nonhomogeneous smear layer completely covering root canal walls

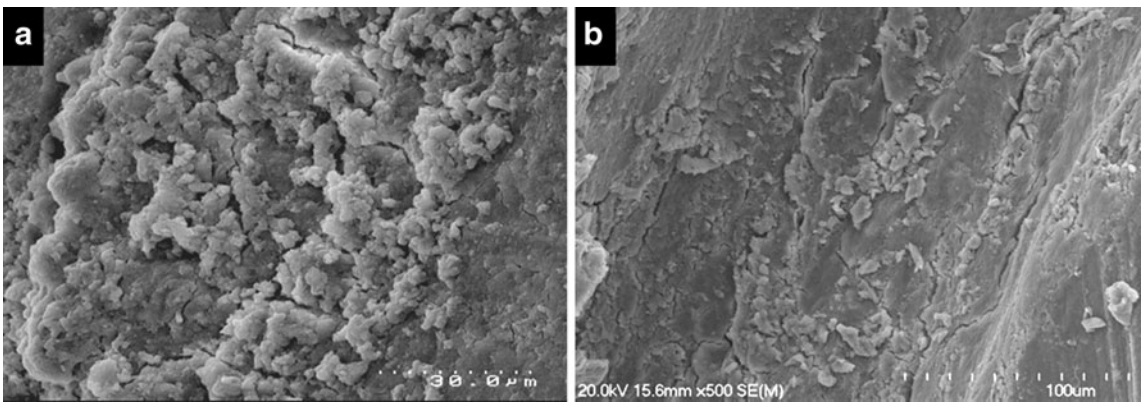

Fig. 2 Group 1. Representative images of a root canal wall (**a** ×1,220. **b** ×300) after a 2-min saline water flush. The canal surface shows a noticeable smear layer and occluded dentinal tubules. Smear layer score 5

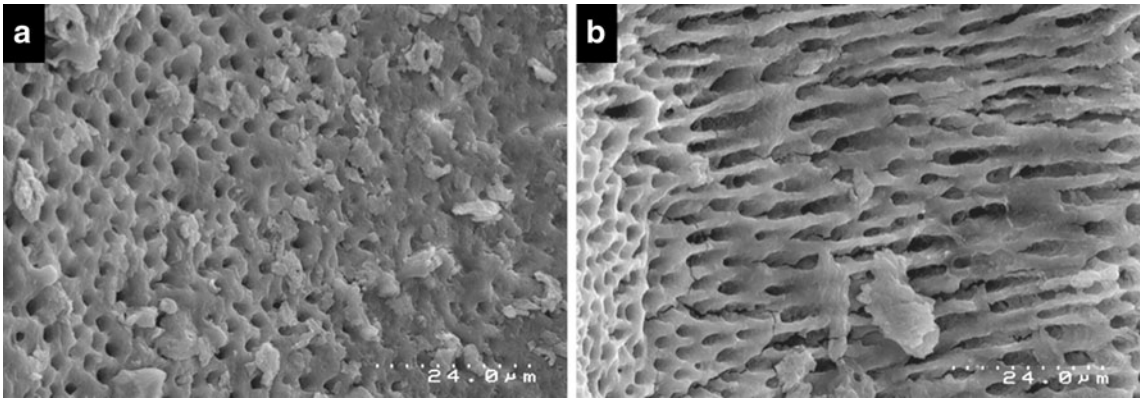

Fig. 3 Group 2. Representative images of a root canal wall (×1,680) after Er:YAG laser irradiation (20 mJ per pulse, 15 Hz, 50 μs pulse duration) for 20 s in sterile distilled water (wet canal). The canal surface shows open tubules, residual debris and a smear layer still present. Smear layer score 3

The resulting data were nonparametric in nature and hence statistical analysis was performed using the Kruskal-Wallis and the Mann-Whitney Wilcoxon U tests; a level of $p<0.05$ was considered statistically significant.

Results

SEM observations

Control group specimens (group 1) consistently exhibited a thick smear layer. SEM examination demonstrated that when only water irrigation was applied, a noticeable smear layer and occluded dentinal tubules remained on the treated surface (Fig. 2). Debris, defined as dentin chips and pulp remnants loosely attached to the internal surface of the root canals, was present in specimens of group 1.

Group 2 specimens treated for 20 s with the Er:YAG laser together with irrigation with sterile distilled water showed improved cleaning compared to group 1 specimens. The root canal surfaces exhibited open tubules, scattered residual debris and a thinner smear layer compared to the group 1 (control) specimens (Fig. 3).

Group 3 specimens treated for 20 s with the Er:YAG laser together with EDTA irrigation showed improves cleaning and debridement compared to group 2 specimens and group 1 (control) specimens (Fig. 4). Group 4 specimens treated for 40 s with the Er:YAG laser together with EDTA irrigation showed the most effective removal of the smear layer from the root canal walls (Fig. 5). SEM images at higher magnifications (from 3600X to 10200X) showed exposed and intact collagen fibers and evidence of an unaltered collagen matrix (Fig. 6). None of the SEM micrographs indicated signs of dentin fusion from excessive heat.

Quantitative evaluation

To quantify the differences in smear layer removal, a five-step scoring method was used. The scores of all laser-treated groups differed significantly from each other and from that of the control group. Group 1, the control group, had the highest (i.e. least acceptable) mean score, and

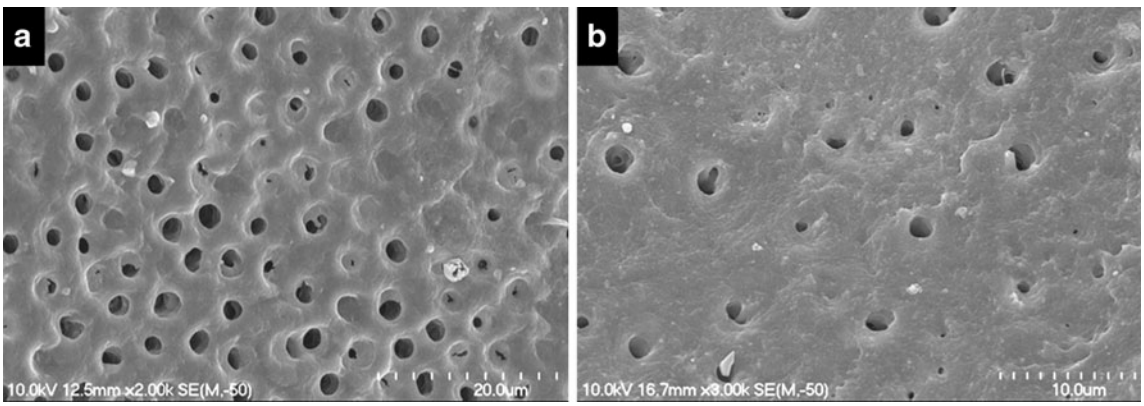

Fig. 4 Group 3. Representative images of a root canal wall (**a** ×1,820, **b** ×2,470) after Er:YAG laser irradiation (20 mJ per pulse, 15 Hz, 50 μs pulse duration) for 20 s in 17% EDTA (wet canal). The root canal surface shows significantly better cleaning and debridement than group 1 (control) specimens. Smear layer score 2

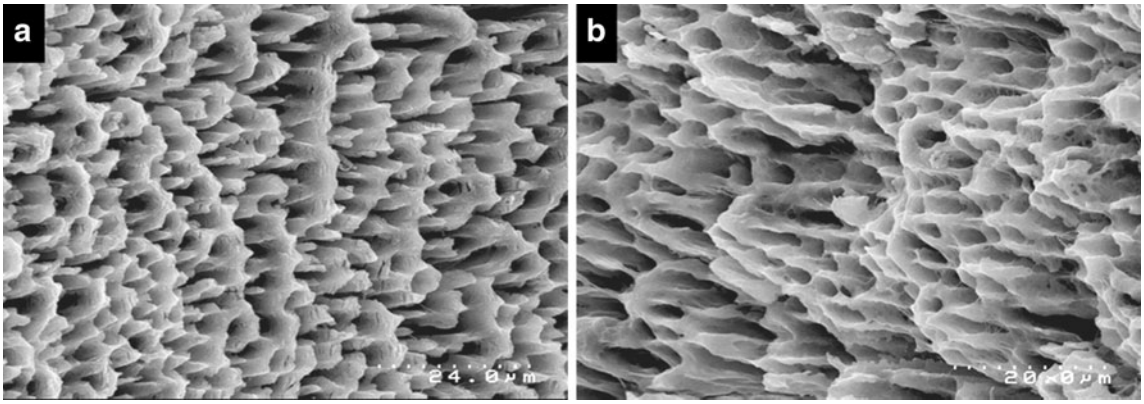

Fig. 5 Group 4. Representative images of a root canal wall (**a** ×1,680, **b** ×1,820) after Er:YAG laser irradiation (20 mJ per pulse, 15 Hz, 50 μs pulse duration) for 40 s in 17% EDTA (wet canal). The root canal surface shows effective removal of the smear layer. Smear layer score 1

groups 2 through 4 had progressively lower (i.e. more acceptable) mean scores (Table 1).

The significance of differences in the cleanliness of the root canal wall between the groups were determined using nonparametric tests (Tables 1 and 2). The Kruskal-Wallis test showed an overall significant difference among the four groups ($p<0.001$). A subsequent pair-wise comparison showed statistical significant differences in smear layer removal from the apical third of root canal walls between the groups ($p<0.001$).

Temperature measurements

Minimal average temperature increases were observed at the root surface during laser irradiation, with increases of 1.2°C and 1.5°C in the 20-s and 40-s irradiation time groups, respectively.

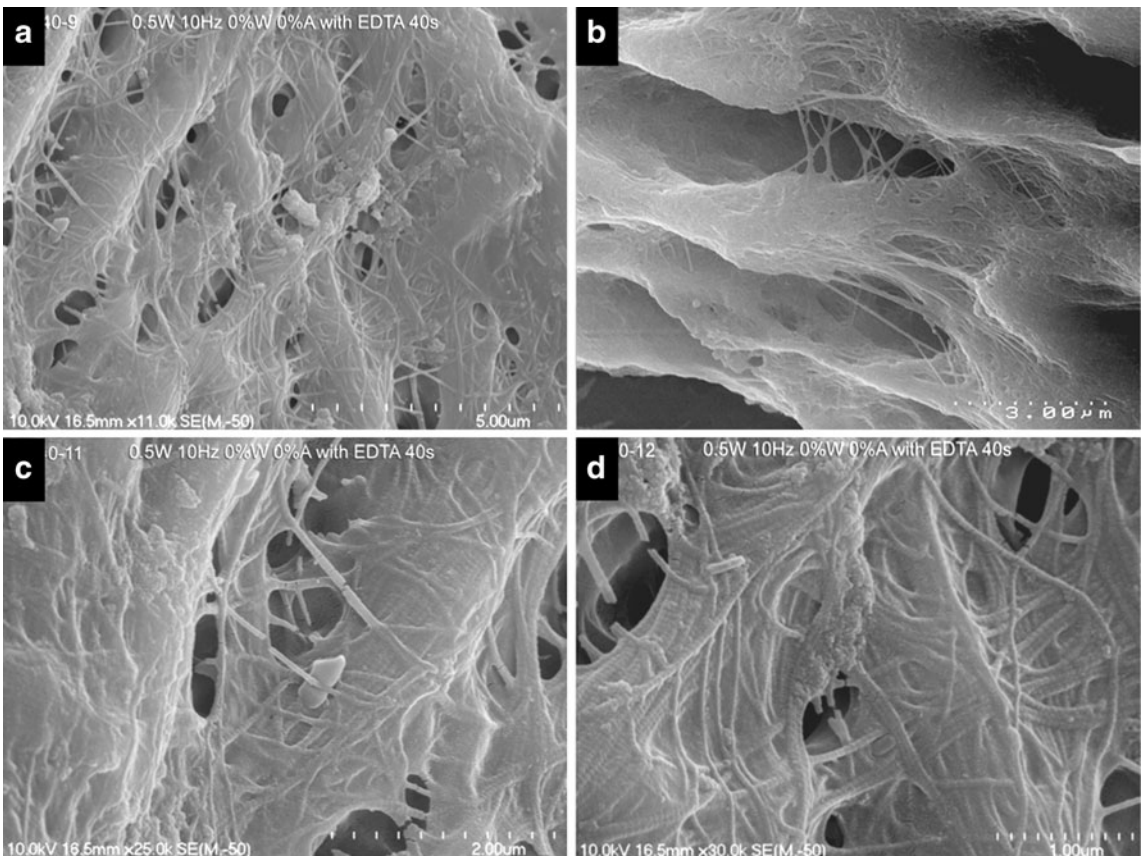

Fig. 6 Group 4, representative sample images at apical third; Er laser irradiation (20 mJ per pulse, 15 Hz, 50ms pulse duration) 40s in 17% EDTA wet canal. SEM at higher magnifications (from 3600X to 10200X) shows exposed and intact collagen fibers and evidence of an unaltered collagen matrix. Smear layer score 1

Table 1 Results of Kruskal-Wallis tests of differences in mean ranks among the study groups

Group	Treatment	*n*	Mean rank of scores	Kruskal-Wallis chi-squared	df	*p*
1 (control)	2-min saline water flush	20	70.45	66.069	3	<0.001
2	Laser irradiation, 20-s cycle in sterile distilled water wet canal	20	46.18			
3	Laser irradiation, 20-s cycle in 17% EDTA wet canal	20	31.68			
4	Laser irradiation, 40-s cycle in 17% EDTA wet canal	20	13.70			

Discussion

Current instrumentation techniques using rotary instruments and chemical irrigation still fall short of successfully removing the smear layer from inside the root canal system. This was confirmed by the results seen in the control group (group 1) where the conventional technique was employed.

The Er:YAG laser used in this investigation was equipped with a novel 400 μm diameter radial and stripped tip. Using subablative parameters (average power 0.3 W, 20 mJ at 15 Hz) proved to be more effective than traditional techniques at removing the smear layer. This finding could be attributed to the photomechanical effect seen when light energy is pulsed in liquid [30–32]. 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 μs), resulted in a photomechanical phenomenon. We speculate that this phenomenon was responsible for the removal of the smear layer in group 2, in which laser irradiation was combined with saline, which alone does not affect the smear layer [10–12]. A profound "shockwave-like" effect is observed when radial and stripped tips are submerged in a liquid-filled root canal. As a result of the very small volume, this effect may remove the smear layer and residual tissue tags and potentially decrease the bacterial load within the tubules and lateral canals [28, 29, 33]. By using lower subablative energy (20 mJ) and restricting the placement of the tip to within the coronal portion of the tooth only, the undesired effects of the thermal energy, previously described in the literature, was avoided [22–26].

In the current study the smear layer and debris were not removed by thermal vaporization, but probably by photomechanical streaming of the liquids, which were laser activated in the coronal part of the tooth. The authors describe this light energy phenomenon as photon induced photoacoustic streaming (PIPS). The effect of irradiation with the Er:YAG laser equipped with a tip of novel design at subablative power settings (0.3 W, 20 mJ) is synergistically enhanced by the presence of EDTA; this leads to significantly better debridement of the root canal contributing to an improvement in treatment efficacy.

The SEM images verified the efficient and minimally disruptive effects on the canal walls, dentinal tubules and even the hydroxyapatite surfaces. No thermal damage was seen in any PIPS-treated samples and temperature increases at the external root surfaces were minimal (<1.5°C). Furthermore, the laser energy activates the EDTA solution, amplifying its surface cleaning action [27]. However, at high magnification, the intertubular dentin around tubular openings appeared to show some signs of erosion with the dentin collagen architecture visible and intact (Fig. 6).

Table 2 Results of pair-wise comparison between the mean ranks of the groups

Pair-wise comparison	*n*	Mean rank	Sum of ranks	Mann-Whitney *U*	Distribution mean	*Z*	*p*
Group 1 (control)	20	30.50	610.00	0.000	200	−5.724	<0.001
Group 2	20	10.50	210.00				
Group 1 (control)	20	30.45	609.00	1.00	200	−5.696	<0.001
Group 3	20	10.55	211.00				
Group 1 (control)	20	30.50	610.00	0.000	200	−5.724	<0.001
Group 4	20	10.50	210.00				
Group 2	20	26.98	539.50	0.000	200	−3.728	<0.001
Group 3	20	14.03	280.50				
Group 2	20	29.70	594.00	16.000	200	−5.236	<0.001
Group 4	20	11.30	226.00				
Group 3	20	28.10	562.00	48.000	200	−4.350	<0.001
Group 4	20	12.90	258.00				

With conventional treatment protocols (without a laser), an irrigation syringe is more effective when the tip is placed closer to the working length. With this new laser system, the laser tip is not placed within the canal itself, but is rather confined to the coronal chamber above the orifice. It is suggested that this allows easy access for the photomechanical effects to occur within the root canal, which may assist in cleaning canals of various shapes.

A standard ISO size #30 file preparation is needed to allow traditional laser tips (200–320 μm) to reach close to the apex [28, 29, 33]. Using the radial and stripped design with PIPS, the apex can be reached without the need to negotiate the tip close to the apex. Correspondingly, this would allow a less-invasive preparation using an ISO size #20/.06 file, according to the method described.

Irrigation with chelating agents following the current conventional instrumentation procedure requires more time to initiate a satisfactory debridement (EDTA placed passively into the prepared root canal) [11, 34]. The PIPS technique resulted in pronounced smear layer removal when used together with EDTA and at the settings outlined.

Published material on endodontic techniques using the Er:YAG laser provides differing operating parameters [35]. These authors recommend the use of higher average power (1.125–1.5 W) delivered through end-firing laser tips. Additionally, these tips need to be placed 1–2 mm from the root apex.

Conclusion

The Er:YAG laser used in this study showed significantly better smear layer removal than traditional syringe irrigation. At the energy levels and with the operating parameters used, no thermal effects or damage to the dentin surface was observed. In this study the Er:YAG laser with the current settings produced a photomechanical effect demonstrating its potential as an improved alternative method for debriding the root canal system in a minimally invasive manner.

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Tissue Dissolution Ability of Sodium Hypochlorite Activated by Photon-initiated Photoacoustic Streaming Technique

Mehmet Burak Guneser, DDS, PhD, Dilara Arslan, DDS, PhD,* and Asliban Usumez, DDS, PhD†*

Abstract

Introduction: The aim of this study was to evaluate the effect of the photon-initiated photoacoustic streaming (PIPS) technique on the pulp tissue-dissolving capacity of sodium hypochlorite (NaOCl) and compare it with the EndoActivator System (Dentsply Tulsa Dental Specialties, Tulsa, OK) and the Er:YAG laser with an endodontic fiber tip. **Methods:** Bovine pulp tissue samples (45 ± 15 mg) and dentin powder (10 mg) were placed in 1.5-mL Eppendorf tubes with 1 mL 5.25% NaOCl (Wizard; Rehber Kimya, Istanbul, Turkey) or distilled water (control) for 5 minutes with activation by the EndoActivator System, the Er:YAG laser with an endodontic fiber tip, and the PIPS technique. Nonactivated NaOCl served as the positive control. All testing procedures were performed at room temperature. The tissue samples were weighed before and after treatment, and the percentage of weight loss was calculated. The differences were statistically analyzed. **Results:** The highest rate of tissue dissolution was observed in the NaOCl + Er:YAG group ($P < .05$). The NaOCl + PIPS group dissolved more bovine pulp tissue than the nonactivated NaOCl group ($P < .05$). There was no statistically significant difference between the rates of tissue dissolution of the NaOCl + EA and the nonactivated NaOCl groups ($P > .05$). **Conclusions:** NaOCl activation with the Er:YAG laser with an endodontic fiber tip was the most effective in bovine pulp tissue dissolution. The PIPS technique also promoted superior tissue-dissolving effects when compared with no activation. However, the EndoActivator System had no direct effect on tissue dissolution. *(J Endod 2015;41:729–732)*

Key Words

Dentin, EndoActivator, Er:YAG laser, photon-induced photoacoustic streaming, pulp tissue dissolution, sodium hypochlorite

From the Departments of *Endodontics and †Prosthodontics, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey.

Address requests for reprints to Dr Mehmet Burak Guneser, Bezmialem Vakif University, İstanbul, Turkey. E-mail address: bguneser@hotmail.com

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Success in endodontic treatment depends mainly on complete removal of the pulpal debridement and bacterial population from the root canal system by means of chemomechanical preparation (1). Sodium hypochlorite (NaOCl) remains the most recommended and popular irrigant for root canal treatment because it has a superior tissue-dissolving activity (2–7) and antimicrobial effect compared with most other irrigants used in endodontics (8, 9). However, the root canal system often has a very complex anatomy, with lateral canals, isthmuses, complex branching, and deltas making complete debridement and disinfection impossible (10). Thus, irrigant activation is suggested to increase the efficacy of irrigant delivery and improve root canal cleanliness (6, 7, 11–15).

The EndoActivator System (Dentsply Tulsa Dental Specialties, Tulsa, OK) has been shown to safely clean the complex root canal system by sonic activation of the root canal irrigants with a flexible, noncutting polymer (16) that does not cause detectable canal transportation. On the other hand, it has been shown to have no effect on necrotic pulp tissue dissolution in simulated accessory canals (17).

Laser-activated irrigation has also been proposed as an alternative to the conventional debridement and disinfection procedures (11–14, 18). Among several laser devices, the Er:YAG laser is promising because of its cleaning mechanism within the root canal, which depends on rapid fluid motion caused by expansion and implosion of laser-induced bubbles (13). The Er:YAG laser with an endodontic fiber tip effectively removes the smear layer and intracanal debris (19), especially on the apical thirds, without causing any structural damage or anatomic alteration inside the root canal or periodontal tissues (20).

Recently, photon-initiated photoacoustic streaming (PIPS), a light energy phenomenon, has been introduced to improve irrigation. The PIPS technique differs from other agitation techniques in that only the tip is placed into the pulp chamber, thereby preventing contact with the root canal wall (12, 21). This technique is attributed to photoacoustic and photomechanical activities, which make it different than other techniques. In this method, an Er:YAG laser is used with a newly designed tapered tip with a radial firing end and 3 mm of the polyamide sheath. When activated in a limited volume of irrigant, the high absorption of the Er:YAG wavelength in water, combined with the high peak power achieved from using subablative parameters (0.3 W, 20 mJ at 15 Hz), results in a photomechanical phenomenon. The strong photoacoustic shock wave promotes 3-dimensional movement of the irrigation solutions (21). Therefore, the PIPS technique shows better root canal debridement than conventional irrigation modalities (11, 12). Peeters and Mooduto (22) reported that using a plain fiber tip in the coronal portion can drive the irrigation solution to the end of the canal without any harmful effects on the apical tissues. In another study, PIPS was more effective in the removal of antibiotic pastes from the root canal compared with the EndoActivator System (23). At present, however, data on its organic tissue-dissolving capacity are lacking. Therefore, the aim of the present study was to compare the effectiveness of the EndoActivator System, the Er:YAG laser with an endodontic fiber tip, and the PIPS technique on the pulp tissue-dissolving capacity of NaOCl.

Materials and Methods

Bovine Pulp Tissue Preparation

Eighty intact, freshly extracted, young bovine maxillary central incisors were used. This investigation was not classified as an animal study because our work had no

influence on the premortal fate of the animals or the slaughtering process. The teeth were extracted within 24 hours after slaughter and immediately placed in glass vials with distilled water. Two longitudinal grooves were cut on the proximal surfaces of teeth with a diamond bur (MANI Inc, Tochigi, Japan), and the teeth were then split in half. Pulp tissue was removed carefully with a cotton plier, washed with distilled water to remove excess blood, and then blotted dry. All pulps were combined to create a random mix of tissue. The pulp tissue samples were adjusted to similar weights of 45 ± 15 mg each with a no. 15 surgical blade.

Dentin Powder

Dentin powder was obtained using spherical dental burs #4 (MANI Inc) inside the root canals of previously split bovine teeth without water coolant and in a low-speed handpiece. All dentin powder was stored in plastic flasks.

NaOCl Solution

A stock solution of 5.25% NaOCl solution (Wizard; Rehber Kimya, Istanbul, Turkey) was tested. The pH of the solution was measured using a pH meter (HI 2211 pH-ORP Meter; HANNA Instruments, Woonsocket, RI) at room temperature (21°C) and adapted to a pH of 12 with 1 N HCl. The amount of final active chlorine content was also verified just before starting each test using an iodine/thiosulfate titration method as previously described (24).

Experiment

The initial weight of each pulp sample was measured with a precision balance (ME204; Mettler-Toledo, Columbus, OH). After the weights were recorded, the pulp samples were randomly divided into 4 experimental groups (n = 10) and 4 control groups (n = 10). The samples were then individually placed in 1.5-mL Eppendorf tubes (volume = 1.5 mL, diameter = 2.5 mm, taper = 4%, length = 25 mm).

The experimental groups (n = 10) were as follows:

1. 5.25% NaOCl + EndoActivator System activation (NaOCl + EA)
2. 5.25% NaOCl + Er:YAG laser with an endodontic fiber tip activation (NaOCl + Er:YAG)
3. 5.25% NaOCl + PIPS activation (NaOCl + PIPS)
4. 5.25% NaOCl + no activation (nonactivated NaOCl, positive control group)

The negative control groups were as follows (n = 10):

1. Distilled water + EndoActivator System activation (distilled water + EA)
2. Distilled water + Er:YAG laser with an endodontic fiber tip activation (distilled water + Er:YAG)
3. Distilled water + PIPS activation (distilled water + PIPS)
4. Distilled water + no activation (nonactivated distilled water)

One milliliter 5.25% NaOCl solution and 10 mg dentin powder were added to tubes containing tissue samples for the experimental groups. For negative control groups, 40 Eppendorf tubes were prepared with an additional 1 mL distilled water and 10 mg dentin powder. All testing procedures were performed at room temperature.

The EndoActivator System Activation. The EndoActivator System was used for passive sonic activation of 5.25% NaOCl. It was performed using the EndoActivator handpiece set at 10,000 cycles per minute with a medium polymer tip (#25/.04).

Er:YAG Laser with an Endodontic Fiber Tip Activation. Er:YAG laser activation was performed with a wavelength of 2,940 nm (Fidelis AT; Fotona, Ljubljana, Slovenia) and an R14 handpiece with a 300-μm endodontic fiber tip (Preciso, Fotona). The fiber tip was used with an output power of 1 W, energy of 50 mJ, and a frequency of 20 Hz as specified by the manufacturer. The water and air on the laser system were turned off.

PIPS Activation. The PIPS protocol was performed with an Er:YAG laser with a wavelength of 2.940 nm (Fidelis AT). A 12-mm-long, 400-μm quartz tip was tapered and had 3 mm of the polyamide sheath stripped back from its end. The tip was applied with 0.3 W, 15 Hz, and 20 mJ per pulse as specified by the manufacturer without water/air spray.

For all devices tested, the tips were immersed in Eppendorf tubes containing irrigating solutions throughout their working length. All samples were activated for 30 seconds, with resting times of 45 seconds after activation. The application was repeated 4 times. In between these activation procedures, the 5.25% NaOCl solution and distilled water in each Eppendorf tube were removed, and 1 mL fresh solution was added. Fresh dentin powder was also added to Eppendorf tubes for each irrigant application. Consequently, the total solution exposure time was 5 minutes with a total volume of 4 mL irrigant and 40 mg dentin powder for each sample in all groups.

After an exposure time of 5 minutes, the pulp samples were removed and washed with distilled water to remove dissolved/suspended tissue remnants or dentin powder. The samples were blotted dry and weighed again. The difference in weights of the tissue sample before and after exposure to 5.25% NaOCl solution or distilled water was divided by the original tissue weight and multiplied by 100 to obtain the percentage of tissue weight loss. The data were then analyzed statistically using 1-way analysis of variance and Tukey post hoc tests with a 95% confidence level (P = .05).

Results

The comparison of the rates of tissue dissolution for all groups with 5.25% NaOCl and distilled water is shown in Table 1. Because no pulp tissue dissolution was observed in any negative control groups with distilled water, statistical analysis was applied only to the NaOCl groups. One-way analysis of variance showed statistically significant differences between the NaOCl groups (P < .05). The highest rate of tissue dissolution was obtained with the NaOCl + Er:YAG group (P < .05). The NaOCl + PIPS group dissolved more bovine pulp tissue than the nonactivated NaOCl group (P < .05), whereas there was no statistically

TABLE 1. Effect of 3 Different Methods of Activation on Tissue Dissolution (% Tissue Weight Loss ± Standard Deviation) by the 5.25% NaOCl Solution and Distilled Water

	EndoActivator	Er:YAG	PIPS	No activation
5.25% NaOCl	47.77 ± 8.17^{bc}	71.59 ± 6.95^{a}	57.29 ± 14.41^{b}	43.41 ± 8.31^{c}
Distilled water	0.17 ± 1.08	0.42 ± 0.83	0.36 ± 0.32	−1.12 ± 1.65

NaOCl, sodium hypochlorite; PIPS, photon-initiated photoacoustic streaming.

Groups identified by different superscript letters are significantly different (P < .05). Groups identified by the same superscript letters are not significantly different (P > .05).

significant difference between the rates of tissue dissolution of the NaOCl + EA group and the nonactivated NaOCl group ($P > .05$).

Discussion

In the current study, the dissolution capacities of the irrigant activation methods were evaluated using a test tube model (2–4, 6) with dentin powder (25), as has been done in many studies. This type of experimental design cannot exactly reflect the clinical conditions; however, it did allow comparative quantitative evaluations regarding hypochlorite activation that are difficult to achieve in natural teeth. The standardization of pulp tissue samples and irrigants prevented the confounding factors related to solution concentration, pH, temperature, volume, mass, and tissue surface area, which are known to influence the results of tissue dissolution studies (3, 6). In addition, the irrigant was regularly exchanged for fresh solution throughout the experiment to simulate the repeated use of irrigant after each file.

This study was based on bovine tissues for ethical reasons and because of the unavailability of human teeth (25). It is also difficult to obtain standardized human pulp tissue samples (3). Bovine pulp tissue is more similar to human pulp than other animal tissues and has been used in previous studies to evaluate the dissolution capacities of endodontic irrigants (2, 7, 17, 24).

It is well-known that dentin has a considerable buffering effect against acid and alkali materials (26) and thereby reduces the tissue dissolution capacity of NaOCl (5). The small particles of dentin powder allow the use of standardized quantities and greater control of the mixture with the irrigants (25). Furthermore, it has been reported that the chemical composition and pH of bovine dentin are not different from human dentin (27). Thus, bovine dentin powder was obtained from root canal walls and added to all test groups to reflect the clinical conditions.

The distance between the tips of tested devices and the pulp samples that could alter the tissue dissolution capabilities (6) was 1 of the limitations of this study. For all devices tested, each tip could be immersed to the depth of its entire length without touching the pulp samples. Therefore, the distance was not the same for all groups because of the different lengths of the tips used. For example, the distance between the end of the PIPS tip and the pulp sample in the test tube was more than the distance of the other tips tested. Nevertheless, it is recommended to place PIPS only into the coronal access opening of the pulp chamber; therefore, the experimental design may somewhat reflect the clinical conditions even in test tubes.

The EndoActivator System is a safe sonic activation method because of its plastic tip against root canal transportation. However, NaOCl + EndoActivator System activation did not significantly improve the bovine pulp tissue dissolution compared with the nonactivated NaOCl irrigation. This is consistent with a recent study (7) that reported that NaOCl solution activated with the EndoActivator System had no effect on tissue dissolution. Al-Jadaa et al (7) suggest 2 possible explanations for the unsatisfactory results with sonic activation:

1. The wavelength of a sonic setup is too long to induce sufficient streaming of the irrigant
2. The sonic energy is too low to activate the irrigant

Thus, we may recommend that the insufficient improvement with sonic activation of NaOCl irrigation for pulp tissue dissolution should be taken into consideration when attempting to dissolve the residual pulp tissue in anatomically complex teeth, such as dens invaginatus, C-shaped molars, and teeth with internal resorption.

Based on our results, the tissue dissolution rate of NaOCl + Er:YAG laser with an endodontic fiber tip activation was superior to the NaOCl + PIPS or NaOCl + EndoActivator System activation methods. The Er:YAG laser has bactericidal activity (28) and the ability to remove the smear layer and dentin debris (19) with minimal side effects in root canals (20). Although the laser is usually used to clean the root canal system after conventional root canal instrumentation, laser systems can also be applied to dissolve the residual pulp tissue remnants (29), especially in areas in which instruments cannot reach, even in well-shaped canals (30). To date, there has been only 1 study evaluating the effect of Er:YAG laser–activated NaOCl irrigant on tissue dissolution. Kuhn et al (29) found that Er:YAG laser activation of NaOCl revealed highly effective soft tissue dissolution, as is consistent with our results. This may be because of the ability of the laser-activated irrigation to create collapse shock waves and rapid streaming caused by laser-induced vapor and bubbles within the irrigant (12, 13). Moreover, our findings suggest that the Er:YAG laser itself has no effect on pulp tissue because the tissue was not dissolved in distilled water activated with the laser. Therefore, the improved tissue dissolution of the Er:YAG laser in conjunction with NaOCl irrigation may have been achieved directly by the activated NaOCl irrigant.

In the PIPS technique at low power, each impulse interacts with the water molecules, creating expansion and profound shock waves that induce the formation of a powerful streaming fluid with no thermal damage on the root surfaces (21). The vapor bubble begins to expand and produce a void in front of the laser light (13) and may increase the efficacy of the tissue dissolution ability of the irrigant. However, in the present study, PIPS did promote pulp tissue dissolution but significantly less so than the Er:YAG laser with an endodontic fiber tip. Similarly, Deleu et al (14) compared the laser-activated irrigation methods on the removal of the smear layer. They showed that the Er:YAG laser was more efficient than PIPS. It has also been shown that smaller fiber diameters and higher pulse energies produce higher fluid flow and might enhance the formation of vapor or a cavity that contains bubbles inside the irrigant (14). Hence, the higher tissue dissolution ability of the Er:YAG laser in this study may be explained by the use of higher pulse energy (50 mJ) and a smaller fiber diameter (300 μm) compared with the parameters and tips of the PIPS technique. Consequently, the cleaning mechanism of PIPS is not yet clarified; therefore, because there are limited data in the literature, our results should be confirmed in future studies.

Conclusions

The results from this *in vitro* study indicate that NaOCl activation with the Er:YAG laser with an endodontic fiber tip was the most effective in bovine pulp tissue dissolution. The tissue dissolution effect of NaOCl could also be enhanced by PIPS when compared with no activation. However, the EndoActivator System had no direct effect on tissue dissolution.

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Efficacy of photon induced photoacoustic streaming (PIPS) on root canals infected with Enterococcus faecalis: A pilot study

Drs. David E. Jaramillo, Raydolfo M. Aprecio, Nikola Angelov, Enrico DiVito, and Thomas V. McClammy explore whether the Er:YAG laser tip is an efficient tool in elimination of Enterococcus faecalis from infected root canals

Abstract

Eradication of microorganisms from an infected root canal before obturation is a primary focus of endodontic treatment, as well as the best predictor for the long-term success of the endodontic therapy. The purpose of this *in vitro* laboratory study was to evaluate the efficacy of a new tapered and stripped Er:YAG laser tip using PIPS (Photon Induced Photoacoustic Streaming) in root canals infected with *Enterococcus faecalis* (ATCC4082). **Methods:** Twenty-four freshly extracted single-rooted human teeth were collected and inoculated with *E. faecalis*. After 4 weeks, the teeth were divided in four groups, two experimental, one positive, and one negative group. Laser treatment was performed for a period of 20 seconds with 6% sodium hypochlorite in groups 1 and 2, while PBS was used for group 3 and 4. Furthermore, dentin debris was produced, and colony-forming units were determined. **Results:** The combinations of 20 seconds laser activated irrigation with Er:YAG Laser and 6% sodium hypochlorite showed 100% inhibition using PIPS of growth of *E. faecalis* compared to 50% inhibition with the combination of Er:YAG Laser and PBS. **Conclusions:** The PIPS technology is efficient tool in elimination of *Enterococcus faecalis* from infected root canals.

Educational aims and objectives

The purpose of this article is to:
Explore whether additional use of lasers can be a valuable addition in removing bacterial load in areas where traditional methods may fail to succeed.

Expected outcomes

Correctly answering the questions on page 33, worth 2 hours of CE, will demonstrate that you can:

- Identify clinical approaches that have been evaluated for disinfection and control of the root canal biofilm during endodontic treatment.
- Realize the efficacy of a new Er:YAG laser tip called PIPS (Photon Induced Photoacoustic Streaming) in root canals infected with Enteroccocus faecalis.
- Realize that despite the fact that traditional chemo-mechanical cleansing measures have shown acceptable results in endodontic outcomes, the additional use of lasers can be a valuable addition in removing bacterial load in areas where traditional methods may fail to succeed.

Introduction

Many clinical approaches have been evaluated for disinfection and control of the root canal biofilm during endodontic treatment[1,2]. The presence of bacteria in root canals has been considered to be responsible for endodontic treatment failure[1,3]. Eradication of microorganisms from an infected root canal before obturation is a primary focus of endodontic treatment[3,4] as well as the best predictor for the long-term success of the endodontic therapy[5]. Location, harboring, and multiplication of bacteria within root canals are the factors most cited for making disinfection of this anatomical structure a clinical problem. Bacteria can colonize and survive in dentinal tubules, lateral canal ramifications, canal isthmuses, and other irregularities in the root canal[1,3], thus making the mechanical instrumentation approach limited in its effectiveness, unless supplemented with antimicrobial solutions that help in reducing the bacterial load[6,7]. *Enterococcus faecalis*, a gram-positive facultative anaerobe is able to resist and adapt to the harshest environmental conditions; this explains its presence and survival in endodontic infections and periradicular lesions[8]. Traditional therapeutic solutions such as sodium hypochlorite and chlorhexidine (either gel or liquid) or combinations of different irrigation vehicles have been shown to be effective in eliminating or reducing the presence of *E. faecalis* from root canals and dentinal tubules[9]. Different techniques have been proposed to improve the efficacy of irrigation solutions, including changes of concentration, temperature, surfactant, and agitation[10]. Despite the fact that traditional chemomechanical cleansing measures have shown acceptable results in endodontic outcomes, several literature reports have suggested that the additional use of lasers can be a valuable addition in removing bacterial load in areas where traditional methods may fail to succeed[1,11,12].

The use of Photo Dynamic Therapy (PDT) added to conventional endodontic treatment leads to a further major reduction of microbial load, proving that PDT is an efficient treatment to kill multi-drug resistant microorganisms[13]. Studies have shown that combined treatments, such as the use of sodium hypochlorite, citric acid, and diode laser energy together have a synergistic effect, increasing treatment efficacy and leading to significantly better decontamination of the root canal[14]. Er:YAG laser is effective in removing debris and smear layer from root canal walls[15]. Standardized instrumentation, followed by a final Er:YAG laser irradiation in wet canals with EDTA irrigation can result in more cleaning of the root canal walls and a higher quantity of open tubules in comparison with the traditional irrigation method[16]. Noetzel et al, evaluated the efficacy of the $CaOH_2$, Er:YAG laser and gaseous ozone in root canal disinfection either alone or combined with mechanical instrumentation and different antimicrobial solutions in canals inoculated with *E. faecalis*[3]. Within the limitation of their study, the authors concluded that Er:YAG laser irradiation does not provide satisfactory reduction of *E. faecalis*-infected root canals when compared to $CaOH_2$ and gaseous ozone, both of which demonstrated higher efficacy. They also reported that the use of antimicrobial solutions such as 1% sodium hypochlorite and 0.2% chlorhexidine showed increased antibacterial efficacy[3]. In addition, Yavari et al, also suggested that even though 1% sodium hypochlorite solution had a more significant antibacterial effect on the microbiological flora present in infected root canals, the use of laser (Er,Cr:YSGG) also showed noticeable antimicrobial effects on the *E. faecalis*[12]. Conversely, De Moor et al, showed that

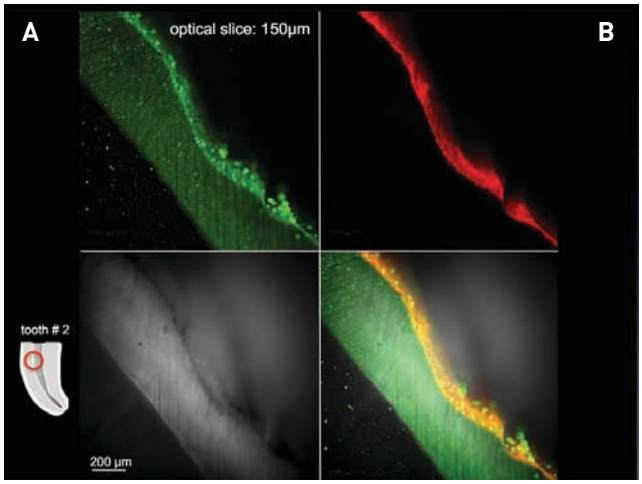


Figure 1: Bacterial growth before laser activated irrigation. BacLight staining, bright green indicates live bacteria (A), bright red indicates autofluorescence (B)

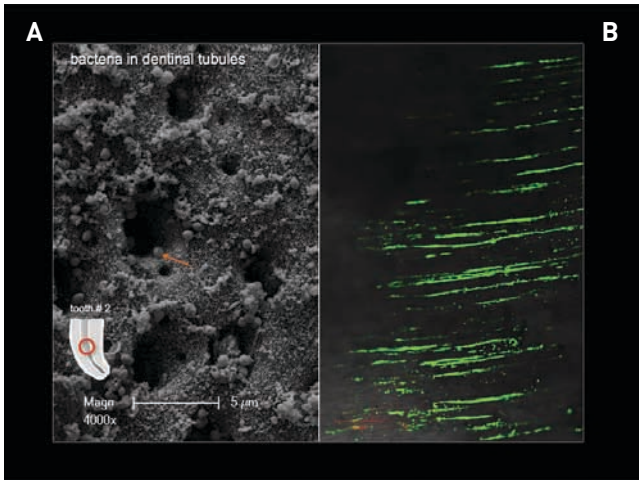


Figure 2: SEM (A) showing bacterial growth before PIPS treatment and confocal image (B) showing live bacterial growth into dentin tubules

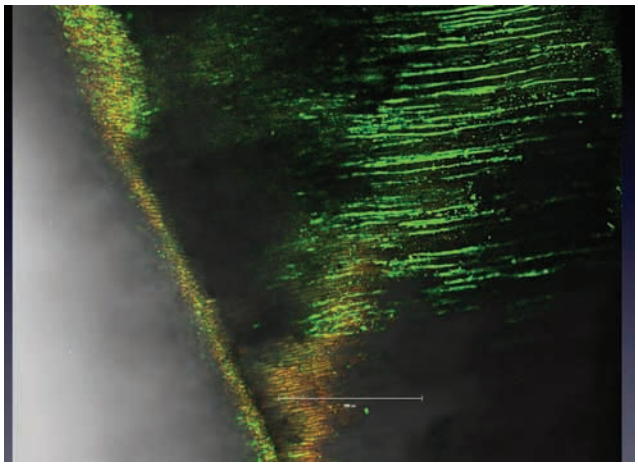


Figure 3: Close up of confocal image showing infiltration of bacteria deep into the dentin tubules

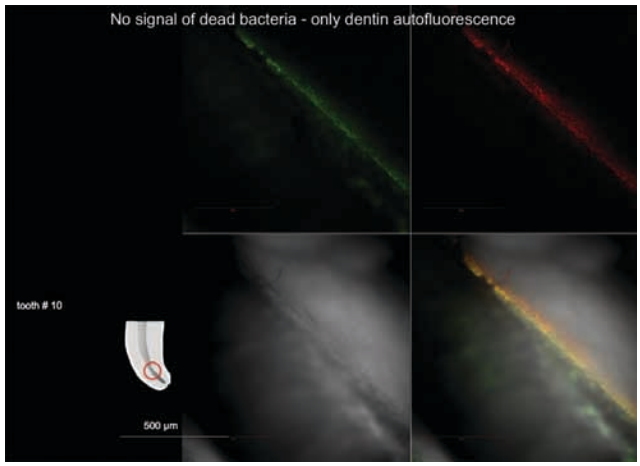


Figure 4: Confocal imaging after 20 seconds of laser activated irrigation with hypochlorites and PIPS. No signal of dead bacteria-only dentin autofluorescence

laser-activated irrigations with Er:YAG laser used for 20 seconds is comparable to the passive ultrasonic irrigation (PUI) with the intermittent flush technique[10]. Lastly, Yasuda et al, concluded that the Er:YAG laser showed high bactericidal effect in straight and curved root canals when compared to the Nd:YAG laser[17]. PIPS (Photon Induced Photoacoustic Streaming) is utilizing extremely low energy levels of laser light to generate a photoacoustic shockwave to stream irrigants throughout the entire root canal system[16]. The purpose of this in vitro laboratory study was to evaluate the efficacy of a new Er:YAG laser tip using PIPS in root canals infected with *Enteroccocus faecalis* (ATCC4082).

Preparation of teeth specimens

A total of 24 freshly extracted single-rooted human teeth were collected and placed in phosphate buffered saline (PBS) solution until use. Radiographs were taken of all the teeth to rule out the possibility of root canal blockage, previous root canal treatment or canal calcifications. Access openings were prepared with a fissure carbide bur (Brasseler). A No. 10 Flexofile (Dentsply) was placed inside the root canal until it was seen at the apical foramen; 1 mm was subtracted from this measurement to establish the working length (WL). A ProTaper® Rotary File system (Dentsply) was used for root canal preparation. The root canal of each tooth was enlarged up to F1 to keep the canal enlargement as small as possible. One milliliter of 6% sodium hypochlorite was used for irrigation between each instrument use. After the canal preparation was completed, 2 ml of 17% EDTA was used as final irrigation to remove debris and smear layer and leave the dentinal tubules open to facilitate bacterial penetration. The teeth were transferred to a flask with deionized water for sterilization by autoclaving for 30 minutes at 121°C with 15 lb. pressure.

Study design

The teeth (n = 24) were divided in 4 groups: 2 experimental (groups A and B), 1 positive control (group C) and 1 negative control group (group D). The first experimental group (group A) consisted of 8 teeth that were all evaluated by Confocal Microscopy Analysis (LSM 5 Pascal Inverted, Carl Zeiss MicroImaging, Inc.). Four of these teeth (teeth 1 to 4; group A1) were previously treated with Er:YAG laser (Fidelis AT, Fotona, Ljubljana, Slovenia) and 6% sodium hypochlorite (6% NaOCl) for 20 seconds; while the remaining 4 teeth (teeth 5 to 8; group A2) were treated with saline instead of 6% NaOCl. All samples (teeth 1 to 8) were stored in PBS solution until the evaluation with the confocal microscopy. The second experimental group (group B; teeth number 9-16) was evaluated by the use of Scanning Electronic Microscope-SEM (XL 30 S, FEG, FEI Company). The first half of group B (teeth 9 to 12; group B1) was treated with Er:YAG laser and NaOCl 6%, while the second half of this group (teeth 13 to 16; group B2) was treated

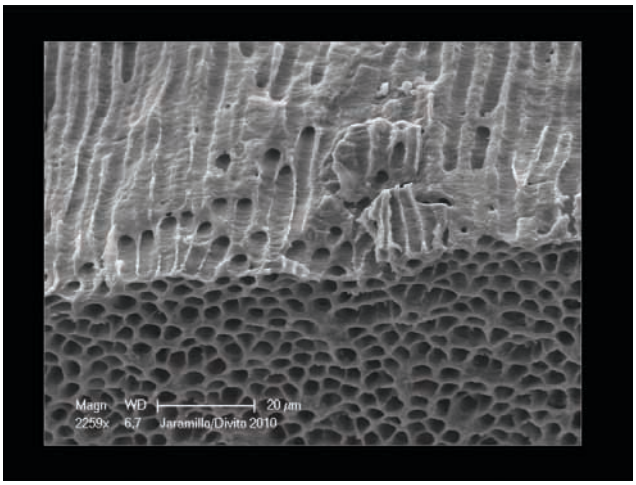


Figure 5: SEM image showing the absence of bacteria in tubules as seen on previous confocal image (Figure 4)

with Er:YAG laser and PBS. All samples (teeth 9 to 15) were stored in 4% formalin until the evaluation with the SEM. The positive control group (teeth 17 to 20; group C) included 4 teeth that were all inoculated with *E. faecalis* (ATCC 4082, Manassas, Virginia). Two teeth (17 and 18; group C1) from this group were stored in PBS and later evaluated using confocal microscopy, while the remaining 2 teeth (teeth 19 and 20; group C2) were stored in 4% formalin and then evaluated using SEM. Finally, the negative control group (teeth 21 to 24; group D) were all coated with clear nail polish to prevent bacterial penetration into the root canal. From this group, the first two teeth (teeth 21 and 22; group D1) were examined using confocal microscopy and teeth 23 and 24 (group D2) using SEM.

Bacteria and culture conditions

The sterilized tooth specimens were inoculated with *E. faecalis* (ATCC) in Brain Heart Infusion (BHI; Becton Dickinson) broth. Specimens were kept at 37°C to allow bacterial growth. The medium was replaced once a week for 4 consecutive weeks. After 4 weeks of inoculation, the teeth were removed from the bacterial culture. The root canal openings were covered with Cavit™ (3M™ ESPE™). Each tooth was wiped with 6% sodium hypochlorite to disinfect the outside of the tooth before further treatment. Cavit was removed, and experimental laser treatment by was completed.

Experimental procedures

The infected canal spaces from sample 1 through 16 were sampled using paper point and then exposed to laser irradiation with an Er:YAG laser (Fidelis) with a wavelength of 2940 nm for 20 seconds in pulse mode (50 microsecond pulse mode) at 0.3 watts (15 HZ, 20 millijules). Er:YAG laser was used to irradiate the root canals after traditional instrumentation. A newly designed quartz tip was used (PIPS, 400 micron 14 mm long). The tip was tapered and had 3 mm of the polyamide sheath stripped back from its end. The co-axial air and water spray feature of the handpiece was set to off. The tip was placed in the coronal portion of the access opening only, remaining stationary and not advanced into the root canal, accompanied by constant irrigation with 6% sodium hypochlorite. After laser treatment, a Hedström file (Maillefer, Switzerland) was used to produce dentin shavings inside all the root canals. The shavings inside the root canals were collected using a sterile paper point and placed in a 2 ml microtest tube containing 1.5 ml Brain Heart Infusion (BHI) broth for colony forming unit (CFU) counts. The tube with paper point was vortexed for 30 seconds and prepared in 1:10 serial dilutions for plating. Each sample was placed in a tube with 2ml of BHI and incubated for 72 consecutive hours. Bacterial growth was determined via visual assessment by two examiners who evaluated turbidity of each sample. Absence of turbidity indicated no bacterial growth, while turbidity was indication of viable bacteria. All non-turbid test samples were vortexed and plated with 25 ml in BHI plates. Two positive and two negative control specimens were also plated on BHI. All plates were incubated for 24 hours and again evaluated for bacterial growth by the same examiners. The presence of white pinpoint colonies indicated growth of *E. faecalis* on agar plates, and microscopic evaluation with gram stain confirmed the presence of gram-positive bacteria. Subsequently, the roots were sliced longitudinally with a diamond disk (Brasseler). The sliced roots were placed in 17% EDTA for 30 seconds to remove sectioning debris. The laser-treated teeth from group A (samples 1-8) were stored in PBS for Confocal Microscopy Analysis (Carl Zeiss MicroImaging). To determine the viability of the cells, a BacLight™ staining kit (Invitrogen™) was used. Live cells were shown as bright green dots, while dead bacteria were stained red. The laser-treated teeth from group 2 (samples 9-16) were stored in 4% formalin solution for fixation for SEM microscopy.

Statistical analysis

Significant differences in bacterial colony forming units were determined using a Friedman's two-way Analysis of Variance by Ranks for comparisons between the three laser treatment groups (before laser treatment, after laser treatment, and dental shavings after laser treatment). The Kruskal-Wallis procedure was implemented to test for differences in the bacterial colony forming units between the four solution groups at each laser treatment level: laser with sodium hypochlorite 6%, and laser with PBS for confocal microscopy, laser with sodium hypochlorite 6% (4% formalin for SEM), laser with PBS (4% formalin for SEM), Appropriate post-hoc comparisons with adjustments for multiple testing were performed at the completion of the Friedman and Kruskal-Wallis procedures if warranted. All hypotheses were two-sided and tested at an alpha level of 0.05. The statistical analyses were conducted with SAS v. 9.1.3 (SAS Institute, Cary, NC).

Statistically significant differences in colony forming units per milliliter were found between the laser treatment groups in two of the four groups; Teeth 1-4, laser with sodium hypochlorite (confocal group) (Friedman test, $p < 0.001$), teeth 9-12, laser with sodium hypochlorite (SEM group) (Friedman test, $p = 0.018$), teeth 13-16, laser with PBS (SEM group) (Friedman test, $p = 0.039$), and bacterial growth in the "before laser treatment" was significantly higher than the after laser treatment in teeth 5-8 ($p = 0.034$) and "after laser treatment dental shavings" teeth ($p = 0.034$) for 9-12 sodium hypochlorite (SEM group). Similar pattern of significance was detected in the PBS (SEM group) group of higher "before laser treatment" bacterial colony forming units when compared to "after laser treatment" ($p = 0.013$) and weaker evidence when compared to "after laser treatment dental shavings" groups. No statistically significant difference in bacterial colony forming units per milliliter was found for teeth in group 5-8 (Table 1).

Two of the three laser treatment groups demonstrated a statistically significant difference in bacterial colony forming units per milliliter: "before laser treatment" (Kruskal-Wallis, $p = 0.042$) and "after laser treatment dental shavings" (Kruskal-Wallis, $p = 0.011$). Significantly less colony forming units per milliliter were observed in the "sodium hypochlorite-PBS" group (1-4) when compared to the "sodium hypochlorite–4% formalin" ($p=0.026$) and "PBS" (5-9) groups ($p=0.019$). No statistically significant difference in bacterial growth was detected between the solutions for the "after laser treatment" group teeth 1-8, (Kruskal-Wallis, $p = 0.181$; Tables 2 and 3).

Table 1

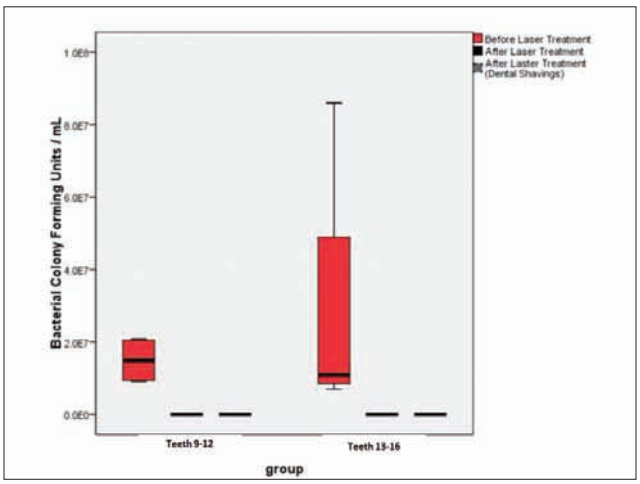
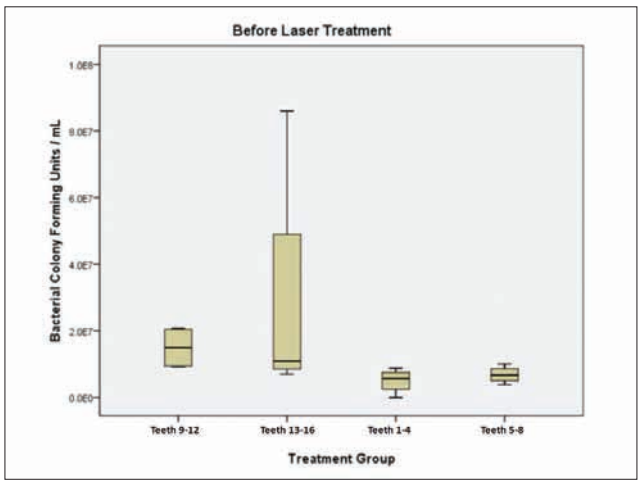


Table 2



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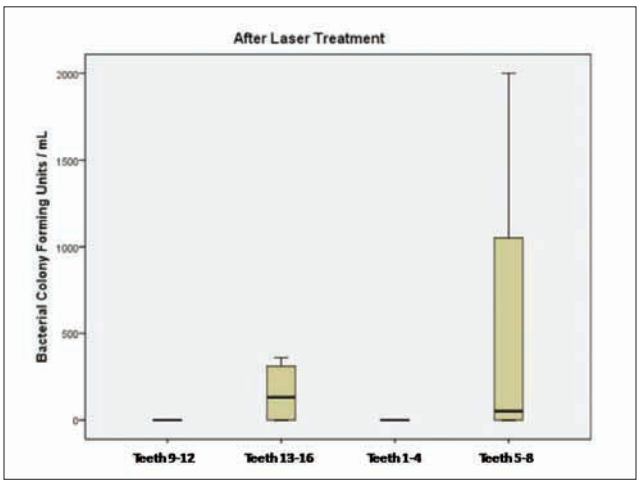

In summary, the combinations of 20 seconds irradiation with Er:YAG laser and 6% sodium hypochlorite completely inhibits the growth of *Enterococcus faecalis* when compared with the combination of Er:YAG laser and phosphate buffered saline. The dental shavings of teeth treated with Er:YAG laser and sodium hypochlorite showed 100% elimination of *Enterococcus faecalis* compared to 50% with the combination of Er:YAG laser and phosphate buffered saline. All untreated (baseline) teeth showed growth of *Enterococcus faecalis* (Figures 1, 2, and 3).

The SEM analysis, as well as the confocal microscopy analysis further confirms our findings. The SEM representative sample (Figure 5) clearly shows that there is no bacteria present on the canal wall of specimens treated with PIPS and sodium hypochlorite, when compared to the PBS control groups. Furthermore, the confocal microscopy analysis demonstrated absence of live bacteria on the canal wall, and even within the dentinal tubules in the treatment group compared to the controls (Figure 4).

Discussion

Persistence of bacteria following endodontic therapy has been identified as a major contributor to endodontic failures. Clinicians and researchers have investigated several ways to eliminate bacteria from the root canal system, including mechanical and chemical techniques[9,10]. Lasers have been used to aid in bacterial decontamination of root canal systems, with varying degrees of success. *E. faecalis* is a well-studied microorganism in the endodontic literature due to its resistance to treatment. It is therefore of remarkable interest to appreciate the results of the present study. Our in vitro pilot study clearly indicates that 20 seconds of laser activated irrigation with sodium hypochlorite using Er:YAG and PIPS technique with 50 microsecond pulse at 15HZ, with 0.3 Watts of power was effective in eliminating *E. faecalis* from in vitro infected root canal systems. This finding could be attributed to the photomechanical effect seen when light energy is pulsed in liquid[18-20]. Other studies have shown; however, that use of laser may not result in optimal bacterial reduction[3]. The possible reasons for differences in the efficacy of laser in endodontic therapy could be due to the different parameters used, including the delivery technique, the time of application within the canal, presence of aqueous solution that would affect the absorption of the laser beam and power of the laser and finally the density of energy delivered. All the previous literature cited were utilizing lasers as a thermal event. Our application was a photoacoustic subablative technique[21,22]. Our study shows that following decontamination and mechanical conventional use of sodium hypochlorite, the single application with PIPS for 20 seconds was sufficient to achieve zero growth of *E. faecalis* within the canal. Most likely, the amount of energy and the motion used during the emission of the laser beam was sufficient to penetrate and disrupt the biofilm created by the *E. faecalis*, and kill bacteria not by a thermal event, but rather via the photomechanical effects of the PIPS tapered and stripped tip. We also opine that the release of hydroxyl radicals as described during a photoacoustic event[22] also lead to the dentin tubule bacterial disinfection seen. The control groups used in this study validate the findings. The fact remains that *E. faecalis* is not the only microorganism involved in endodontic infections, so it may be beneficial to design future studies that will explore the effect of this treatment on other endodontic pathogens.

Our results are in accordance with previous studies that confirmed the efficacy of lasers in decontamination of the endodontic canals[16-20]. We speculate the low energy laser light generates a photoacoustic shockwave that pulses its way three dimensionally to all the internal aspects of the root canal system, effectively disrupting the biofilm. This may be of particular interest to the clinical practice of endodontics, allowing the clinician to use the PIPS method for effective disinfection of the entire root canal system, without unnecessary over-instrumentation, especially in the apical third of the canal.

Conclusions

Within the limitation of this study, we can conclude that the combinations of 20 seconds irradiation with Er:YAG laser via a

photoacoustic delivery system and 6% sodium hypochlorite is very effective in inhibiting *Enterococcus faecalis* growth. The results are encouraging in terms of the possible application of the Er:YAG laser in endodontics. The PIPS technology can be used as an efficient additional tool in the decontamination of infected root canals during endodontic treatment. EP

David E. Jaramillo, DDS, completed dental school and an advanced program in endodontics in Mexico in 1990 and has been teaching since then. He has also successfully practiced endodontics for 15 years. In 2004, he accepted the faculty position at USC Endodontic advanced program. In 2005, he started working at the Center for Biofilms at USC, run by Dr. Bill Costerton where he got trained in the use of SEM (Scanning Electron Microscopy) and Confocal (Laser Scanning Microscopy). In 2006, he accepted a full-time position at Loma Linda University School of Dentistry where he is an associate professor and the clinical director of endodontics. Dr. Jaramillo also works at the Center for Dental Research LLUSD. He teaches at six different advanced endodontic programs in Mexico along with USC and Long Beach VA Hospital. He has lectured in the US, Mexico, Europe and South America and has been published in numerous journals. He has co-authored several papers with his colleagues from the Department of Electrical Engineering-Electrophysics, Viterbi School of Engineering University of Southern California on the use of new plasma technology for biofilm removal from root canal system, as well as a chapter in the 6th edition of *Ingle's Endodontics*. Dr. Jaramillo has donated scanning electronic microscopy images for illustration for a chapter entitled "The Microbiology of the Necrotic Pulp" for Textbook of *Endodontology* by Dr. Gunnar Bergenholtz- 2nd edition. Dr. Jaramillo has done extensive research in the field of biofilms and has two temporary patents filed through the university patent office.

Raydolfo M. Aprecio Sr., BS, OD, is a Research Associate with extensive research experience and expertise in microbiology. Before joining the Center for Dental Research, he was a Senior Research Assistant with the Department of Microbiology and Molecular Genetics, LLU School of Medicine for 25 years. He has worked on numerous research projects in the areas of endodontics and periodontics and helped numerous graduate students and residents from Departments of Endodontics, Pediatrics and Implant Dentistry for their thesis research projects. He has served as a mentor to dental and hygiene students for their table clinic research. Each year, his student table clinics have won awards during the annual Student and Alumni Convention, California Dental Association and national meetings. He was an original member of the research team that developed the MTA and MTAD.

Nikola Angelov, DDS, MS, PhD, is a professor and a Program Director of Undergraduate Periodontics at Loma Linda University School of Dentistry. Dr. Angelov was born in Skopje, Macedonia in 1968. He earned his DDS, MS and PhD degree from St. Cyril and Methodius University and has been teaching for the Department of Oral Pathology and Periodontology in Macedonia since 1993. In 2001, he received a postdoctoral fellowship at the National Institute of Health, National Institute of Dental and Craniofacial Research (NIH/NIDCR). At NIH/NIDCR, Dr. Angelov successfully developed a new model of oral mucosal wound healing and was also involved in collaborative clinical studies, clinical trial evaluations, as well as other studies. Dr. Angelov also holds a Certificate of Specialty in Periodontics and is a Diplomate of the American Board of Periodontology. In 2008, Dr. Angelov received the prestigious annual Educator of the Year Award from the American Academy of Periodontics for outstanding results in teaching in the area of Periodontics. Dr. Angelov has several other academic awards and is actively involved in teaching and research. His research area includes oral infection and immunity, mucosal wound healing, periodontal microbiology, as well as dental implants. Dr. Angelov has extensively been published in peer review articles, and has been an invited speaker to prominent professional and research meetings. He is a member of numerous professional societies in the USA and abroad. Dr. Angelov also serves as a reviewer on the Journal of Periodontology, an official journal of the American Academy of Periodontology, as well as a School of Dentistry representative to the Institutional Review Board, and the Institutional Animal Care and Use Committee at Loma Linda University in California.

Dr. Enrico DiVito formed his dental practice in 1980 in Scottsdale. In 2004, he formed the Arizona Center for Laser Dentistry. He is also the founder and director of the state-accredited Arizona School of Dental Assisting (ASDA). In addition to teaching at ASDA, DiVito is also a clinical instructor at the Arizona School of Dentistry and Oral Health and is responsible for helping to create the Department of Laser Dentistry. He earned his undergraduate degree from Arizona State University in 1980 and is a graduate from the University of the Pacific, Arthur A. Dugoni School of Dentistry with honors receiving several clinical excellence awards. He can be reached at edivito@azcld.com.

Thomas V. McClammy, DMD, MS, graduated from Oregon Health Sciences University, School of Dentistry, in 1982. During the next 15 years, he created a remarkable general practice in Oregon and passionately pursued continuing education courses to fulfill his insatiable desire to learn. In 1996, Dr. McClammy was accepted as a post graduate endodontic resident at Boston University's Goldman Graduate School of Dentistry. He earned a Master's degree in 1999 from his mentor, Dr. Herbert Schilder, who is widely recognized as the "father of modern day endodontics." Currently, Dr. McClammy has a full-time practice dedicated to saving teeth with endodontics and implants in Scottsdale, Arizona, and is well known for his expertise, passion, and extraordinary teaching skills.

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Original Research

Effectiveness of Sonic, Ultrasonic, and Photon-Induced Photoacoustic Streaming Activation of NaOCl on Filling Material Removal Following Retreatment in Oval Canal Anatomy

Shan Jiang, MDS,[1] Ting Zou, MSc,[2] Dongxia Li, DDS,[2,3] Jeffery W.W. Chang, BDS, MDS,[2] Xiaojing Huang, DDS, PhD,[1] and Chengfei Zhang, DDS, PhD[2]

Abstract

Objective: This study aimed to assess the effectiveness of sonic, ultrasonic and laser [photon-induced photoacoustic streaming (PIPS)] irrigation activation in removing filling remnants from oval root canals after standard canal retreatment procedures with the ProTaper universal rotary retreatment system. ***Methods:*** Twenty-eight maxillary first premolars were instrumented with ProTaper NiTi rotary instruments and obturated with gutta-percha and AH Plus sealer using the continuous wave of condensation technique. After storage at 37°C and 100% humidity for 1 week, the specimens were retreated with the ProTaper universal retreatment system for the removal of filling material. Teeth were then randomly assigned into four groups ($n=7$): group 1, positive control; group 2, retreated with sonic irrigation; group 3, retreated with ultrasonic irrigation; and group 4, retreated with laser irradiation. The specimens were scanned using micro-CT before instrumentation, after obturation and mechanical retreatment, and after additional activation procedures. The percentage volume of the filling remnants was measured. Specimens were split longitudinally after micro-CT scan, canal walls were examined using scanning electron microscopy (SEM), and the amount of residual filling material was scored. ***Results:*** The filling materials' removal efficacy in the three experimental groups was higher than that of the control group ($p<0.05$), whereas filling materials ranging from 1.46 ± 0.30 to $2.21\pm0.46\,mm^3$ remained in the canal in all three experimental groups. Additionally, there was a significantly greater reduction in the amount of filling remnants in the PIPS group than in the sonic and ultrasonic groups (both $p<0.05$), and significantly greater reduction in the ultrasonic group than the sonic group ($p<0.05$). ***Conclusions:*** Activation of NaOCl with PIPS showed significantly better performance than sonic and ultrasonic techniques in removing the filling remnants following mechanical retreatment of oval root canals. The ultrasonic technique also performed better than the sonic technique. However, none of the additional activation procedures was able to completely eliminate the filling remnants.

Introduction

Endodontic retreatment aims at completely removing the previous root canal filling materials (gutta-percha and endodontic sealer) and creating the necessary pathways, which will facilitate further shaping, cleaning, re-disinfection and re-obturation of the canal system to establish healthy periapical tissues.[1] The remnants of the infected root canal filling materials could compromise the effectiveness of cleaning and disinfecting using mechanical and chemical methods.[2] Therefore, the complete removal of obturation materials from previously filled root canals may be considered an important step for endodontic retreatment outcomes. With appropriate instruments and their corresponding procedures to enhance the removal of gutta-percha and endodontic sealer, an improved disinfection of the root canal system could be achieved.[3]

Traditionally, numerous strategies including mechanical (stainless steel hand files, NiTi rotary systems), chemical (solvents), and thermal (heat carrying instruments) techniques,

[1]Department of Endodontics and Operative Dentistry I, School and Hospital of Stomatology, Fujian Medical University, Fujian, China.
[2]Comprehensive Dental Care, Endodontics, Faculty of Dentistry, The University of Hong Kong, Prince Philip Dental Hospital, Hong Kong SAR, China.
[3]Department of Stomatology, Air Force General Hospital of the PLA, Haidian District, Beijing, China.

and the combination of these techniques have been reported to eliminate root filling materials.[4,5] However, none of these techniques alone or in combination can completely remove the filling materials from the root canals.[6–11] In addition, these methods may present some serious side effects, such as periapical tissue irritation, periapical inflammation, and postoperative flare-ups caused by excessive apical extrusion of root filling materials.[12] Therefore, more effective techniques are still requiring future investigations.

Additional methods, such as sonic, ultrasonic, and some laser devices, have been reported to improve the removal efficacy of root canal filling materials.[13–15] Within laser-activated approaches, photon-induced photoacoustic streaming (PIPS) is a technique that was primarily developed for cleaning and debriding the root canal system.[16] This system uses a short pulse rate (50 ms) to create peak power spikes, which do not seem to cause thermal damage.[16] Previous studies showed that PIPS was significantly better than traditional techniques in debriding the root canal and removing calcium hydroxide paste medication.[16–18] Therefore, PIPS may effectively remove filling remnants after the standard retreatment procedures.

To our knowledge, no studies have investigated the efficacy of PIPS in removing filling material residues from oval root canals. Therefore, the aim of this *in vitro* study was to evaluate the efficacy of PIPS, EndoActivator (sonic), and an ultrasonic technique for removal of gutta-percha and endodontic sealer after mechanical retreatment of oval root canals, using high-resolution micro-CT and scanning electron microscopy (SEM).

Materials and Methods

Twenty-eight freshly extracted human maxillary first premolars with completely developed apices and a single straight, oval-shaped root canal were selected and stored in a 0.1% thymol solution until further processing. Periapical radiographs were taken in the buccolingual and mesiodistal directions at 80 kV and 100 mA to confirm the presence of a single straight root canal and calculation of the canal diameter ratio. The oval root canal was defined as a cross-section ratio of long (buccolingual): short (mesiodistal) diameter ≥ 2.5 at 5 mm from the apex.[19] Teeth that presented previous endodontic treatment or fracture lines were excluded.

Canal instrumentation

The selected teeth were decoronated using round diamond burs in a high-speed hand piece at a length of $\sim$16 mm. A stainless steel size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was inserted into the canal until the tip of the file just reached the apical foramen. The working length (WL) was determined to be 0.5 mm shorter than this length. All canals were prepared using the crown-down technique, using ProTaper NiTi rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) following manufacturer's instructions. The canal instrumentation was completed with an F2 ProTaper file. Canals were irrigated with 2 mL of 3% sodium hypochlorite solution (NaOCl) using a 30-gauge blunt-tip needle (Terumo Corporation, Leaven, Belgium) at every change of file. After completion of canal instrumentation, canals were irrigated with 6 mL of 17% ethylenediaminetetraacetic acid (EDTA) solution, followed by flushing with 2 mL of 3% NaOCl. After aspiration of irrigation solution in the pulp chamber, the canals were dried using sterile paper points (Dentsply/Herpo, Petrópolis, Rio de Janeiro, Brazil).

Canal filling

Obturation of all root canals was performed using the continuous wave of condensation technique and the Touch'n Heat device (SybronEndo, Orange, CA), according to manufacturer's specifications. Briefly, a size 35 taper 0.06 gutta-percha master point (Dentsply, Rio de Janeiro, Brazil) coated with AH Plus sealer (Dentsply De Trey, Konstanz, Germany) was fitted with tug-back to the WL. The root canals were subsequently filled with Obtura II (SybronEndo). To obtain approximately the same volume of gutta-percha filling, a 14 mm length was uniformly filled from the apex of the root in each canal. The quality of the root canal filling was assessed using both mesiodistal and buccolingual direction radiographs. Specimens showing any voids in the obturation mass were discarded and replaced. The access cavities were sealed with Caviton (GC, Tokyo, Japan). All specimens were stored at 37°C and 100% relative humidity for 1 week to ensure that the sealer was completely set.

Removal of canal filling material

After the temporary fillings were removed, the mechanical re-instrumentation of all root canals was removed using a standard protocol. First, the 3 mm length of filling materials from the cervical part of the root canal was removed using Gates Glidden burs size #3 (Dentsply, PA, USA). Then, the ProTaper universal retreatment system (Dentsply Maillefer, Ballaigues, Switzerland) was used to remove the filling material. The D1 (ISO 30, 0.09 taper), D2 (ISO 25, 0.08 taper) and D3 (ISO 20, 0.07 taper) files were sequentially used for the coronal, middle, and apical thirds, respectively. The ProTaper Ni-Ti rotary retreatment files (Dentsply Maillefer, Ballaigues, Switzerland) were used at 300 rpm and with a torque of 2 N/cm in a crown-down motion. The canals were irrigated between files with 2 mL of 3% NaOCl, followed by final irrigation with 5 mL of 17% EDTA. The criteria for completion of mechanical retreatment were as following: (1) the last file D3 reached the full WL, (2) no filling material covered the flutes of the files, and (3) the final irrigation solution was free of visible debris.[20] The specimens were then randomly divided into four groups of seven teeth each, and processed as follows.

Group 1 ($n=7$): Control group. No further procedure was performed, and samples were ready for evaluation.

Group 2 ($n=7$): PIPS procedure. Each specimen was irradiated using a 2940 nm Er.YAG laser (Fidelis AT, Fotona, Ljubljana, Slovenia), 1 W, 20 Hz, and 50 mJ per pulse with a 14 mm long and 300 μm diameter quartz tip. The pulse duration was 50 μs. The water and air spray of the laser units were turned off. The laser tip was fixed in place in the coronal part of the canal without touching the inner surface of the main canal wall and activated for 20 sec (3×20 sec).

Group 3 ($n=7$): Sonic (EndoActivator) procedure. Each specimen was activated using EndoActivator (setting: headpieces 10,000 cycles/min) with a sonic tip (size 20, taper 0.02)

(Dentsply Tulsa Dental Specialties, Tulsa, OK). The sonic tip was placed into the canal 1 mm short of the WL without touching the walls and activated for 20 sec (3×20 sec).

Group 4 ($n=7$): Ultrasonic procedure. Each root canal was activated using an ultrasonic device on a 25% power setting in E mode 28 kHz (EMS, Le Sentier, Switzerland) and delivered using an ultrasonic tip (size 20, taper 0.02) (ESI Instrument, EMS, Le Sentier, Switzerland). A smooth ultrasonic file was placed into the canal to 1 mm short of the WL without touching the walls and activated for 20 sec (3×20 sec).

Irradiation and activation

Before irradiation or activation in groups 2, 3, and 4, the root canal was filled with 2 mL of 3% NaOCl solution. During irradiation or activation, the pulp chamber was refreshed using 3% NaOCl solution when the coronal reservoir level became low. The above-described irradiation/sonic/ultrasonic procedures were repeated three times for a total of 60 sec. All procedures were performed by the same endodontist.

Micro-CT measurement and evaluation

A high-resolution micro-CT (SkyScan 1172, Aartselaar, Belgium) was used at 80 kV, 100 mA and an isotropic resolution of 20 μm to scan the sample before instrumentation (scan 1), after gutta-percha filling (scan 2), after mechanical re-instrumentation (scan 3) and after a second re-instrumentation (scan 4). Each sample was placed in to a microcentrifuge tube (SPL Life Sciences, Pocheon-Si, Korea) that served as a sample container during the scanning procedure. A series of cross-section images were acquired with 20 μm pixel sizes. The region of interest was selected from the cementoenamel junction to the apex of the root. The original gray scale images in TIFF format were then processed using NRecon software (Version 1.6.9.18 Bruker micro-CT, Kontich, Belgium) to build a three-dimensional (3D) reconstruction of the sample. The reconstructed images in BMP format were then further processed using the SkyScan Analyzer software package (Bruker micro-CT, Kontich, Belgium) including a CT-analyzer program (CTAn, Version 1.14.4.1) for 2D and 3D quantitative analysis of reconstructed volumes, and a CT-volume program (CTVol, Version 2.2.3.0) for 3D visualization of scanned objects. The volume (in mm^3) of the root canal, the filling materials after canal filling, the remaining filling materials after mechanical retreatment, and the remaining filling materials after additional irrigation/irradiation procedures were obtained from scans 1, 2, 3, and 4, respectively. The cleaning volume for the filling materials used in the additional irrigation/irradiation procedures was calculated by subtracting the volume of the remaining filling materials after the additional irrigation/irradiation procedures from the volume of the remaining filling materials after the mechanical retreatment.

SEM evaluation

After micro-CT scanning, all samples were grooved longitudinally in a buccolingual direction using a diamond disc and a high-speed hand piece, and then root canals were split into halves using a bone hammer. Samples were then dehydrated using increasing ethanol concentrations, dried at the critical point and sputter-coated with gold (Magnetron Ion Sputter Metal Coating Device, Msp-2S, IXRF System, Inc. MA, Japan). The presence of sealer remnants in the coronal, middle and apical thirds of each sample were evaluated using SEM (Hitachi, Tokyo, Japan) at 1000× magnification. The SEM images were rated by two calibrated examiners using the following scale: 0, no residue; 1, small amount of residue (≤20% of the surface covered); 2, moderate amount of residue (20–60% of the surface covered); and 3, large amount of residue (>60% of the surface covered).[21]

Statistical analysis

Statistical analysis was conducted using SPSS software (SPSS 20.0 for Windows, SPSS, Chicago, IL). The normality and the equality of the data's variance were evaluated using the Shapiro–Wilk test and Levene's test, respectively. The effectiveness of retreatment among the groups was compared using Kruskal–Wallis H and Mann–Whitney U tests. The differences of the remaining filling material before and after additional activation techniques within each group was compered using Wilcoxon signed rank test. The level of significance was set as $p < 0.05$.

Results

Micro-CT imaging and evaluation

The percent volume of the remaining filling materials in the full root canal length and all thirds (coronal, middle, and apical) are shown in Table 1. The filling material volume reductions are summarized in Table 2. Overall, the PIPS technique was superior in removing filling materials compared with the sonic (EndoActivator), ultrasonic, and control groups ($p < 0.05$). However, none of the retreatment techniques completely eliminated all filling materials from the root canal (Fig. 1).

Table 1. Remaining Filling Materials Volume (mm^3; Mean ± SD) for Overall and Each Third of the Canal After Mechanical Retreatment and Additional Activation Techniques

	Mechanical retreatment				*Activation techniques*			
	Control	*Sonic*	*Ultrasonic*	*PIPS*	*Control*	*Sonic*	*Ultrasonic*	*PIPS*
Overall	2.43 ± 0.56	2.37 ± 0.49	2.46 ± 0.57	2.39 ± 0.48	2.43 ± 0.56^{IV}	2.21 ± 0.46^{III}	1.98 ± 0.39^{II}	1.46 ± 0.30^{I}
Coronal	1.12 ± 0.28	1.09 ± 0.25	1.15 ± 0.31	1.12 ± 0.29	1.12 ± 0.28^{IV}	1.02 ± 0.23^{III}	0.90 ± 0.25^{II}	0.76 ± 0.19^{I}
Middle	0.93 ± 0.23	0.92 ± 0.24	0.94 ± 0.22	0.89 ± 0.25	0.93 ± 0.23^{IV}	0.86 ± 0.21^{III}	0.78 ± 0.19^{II}	0.65 ± 0.17^{I}
Apical	0.38 ± 0.11	0.36 ± 0.12	0.37 ± 0.10	0.38 ± 0.13	0.38 ± 0.11^{III}	0.33 ± 0.10^{II}	0.26 ± 0.12^{I}	0.25 ± 0.09^{I}

$^{I\text{-}IV}$Ranking: there were significant differences ($p < 0.05$) between groups with different ranks at the same level.
PIPS, photon-induced photoacoustic streaming.

Table 2. Volume (mm^3) of Filling Materials Removed (Mean ± SD) Overall and in Each Third of the Canal Following Each Activation Technique

	Sonic	*Ultrasonic*	*PIPS*
Overall	0.16 ± 0.07^{III}	0.48 ± 0.10^{II}	0.93 ± 0.14^{I}
Coronal	0.07 ± 0.04^{III}	0.25 ± 0.09^{II}	0.56 ± 0.11^{I}
Middle	0.06 ± 0.03^{III}	0.14 ± 0.06^{II}	0.26 ± 0.08^{I}
Apical	0.03 ± 0.01^{II}	0.09 ± 0.03^{I}	0.11 ± 0.04^{I}

$^{I\text{-}III}$Ranking: there were significant differences ($p < 0.05$) between groups with different ranks at the same level.
PIPS, photon-induced photoacoustic streaming.

After the mechanical retreatment (ProTaper universal retreatment system), the remaining filling materials ranged from 2.37 ± 0.49 to $2.46 \pm 0.57\,mm^3$, and there were no differences among the four groups ($p > 0.05$). This was followed by the additional activation procedures, and more filling materials were removed in the sonic, ultrasonic, and PIPS groups in all thirds than in the control group ($p < 0.05$). In all four groups, the remaining filling materials located mostly along the long axis of the oval canals (Fig. 2). In addition, in the coronal and middle thirds, the remaining filling materials in the PIPS group were significantly lower than those in the sonic and ultrasonic groups (both $p < 0.05$), and the amount remnants were also significantly lower in the ultrasonic group than in the sonic group ($p < 0.05$). In the apical third, significantly more filling materials were removed in the both PIPS and ultrasonic groups (0.11 ± 0.04 and $0.09 \pm 0.03\,mm^3$, respectively) than in the sonic group ($0.03 \pm 0.01\,mm^3$, both $p < 0.05$), whereas there was no significant difference between the PIPS and the ultrasonic groups ($p > 0.05$). The Wilcoxon signed rank test showed that in the PIPS and ultrasonic groups, the amount of the remaining filling materials in all thirds was significantly less after additional activation technique compared with before additional activation techniques (all $p < 0.05$), and that there were no significant differences in the remaining filling materials between before and after sonic irrigation technique (all $p > 0.05$).

SEM observation and evaluation

The distribution of the remaining filling material scores in all thirds is presented in Table 3. There was low interexaminer variability in the SEM image evaluation (κ value = 0.95). In the coronal and middle thirds, the mean score of the residual filling material in the PIPS group was significantly lower than that in the ultrasonic and sonic (EndoActivator) groups (both $p < 0.05$), and it was significantly lower in the ultrasonic group than in the sonic group ($p < 0.05$). In the apical third, there was a significantly lower residue score for both the ultrasonic and PIPS groups (1.65 ± 0.33 and 1.76 ± 0.26, respectively) than for the sonic group (2.61 ± 0.43, both $p < 0.05$), but there was no statistical difference between the ultrasonic and PIPS groups ($p > 0.05$). All three experimental groups were significantly different from the control groups in the apical, coronal, and middle thirds (all $p < 0.05$). No groups demonstrated complete filling material removal from the canals (Fig. 3).

Discussion

Complete elimination of gutta-percha and sealer used in previous fillings is essential, and it ensures thorough disinfection and sterilization of the root canal systems, which is a crucial step in successful endodontic retreatment.[22] Infected pulp tissue,

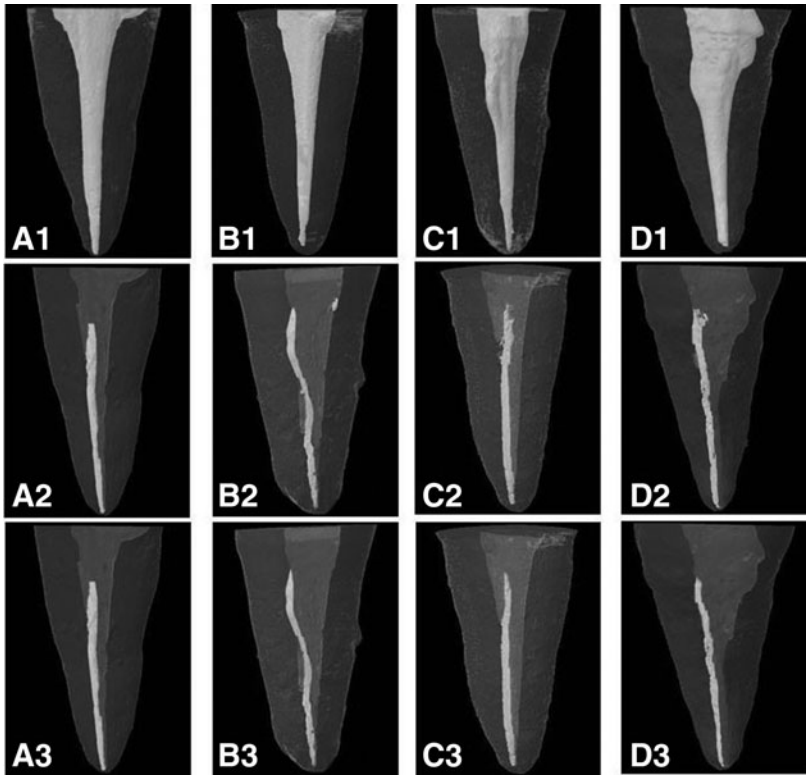

FIG. 1. Three-dimensional reconstruction of micro-CT scans showing filling material after obturation, mechanical retreatment, and additional activation of NaOCl with different devices. Control (A1, after obturation; A2, after mechanical retreatment; A3 after additional irrigating procedure); EndoActivator (B1, after obturation; B2, after mechanical retreatment; B3, after additional irrigating procedure); ultrasonic (C1, after obturation; C2, after mechanical retreatment; C3, after additional irrigating procedure), and photon-induced photoacoustic streaming (PIPS) (D1, after obturation; D2, after mechanical retreatment; D3, after additional irrigating procedure).

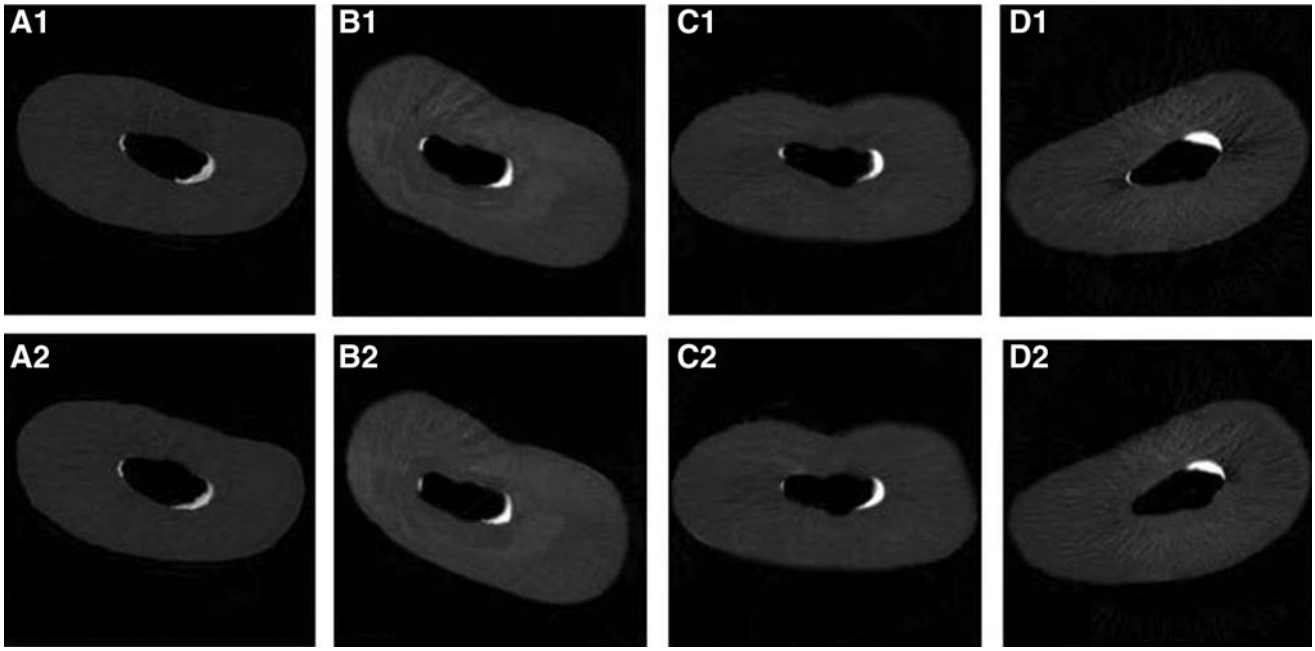

FIG. 2. Cross-sectional micro-CT image of residual filling material before and after additional activation of NaOCl with different devices. Control (A1, before irrigation; A2, after irrigation); EndoActivator (B1, before irrigation; B2, after irrigation); Ultrasonic (C1, before irrigation; C2, after irrigation), and photon-induced photoacoustic streaming (PIPS) (D1, before irrigation; D2, after irrigation).

bacteria, or their products, which may be hidden beneath the residual canal filling material or entangled with the remnants, may largely reduce the cleaning and disinfecting capacities of the mechanical and chemical procedures.[23] Consequently, the unremoved pulp tissue, bacteria, and their products are major causes of persistent periapical infection.[24] Therefore, mechanical (stainless steel hand files, NiTi rotary systems), chemical (solvents), and thermal (heat carrying instruments) techniques and then combinations are used clinically. To date, there are no treatment regimens that could produce canal walls completely free of all root-filling residue.[25] Compared with hand files (Hedstrom files and K files) and other NiTi rotary instruments (ProFile, Mtwo and D-RaCe), the ProTaper universal rotary retreatment system can remove root canal filling material more quickly and effectively, but it is not able to completely eliminate filling materials.[26–31,] Additionally, high anatomical variability and complexity of oval-shaped root canals, obviously increasing the difficulty of the root canal cleaning and shaping, represents a major challenge and requires additional procedures in root canal retreatment.[10,32,33] In the present study, sonic (EndoActivator), ultrasonic and laser (PIPS) activation were examined as additional methods for removing remnant fillings from the oval-shaped root canal of the maxillary first premolar after the ProTaper universal rotary instrumentation.

Various techniques have been used to evaluate the residual filling materials left in the root canal after retreatment. SEM can often provide direct topographical and morphological data on the filling materials, especially the presence of sealer on the surface of the root canal walls.[34] Micro-CT can provide 3D information and accurate quantification data (volume) of the remaining filling materials.[20] There are two major techniques that are used for this type of study, as they are complementary and can provide sufficient data for analyzing the removal of filling materials from the root canal system. Therefore, the present study used both SEM and micro-CT to assess the effectiveness of the three additional activation techniques on removing the remaining filling materials.

SEM and micro-CT analysis results showed that additional sonic (EndoActivator), ultrasonic, and laser (PIPS) procedures significantly eliminated the remaining filling materials from the maxillary first premolars compared with the control group. Among the three experimental techniques, the laser (PIPS) activation procedure was the most effective of the three techniques at removing the filling remnants from the canal walls. Similarly, previous studies have shown that PIPS was effective at debriding and cleaning the root canal surfaces.[35,36] For example, Lloyd et al. found that PIPS was more effective at eliminating organic debris from the canal than was standard needle irrigation.[17] Our recent study also showed that PIPS irrigation techniques obtained a greater reduction of $Ca(OH)_2$ and better cleanliness of the isthmus area than EndoActivator and needle irrigation.[18] All of these findings indicated that PIPS could be a highly promising laser application in endodontics and other areas of dentistry.

Table 3. Sealer Residue Scores in Each Root Canal Third After Each Activation Technique (Mean ± SD)

Group	*Coronal*	*Middle*	*Apical*	*Overall*
Control	2.08 ± 0.27^{IV}	2.56 ± 0.32^{IV}	2.95 ± 0.40^{III}	2.53 ± 0.51^{IV}
Sonic	1.31 ± 0.29^{III}	1.89 ± 0.36^{III}	2.61 ± 0.43^{II}	1.94 ± 0.46^{III}
Ultrasonic	0.84 ± 0.32^{II}	1.35 ± 0.36^{II}	1.76 ± 0.26^{I}	1.32 ± 0.26^{I}
PIPS	0.35 ± 0.32^{I}	0.75 ± 0.31^{I}	1.65 ± 0.33^{I}	0.92 ± 0.22^{I}

[I-IV] Ranking: there were significant differences ($p < 0.05$) between groups with different ranks at the same level.

PIPS, photon-induced photoacoustic streaming.

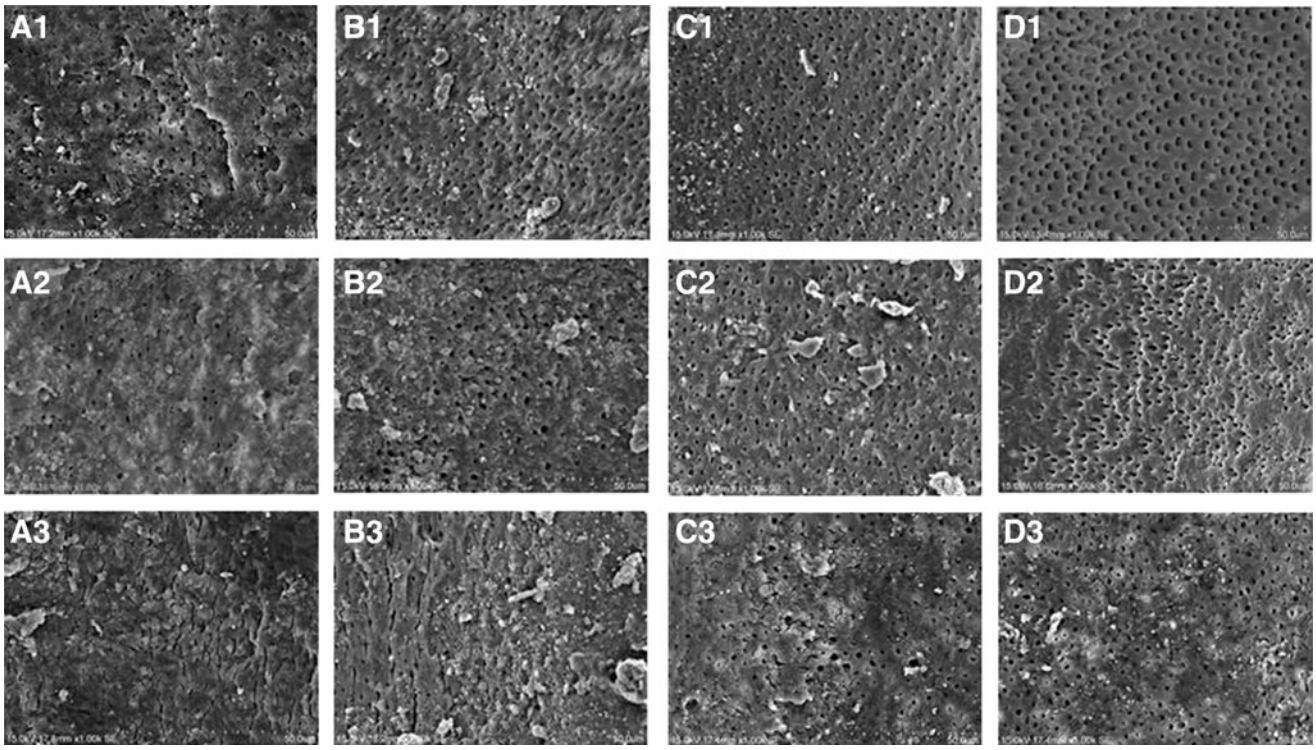

FIG. 3. Scanning electron microscopy (SEM) images of residual filling material in the coronal, middle, and apical thirds of the root canal after additional activation of NaOCl. Control (A1, coronal third; A2, middle third; A3, apical third); sonic (B1, coronal third; B2, middle third; B3, apical third); ultrasonic (C1, coronal third; C2, middle third; C3, apical third), and photon-induced photoacoustic streaming (PIPS) (D1, coronal third; D2, middle third; D3, apical third).

The PIPS mechanism in endodontics consists of an Er:YAG laser (wavelength of 2940 nm) with a 14 mm long and 300 μm diameter quartz tip at the pulp chamber.[16] This technique is mainly based on photoacoustic and photomechanical effects rather than on photothermal effects.[37] Therefore, it is reasonable to speculate that the better performance of PIPS in removing the residue from filling material was a result of the cavitation effect through formation of explosive vapor bubbles.[36] Moreover, Er:YAG laser energy exhibits the highest absorption rate in water and hydroxyapatite, which causes evaporation of fluid to allow for movement of the of fluid through the root canal system.[38,39] In addition, interaction of each impulse with the water molecules creates successive shock waves that result in the formation of a powerful streaming fluid,[16] which might facilitate the effectiveness of PIPS in removing the filling materials.

Results from the present study showed that an ultrasonic technique was superior to the sonic (EndoActivator) technique in removing filling material residue, especially in the coronal and middle thirds of the root canal. This result is in agreement with previous studies reporting that ultrasonic activation was significantly better at eliminating dentin debris than was sonic activation.[40] Theoretically, the mechanism of ultrasonic cleaning is based on the transmission of acoustic energy from an ultrasonically vibrating file to an irrigant, which means that high-frequency ultrasonic waves cause acoustic streaming and cavitation of the irrigant to remove the filling materials on root canal walls.[41] EndoActivator works by carrying a sonically driven tip to activate the irrigant to remove the filling materials.[42] A higher frequency wave results in a higher irrigant flow rate.[43] Therefore, a higher frequency ultrasonic system is expected to be more effective at removing filling materials than a sonic device.

In the present study, the additional use of PIPS and ultrasonic techniques after using the ProTaper Universal retreatment system resulted in a significant improvement in removing the remaining filling materials. Similarly, the additional effect of removing the residual filling materials were also observed by the use of the self-adjusting file (SAF) after ProTaper Universal retreatment system and R-Endo retreatment rotary instruments.[44,45] In contrast, arques da Silva et al. reported that the additional use of ProTaper F4 after using the ProTaper Universal retreatment system did not produce significant improvement in removing the remaining filling materials.[31] In addition to the laser or mechanical additional applications, Bodrumlu et al. reported that the combination of Gates Glidden drills (size 4) and Hedstrom files (size 30) could also be effectively eliminated the filling materials, especially for the straight root canal.[46] However, compared with these mechanical or manual additional techniques, PIPS, worked by only inserting the laser tip into the coronal third of the canal, might have a lower risk of instrument fracture or other complications.

None of the experimental techniques completely eliminate filling material residues from the maxillary first premolar root canals; filling materials in amounts ranging from 1.46 ± 0.30 to $2.21 \pm 0.46\,mm^3$ remained in the canals. This finding is in harmony with those of previous studies.[6–8,30] Moreover, all three experimental techniques showed better efficacy in removing filling materials from the coronal and

middle thirds compared with the apical third of the canal. Conversely, Abramovitz et al. reported that a self-adjusting file was more effective in removing residual gutta-percha from the apical section than the coronal and middle sections of the mesial canals of mandibular molar after using ProTaper Universal retreatment files.[30] This is likely because of the different techniques applied. When the PIPS tip was placed only in the coronal part, its photoacoustic shock wave may weaken over the distance to the apical one third, and, therefore, the effectiveness of PIPS in removing filling materials from the apical part was decreased. Similarly, Zhu et al. found that PIPS was more effective in removing the smear layer and debris in the coronal and middle thirds than in the apical third.[47] It is expected that ultrasonic and sonic techniques will be less effective in removing filling material in the apical region, and this was likely a result of the reduction in the acoustic microstreaming and/or cavitation effect after the ultrasonic file or sonic tips entered the apical vapor lock.[41,42] As mentioned, the optic tip of the PIPS device was only placed in the coronal third of the canal, which was likely to preserve the root structure.[16] To sufficiently remove the filling materials in the apical third of the root canal, further studies shoud be conducted to optimize PIPS parameters and the PIPS tip location in the root canal.

Limitations

Limitations of the present study should be noted. First, this study was conducted on teeth with straight oval root canals, and the findings cannot be directly applied to teeth with curved root canal systems, because root curvature is a crucial factor in affecting the efficacy of root canal instrumentation.[9] Second, similar to other similar studies,[24,31,35] decoronating teeth, which is impossible in the daily practice, were done in the present study in order to standardize the specimens; therefore, the conclusion of the current study cannot be directly extended to clinical conditions. Further research is needed to complement the results of the present study.

Conclusions

The additional use of PIPS for the activation of NaOCl was superior to sonic and ultrasonic techniques in removing the remaining filling materials after standard retreatment procedures using the ProTaper universal retreatment system. However, none of the experimental techniques completely removed the filling remnants from the root canal of the maxillary first premolars.

Author Disclosure Statement

The authors declare no competing financial interests.

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Address correspondence to:
Chengfei Zhang
Comprehensive Dental Care, Endodontics
Faculty of Dentistry
The University of Hong Kong
Prince Philip Dental Hospital
34 Hospital Road, Sai Ying Pun
Hong Kong SAR, 999077
China

E-mail: zhangcf@hku.hk

or

Xiaojing Huang
Department of Endodontics and Operative Dentistry I
School and Hospital of Stomatology
Fujian Medical University
246 Yangqiao Zhong Road
Fuzhou, Fujian 350002
China

E-mail: hxiaoj@163.com

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SHORT COMMUNICATION

Irrigant flow during photon-induced photoacoustic streaming (PIPS) using Particle Image Velocimetry (PIV)

Jon D. Koch[1] · **David E. Jaramillo**[2] · **Enrico DiVito**[3,4] · **Ove A. Peters**[5]

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Abstract
Objectives This study aimed to compare fluid movements generated from photon-induced photoacoustic streaming (PIPS) and passive ultrasonic irrigation (PUI).
Materials and methods Particle Image Velocimetry (PIV) was performed using 6-μm melamine spheres in water. Measurement areas were 3-mm-long sections of the canal in the coronal, midroot and apical regions for PIPS (erbium/yttrium-aluminium garnet (Er:YAG) laser set at 15 Hz with 20 mJ), or passive ultrasonic irrigation (PUI, non-cutting insert at 30 % unit power) was performed in simulated root canals prepared to an apical size #30/0.04 taper. Fluid movement was analysed directly subjacent to the apical ends of ultrasonic insert or fiber optic tips as well as at midroot and apically.
Results During PUI, measured average velocities were around 0.03 m/s in the immediate vicinity of the sides and tip of the ultrasonic file. Speeds decayed to non-measureable values at a distance of about 2 mm from the sides and tip. During PIPS, typical average speeds were about ten times higher than those measured for PUI, and they were measured throughout the length of the canal, at distances up to 20 mm away.
Conclusions PIPS caused higher average fluid speeds when compared to PUI, both close and distant from the instrument. The findings of this study could be relevant to the debriding and disinfecting stage of endodontic therapy.
Clinical relevance Irrigation enhancement beyond needle irrigation is relevant to more effectively eradicate microorganisms from root canal systems. PIPS may be an alternative approach due to its ability to create high streaming velocities further away from the activation source compared to ultrasonic activation.

Keywords Particle image velocimetry · Photon-induced photoacoustic streaming · Ultrasonics · Endodontics · Irrigation

Introduction

An important aim of root canal treatment is the elimination or prevention of periradicular periodontitis; it is well established that bacteria and their toxins are the cause of this disease [13] and therefore eradication, or at least reduction to a biologically acceptable number, of intracanal microorganisms is required [23]. Enlargement of root canals with current root canal instruments reduces bacterial counts even in the case of buccolingually wide root canals [24]. 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 [2, 11]. Different agitation techniques have been proposed to improve the efficacy of irrigation solutions, including agitation with hand

✉ Ove A. Peters
opeters@pacific.edu

[1] Department of Mechanical and Aerospace Engineering, Allen School of Engineering, Trine University, Angola, IN, USA

[2] Department of Endodontics, University of Texas Health Science Center at Houston, School of Dentistry, Houston, TX, USA

[3] Private Practice, Arizona Center for Laser Dentistry, Scottsdale, AZ, USA

[4] Arizona School of Dentistry and Oral Health, Mesa, AZ, USA

[5] Department of Endodontics, University of the Pacific Arthur A. Dugoni School of Dentistry, 155 5th St, San Francisco, CA 94103, USA

files, gutta-percha cones, plastic instruments and sonic and ultrasonic devices [9].

Lasers have been explored as a means to enhance endodontic treatment procedures in a variety of ways in addition to enhance irrigation efficacy. One of the more recent suggestions is the use of laser energy to enhance irrigation. Lasers may be used directly to irradiate radicular walls [28] or to activate photosensitizers that associate with bacteria [8]. Both of these approaches have failed to consistently promote bacteria-free root canal systems in vitro [19, 28].

More recent work proposed to activate irrigation solutions by the transfer of pulsed laser energy [3, 7]. Irrigation enhanced by erbium/yttrium-aluminium garnet (Er:YAG) laser light appears to be effective in removing dentin debris [3] and smear layer [5]. Specifically, the action of a pulsed Er:YAG laser via photon-induced photoacoustic streaming (PIPS) has been shown to be effective in root canal debridement [5, 10, 21].

However, to the best of our knowledge, the detailed nature of the streaming patterns that permit PIPS to disinfect root canals has not been established.

Particle Image Velocimetry (PIV) is a non-intrusive technique for the measurement of a velocity field [22]. The displacement of small tracer particles added to a fluid is recorded by high-speed imaging and analysed using statistical correlation methods to extract the velocity distribution in the examined plane [22].

The aim of the present study was to compare differences in fluid movement generated from PIPS and passive ultrasonic irrigation (PUI) in a curved simulated root canal. Particle Image Velocimetry measurements were performed to assess the magnitude and distribution of the irrigant flow velocity stimulated by PIPS device inside a simple specimen and to compare flow created with an ultrasonic device under experimental conditions that simulated clinical use.

Materials and methods

An acrylic endodontic training block (Viade Products, Camarillo, CA, USA) with a simulated root canal was instrumented to a size #30/0.04 by using Vortex rotary files (Dentsply Tulsa Dental, Tulsa, OK, USA) in a crown-down technique. The canal was irrigated with distilled water by using a syringe and a 30-g irrigation needle and then dried with paper points. The prepared block was clamped to a translation stage to control its location. After needle injection of the water/particle mixture as described below, one of the two tested irrigation devices was placed into the canal (PIPS laser fibre or passive ultrasonic irrigation tip as described below). The respective tip was secured in position using a clamping device; the tip of the PIPS fibre was inserted to a depth of about 3 mm below the top of the plastic block while the ultrasonic instrument (K file size #15, Satelec-Acteon, Merignac, France) was inserted 3 mm below the top of the orifice of the canal with the tip being visible (section a, Fig. 1). This depth was sufficient to ensure the activation of the fluid within the canal but not so deep as to allow the instrument to bind against the canal wall. The location of each instrument is indicated by the outlines in Figs. 1 and 2. The ultrasonic instrument was connected to a Spartan USA Endo-1 unit (Obtura Spartan, Algonquin, Chicago, IL, USA) at an intensity setting of 3 (on a scale of 0 to 10). The light source for the PIPS tip was an Er:YAG laser (2940 nm) set to 20 mJ, 15 Hz and a 50-μs pulse duration. The fibre tip was tapered and stripped; it had a diameter of 600 μm (LightWalker AT, Fotona, Ljubljana, Slovenia).

The PIV configuration is described in detail elsewhere [15]; in brief, a double-pulsed, frequency-doubled YAG laser (Solo PIV; New Wave Research, Sunnyvale, CA, USA) was used as the excitation source. The beam was expanded, steered and focused through a ×5 microscope objective onto the root canal model that was filled with a mixture of dye-coated microparticles (6-mm melamine resin particles coated with rhodamine B, Fluka, #74,097, Buchs, Switzerland) and distilled water. Fluorescence was captured through the objective lens and focused onto a CCD camera (LaVision Imager

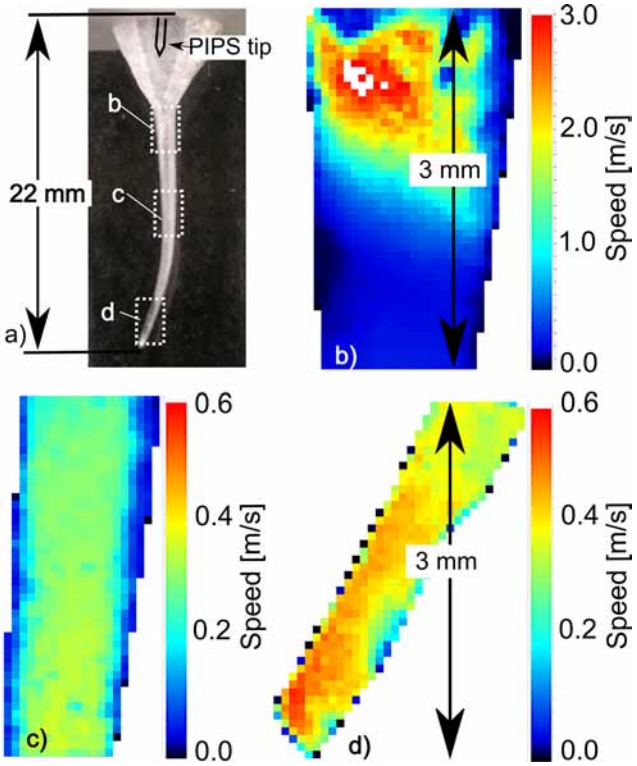

Fig. 1 Image (**a**) at *top left* shows entire canal model and locations where PIV measurements were made with methods detailed in an earlier publication [24]. Images (**b**), (**c**), (**d**) show average speeds from at least 100 PIV measurements taken 0.8 ms after a 15 mJ PIPS laser pulse in the locations indicated by boxes in (**a**). The velocity scale for each image is indicated by the *colour bar* on the right. The PIPS tip was located at the top of the coronal third for each measurement as outlined in the *left hand image*

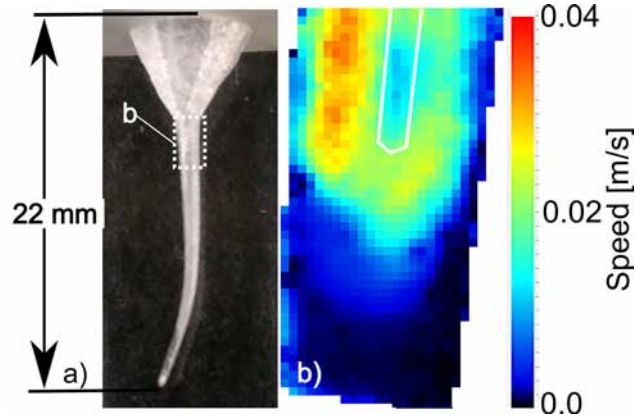

Fig. 2 Image at left (**a**) shows the canal model and in box (**b**) the location where velocity measurements were made with the ultrasonic file inserted into the top of the canal. The position of the end of the non-activated file (behind the PIV image plane) is shown as a *white outline* in the image on the right. Image on the right (**b**) shows the average speed around the file from 200 PIV measurements taken during activation of the file in a static position. Average speeds immediately around the file are approximately 0.03 m/s. The average speed decays to less than 0.01 m/s within a distance of 2 mm below the file (the *bottom* of the image on the right). There was no observable fluid movement in lower sections of the canal model

Pro X 2 M; LaVision, Göttingen, Germany). Non-fluorescent light was rejected using a dichroic reflector and band-pass filter.

When the ultrasonic file was studied, images were separated in time by 150 μs. This time corresponds to slightly more than four periods of ultrasonic oscillation; the measurements are thus suitable for capturing the average velocities around the file but not the velocity of the file itself or of the oscillatory components that have been noted in the area immediately adjacent to the file [27]. The ultrasonic file was turned on approximately 1 s before images were acquired in a free-running mode without regard to synchronization. When the PIPS tip was studied, images were separated by 75 μs in the lower section of the canal (Fig. 1, sections c and d) but separated by 15 μs in the upper section of the canal because the velocities in the upper section were found to be much faster than other locations. All PIPS images were synced to a time that was 0.8 ms after the firing of the laser. Synchronization between the image acquisition and the PIPS laser was accomplished by detecting a signal from the PIPS laser flashlamp using a fast photodiode (Thorlabs, DET10A, 2 ns response time, Newton, NJ, USA) and monitoring on an oscilloscope. The period and delay of the PIV system, which was also monitored on the oscilloscope, was then adjusted to match the free-running PIPS laser.

Sets of image pairs were cross-correlated using DaVis 7.1 (LaVision). Initial interrogation windows were 128 pixels on a side. Based on 1/4 of this window size, the maximum measureable velocity component was approximately 0.4 m/s for PUI and 0.8 m/s for PIPS (4 m/s in the upper region of Fig. 1a). The minimum measurable velocity component based on a conservative 0.5 pixel detectable displacement was about 0.005 m/s for PUI and 0.01 m/s for PIPS (0.05 m/s in the upper region). These minimum measureable velocities serve as estimates of the measurement uncertainty. Multipass processing was used, ultimately resulting in vectors corresponding to 64-pixel windows with 50 % overlap, or 1 vector every 0.06 mm. The results of 200 vector images were averaged for PUI irrigation (Fig. 2, discussed below), while the PIPS measurements were averaged over at least 100 vector images (Fig. 1, discussed below). Syncing the PIV measurement with the PIPS laser required to start recording images in a non-synced mode while adjusting the PIV-PIPS timing; these non-used images consumed some of the available RAM in the computer memory, resulting in fewer images available for PIPS measurements.

Reynolds number

The measured speeds in each region were averaged over space and time to obtain a single ensemble-averaged velocity. A Reynolds number, *Re*, was then calculated based on the density ($\rho = 998$ kg/m^3) and viscosity ($\mu = 0.955$ g/m s) of water at 22 °C and the ensemble-average speeds (v) in each PIPS region as follows:

$$Re = \frac{\rho v D}{\mu}$$

The characteristic dimension, D, was the average canal diameter in each imaged region.

Results

Photon-induced photoacoustic streaming

Figure 1 shows that large average fluid speeds (> 3 m/s) are present in the coronal portion of the canal (Fig. 1b). Average fluid speeds are somewhat slower in the middle of the canal (Fig. 1c) but still significant (approx. 0.3 m/s) and fairly uniform over the imaged region. Average speeds in the apical region (Fig. 1d) are nearly the same as in the middle of the canal (0.3–0.5 m/s). Ensemble (spatial and temporal) averaging was performed to obtain characteristic speeds at each measured location as shown in Fig. 1. From top to bottom, the spatially and temporally averaged speeds were 1.02, 0.25, and 0.43 m/s, respectively. These correspond to Reynolds numbers of approximately 1670 (coronal third), 290 (midroot) and 280 (apical), respectively. As a means of describing the width of the speed distribution, the standard deviations of the measured speeds in each region were 1.25 m/s in the coronal region, 0.19 m/s in the midroot and 0.28 m/s in the apical region.

Individual images indicate the presence of large-scale vertical motions in the mid and lower portions of the canal. Example instantaneous images of the PIPS flow in the apical region are shown in Fig. 3. At the moment of measurement, these flow fields could be dominated by flow in either the upward (Fig. 3a, c) or the downward (Fig. 3b, d) direction, likely a result of the highly unsteady nature of the flow and the variability in the timing between the fluid motion and the flashlamp output. Occasional vortical structures (top of Fig. 3a) and regions of apparent low velocities were also noted; areas of large changes in velocity over a short distance indicate the presence of shear stresses.

Passive ultrasonic irrigation

For PUI, Fig. 2 shows the average speed around the file from 200 PIV measurements taken during activation of the file in a static position (Fig. 2a). Average speeds around the file are approximately 0.03 m/s, only about one tenth the value of those observed throughout the canal during the PIPS experiments. Furthermore, the average speed decays to less than 0.01 m/s within a distance of 2 mm below the file (the bottom of the image on the right). There was no observable fluid movement in lower sections of the canal model.

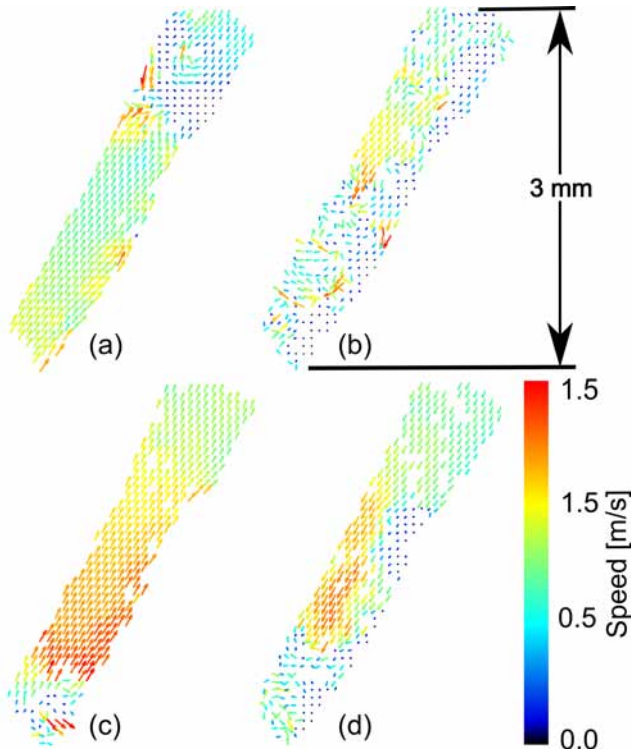

Fig. 3 Single-measurement images of PIPS velocity fields in the apical region. Large-scale axial flows are occasionally present and are noted to be travelling in each direction (**a**, **c**). Some vortical motions are noticeable (top of **a**). Jet-like structures are also found (**b**, **d**)

Discussion

In this study, irrigant fluid speeds were assessed by PIV, comparing laser-activated irrigation (PIPS) and passive ultrasonic irrigation (PUI). Fluid movement measured distant from the PIPS tip was evident, compared to the lack of obvious movement much past 1 mm below an ultrasonic tip. Since the estimated measurement uncertainty of a PIPS PIV data point was ±0.05 m/s while that of the PUI experiment was ±0.005 m/s, the differences between the ensemble-averaged velocity in the midroot region (0.25 m/s) and the speed near the PUI instrument (0.03 m/s) are significantly outside the measurement uncertainty. Hence, PIPS was associated with a significantly greater average speed than PUI by at least a factor of 10.

Notably, at small distances below the tip the overall fluid velocities with PIPS were about ten times higher than those predicted from numerical simulation [1, 25] for side-vented needles, at similar distances from the needle tip. These velocities relate to the experimental conditions, with plastic rather than dentin as surface and absolute numbers in actual root canal systems will likely vary.

The Reynolds numbers, if compared to transitional regimes in pipe flow, would tend to indicate laminar flow; however, given the unsteady nature of this flow and the oscillations noted in single images, we would suggest a classification of a transitional flow for at least a portion of the time after the PIPS laser pulse.

It is interesting to note that the ensemble-averaged speed appears to increase in the apical region relative to the midroot section (0.43 m/s in the apical compared to 0.25 m/s in the midroot). Passive ultrasonic irrigation typically results in a decay in the velocity field as one moves away from the excitation source [26]. Possible reasons for this observation include the existence of localized resonance between the excitation field and the flow field inside the cavity as well as statistical uncertainty in the samples collected. Resonance may exist in some areas inside the canal due to the superposition of pressure waves and their impact on the velocity field.

It is also possible that the samples, obtained via the two-dimensional interrogation of a three-dimensional flow field that oscillates rapidly in time, are not sufficiently representative of the averaged ensemble speed. As indicated by the substantial standard deviations of 0.19 m/s in the midroot and 0.28 m/s in the apical region, however, there is significant overlap between the distributions. Further studies on how the flow field evolves after a single PIPS laser pulse are needed to address this question.

Ultrasonic irrigation is thought to act via a process commonly referred to as acoustic streaming; recently, it was suggested that some cavitation may also occur [18]. Regardless, its efficacy depends on the ability of the activated instrument to oscillate [26] freely within the canal, and its action may thus be restricted or reduced in curved or small canals. The

inability to freely oscillate may be a reason that PUI had only limited effect in removing bacterial burden from bacterially contaminated small canals in vitro [21]. In the present experimental configuration, the PUI file was inserted only to a distance of 3 mm into the curved root canal in order to ensure there would be no binding of the file against the side. The induced flow field around the tip of the file is thus thought to be uninhibited by binding effects and is qualitatively similar to studies where the file has been fully inserted into a model canal in a non-binding configuration.

PIPS is believed to cause fluid motion via pressure waves that propagate outward from expanding and collapsing cavitational bubbles at the PIPS tip [6]. The size, shape and dynamic behaviour of the bubble are controlled by the shape of the tip and settings of the Er:YAG laser [5]. In this study, sub-ablative parameters were used to produce the cavitational bubble [3–5]. That is to say, the high absorption of the Er:YAG wavelength by water combined with the high peak power derived from the short pulse (50 μs) resulted in the rapid formation, expansion and subsequent collapse of a vapour bubble at the tip rather than any direct material removal at the edge of the canal. The rapid movement of the fluid around the tip propagates to the surrounding fluid, thus causing the PIPS effect.

The data in this study indicate that PIPS induces larger average speeds in the fluid within a model canal, compared to ultrasonic activation [16] and it does so at a significant distance from the instrument. Effective clinical irrigation is thought to originate from a combination of fluid dynamic or mechanical forces and chemistry. The velocity field is arguably the most important variable needed to characterize the shear stresses and bulk transport within the canal from a fluids perspective. Hence, the large difference in speeds between the two instruments is suggestive of a clinical advantage for the disinfection and possible biofilm detachment in the main root canal. However, this does not address dentinal tubule contamination and different experiments will be necessary to test the effect of irrigation activation in this condition.

To the best of our knowledge, direct clinical comparisons between the two irrigation techniques tested here in vitro are not yet available. However, other studies can lend some insight; it has been reported that shear forces of up to 2.8 N/m^2 were generated with plastic finishing files designed for irrigation enhancement [14]. This is comparable to findings on continuous application of ultrasonic activation [16]. Of note, these values would appear to be too low to completely detach biofilm from substrate; for example, Huang at al. [12] indicated that up to 46 N/m^2 is needed to detach *Pseudomonas aeruginosa* from silicone material. Layton et al. found that *Enterococcus faecalis* biofilm in plastic models was reduced but not eliminated after continuous ultrasonic activation [16].

Shear-stress measurements are not available for PIPS, but Ordinola-Zapata et al. demonstrated the superior ability of PIPS-supported irrigation to disrupt biofilm on dentin substrate [20]. Likewise, debris was more effectively removed from root canal systems in vitro with laser-activated PIPS irrigation, compared to needle irrigation [17]. Further experiments could be designed to test different canal shapes that, within the limits of canal anatomy and available preparation techniques, could promote optimized fluid movement.

In conclusion, our findings suggest that PIPS-induced fluid movement is more prominent at a distance compared to ultrasonic irrigation. To extend the findings of this using PIV, more experiments under varied conditions should be performed to better understand streaming patterns of current irrigation modalities under clinical conditions.

Conflict of interest This project was financially supported by Medical Dental Advance Technologies Group, of which Dr. DiVito is a partner.

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Elimination of Intracanal Tissue and Debris through a Novel Laser-activated System Assessed Using High-resolution Micro–computed Tomography: A Pilot Study

Adam Lloyd, BDS, MS, Jonathan P. Uhles, DDS,* David J. Clement, DDS,* and Franklin Garcia-Godoy, DDS, MS, PhD, PhD†*

Abstract

Introduction: Laser-activated irrigation to remove organic debris from canal isthmuses was investigated using x-ray microfocus computed tomographic imaging. **Methods:** A total of 14 extracted human mandibular molars were used. The mesial canals were prepared using a standardized instrumentation protocol. Two groups (n = 7) underwent final irrigation using either standard needle irrigation (SNI) or photon-induced photoacoustic streaming (PIPS). After enlarging canals to 30/.06, canal volumes were reconstructed from micro–computed tomographic scans before and after irrigation to assess removal of organic tissue and inorganic debris by quantitative analysis of the superimposed volumes. Comparisons of the volumes were made using 2-way analysis of variance and Tukey method, with statistical differences considered significant at the alpha = 0.05 level. **Results:** Debris removal and an increase in root canal system volume for the laser-activated PIPS group was more significant ($P < .001$) than for the SNI group ($P = .04$). Irrigation using PIPS increased the canal volume and eliminated debris from the canal system 2.6 times greater than SNI. **Conclusions:** Eliminating debris from complex canal spaces found in mandibular molars was achieved at a significantly greater level using laser-activated PIPS irrigation compared with SNI. *(J Endod 2014;40:584–587)*

Key Words

Er:YAG laser, photon-induced photoacoustic streaming, root canal disinfection

From the *Department of Endodontics and †Bioscience Research Center, College of Dentistry, University of Tennessee Health Science Center, Memphis, Tennessee.

Address requests for reprints to Dr Adam Lloyd, Department of Endodontics, University of Tennessee Health Science Center College of Dentistry, 875 Union Avenue, Memphis, TN 38163. E-mail address: alloyd@uthsc.edu

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Essential to the success of endodontic treatment for teeth with apical pathosis is the elimination of microorganisms and their byproducts from the root canal system (1, 2). It would appear that a limiting factor in canal disinfection is the inability to adequately have irrigant enter canal isthmuses because they are blocked by hard tissue created during mechanical preparation (3) or infected pulp tissues and their associated microbes. Additionally, the blockage of canal isthmuses prevents fluid interchange regardless of the volume of irrigant (4–6). Examination of the intricacies associated with molars, the most commonly treated teeth even among general dentists (7), has shown that the isthmus remains untouched during canal preparation (8). Examination of the apical 5 mm of molars found the incidence of canal isthmuses and complex systems to range from 17.25% to 80% (4, 5, 9, 10), which if left untreated could account for post-treatment disease (11). Enlarging canals to include isthmus preparation would result in gross enlargement and likely root perforation while not significantly increasing the amount of contact of the instrument to dentin in the canal (12).

The elimination of microbes has been shown to be enhanced when using lasers in canal disinfection (13). Recently, canal disinfection protocols such as photodynamic therapy have been investigated to decrease the intracanal bacterial load (14). Targeted delivery of photosensitizer into the root canal system complexities still proves challenging, and elimination of bacteria is not guaranteed. Another laser-activated approach, photon-induced photoacoustic streaming (PIPS) involving agitation of standard intracanal irrigants, has been shown to create explosive vapor bubbles with secondary cavitation effects, enhancing fluid interchange and the removal of debris (15–17). Laser activation using a modified tip design has shown the removal of the smear layer in the presence of EDTA (18, 19).

A novel 9-mm-long, 600-μm quartz tip for use in an Er:YAG laser has been developed that transfers energy into the irrigant causing removal of organic debris with only a minor increase in tooth temperature. PIPS is a nonthermal subablative phenomenon that has also been shown to eliminate the smear layer in the presence of EDTA (19) and provide more negative bacterial samples than ultrasonic activation (20). The tip is tapered and stripped of its polyamide sheath 3 mm from its end. PIPS has the potential to remove tissue from intricate canal anatomy and, with the use of an appropriate irrigant, provide better canal disinfection (19). The aim of this study was to assess the change in root canal system volume by the removal of tissue from the radicular pulp space using PIPS laser activation assessed using micro–computed tomographic (micro-CT) scanning.

Materials and Methods

For the purpose of this study, 16 recently extracted vital human mandibular molars had only their mesial roots prepared. Tooth length was standardized by grinding the occlusal plane flat and embedding each tooth in a matrix that allowed precise positioning on the micro-CT stage. Scanning of all specimens before access cavity preparation was performed using micro-CT imaging (Varian Medical Systems, Palo Alto, CA) at 75 kV and 100 μA through 180° of rotation around the vertical axis and a single rotation step of 0.9° during a 15-minute scan with a source to object distance of 300 mm and

a cross-sectional pixel size of approximately 30 μm. Each slice was a 16-bit addressable 1,024 × 1,024 area that was used to create a 1-K 3-dimensional image volume-rendered representation to screen for the presence of an isthmus (VG Studio Max 2.2; Volume Graphics GmbH, Heidelberg, Germany).

Access cavity preparation was performed. The working length was established by visualizing an ISO #10 file at the canal terminus and subtracting 0.5 mm, which provided the canal preparation length. Instrumentation was completed to size 30/.06 (ProFile Vortex; Dentsply Tulsa Dental Specialties, Tulsa, OK) in a crown-down fashion. The apical size determination was dictated by the ability to irrigate to within 1 mm of the working length using a 30-G side-vented Luer-Lok needle (ProRinse, Dentsply Tulsa Dental Specialties). Samples were irrigated using 10 mL 6% sodium hypochlorite (The Clorox Co, Oakland, CA) during canal preparation, and canal patency was maintained. The pulp chamber was flooded with sodium hypochlorite and replenished with 1 mL irrigant after each instrument. The samples underwent a definitive scan with a slice thickness of 16.84 μm, designated the primary scan, from which the root canal system's initial volume was calculated. Teeth were randomly divided into the following experimental groups:

Group 1 (*N* = 7): Standard Needle Irrigation

The standard needle irrigation (SNI) protocol after canal preparation involved irrigation with a 30-G side-vented Luer-Lok needle delivering 4 mL 17% EDTA (Roth Drug Co, Chicago, IL) over a period of 60 seconds at a distance 1 mm short of the working length. This was followed by SNI of 10 mL 6% sodium hypochlorite delivered over 30 seconds.

Group 2 (*N* = 7): PIPS Laser-activated Irrigation

The PIPS protocol was followed exactly according to the manufacturer's instructions by a clinician proficient with the PIPS protocol. A 2,940-nm wavelength Er:YAG laser was used (Fidelis; Fotona, Ljubljana, Slovenia) at 15 Hz and 20 mJ using a 9-mm-long, 600-μm diameter endodontic fiber with the polyamide tip stripped back 3 mm. The tip was placed into the access cavity only and activated with each of the following irrigating solutions as they were applied into the access cavity with a 28-G irrigating needle:

1. Three 30-second cycles of continuous flow sodium hypochlorite
2. A 30-second cycle of water
3. A 30-second cycle of EDTA
4. Three 30-second cycles of water

The total volume of sodium hypochlorite and EDTA was the same as for the SNI group.

Controls (*N* = 2)

Two samples served as controls and underwent canal preparation to size 30/.06 (ProFile Vortex, Dentsply Tulsa Dental Specialties), irrigating with 6% sodium hypochlorite but without any postpreparation irrigation or PIPS.

A second micro-CT scan was performed on both groups and controls using the same parameters as the first scan. The pre- and post-backscatter projections were geometrically aligned with pin registration, and the 3-dimensional data sets were superimposed. The total volume from each scan was derived from voxels with black color interpreted as soft tissue, liquid, or air. Opaque (bright) voxels were interpreted as debris within the confines of the radiopaque canal walls. The removal of opaque voxels from each sample following irrigation protocols created a second canal volume. The data derived from the difference between the pre- and postirrigation regimens was recorded and analyzed.

Measuring the same teeth both before and after different irrigation regimens allowed the use of repeated measures statistical tests, thereby increasing statistical power by controlling for the variability in canal volume between teeth. The measurements in pre- and post-SNI and PIPS groups passed the Shapiro-Wilk test for normality (P = .44) and had equal variances (P = .91), so repeated measures 2-way analysis of variance was used to detect the interactive effects and Tukey HSD (α = 0.05). The power calculation for the sample size in the present study was 0.998. SigmaPlot 12.3 (Systat Software Inc, Chicago, IL) was used to conduct statistical analyses.

Results

No significant difference in the initial root canal system volumes between each group existed. There were significant differences between the SNI and PIPS irrigation groups and the time of measurement (pre- or post-irrigation) factors (P = .02). Although both treatments resulted in a significant increase in root canal system size, the interaction in the increase in debris removal was more significant for PIPS (P < .001) than for SNI (P = .04). The mean increase in the canal volume for PIPS (1.51 mm^3) was × 2.6 greater than for SNI (0.58 mm^3). Control samples represented no effective change in the root canal system volumes between scans.

Discussion

This *in vitro* study attempted to examine the effectiveness for intracanal debris removal using SNI and PIPS laser-activated irrigation assessed using micro-CT imaging from the mesial roots of freshly extracted vital mandibular molars. All samples exhibited complex root canal systems that included the presence of cul-de-sacs and isthmuses, which act as an impediment to allowing interchange of irrigants or intracanal medicaments when occupied by organic tissue and microbes or blocked by inorganic debris from instrumentation techniques. Given the anatomic complexities and the inability to remove hard and soft tissue debris, it is logical that the success rate for mandibular molar endodontics has been shown to be lower compared with other teeth (21). A previous study of complex root canal systems found isthmuses and regions secondary to the main canals to account for almost half the canal system, with the investigators unable to eliminate debris from the isthmus area using SNI (22). This finding is in agreement with a previous investigation that showed hard tissue accumulation in the canal system isthmus after canal preparation (3). However, no irrigation was used in the study. In the current investigation, there was a tendency for debris to be retained in the isthmus area because instrumentation debris was packed laterally into the isthmus. We found that this packed organic and inorganic debris was removed 2.6 times greater volumetrically using the PIPS irrigation protocol (Fig. 1*A–I*) as compared with SNI (Fig. 2*A–C*).

Some authors have suggested the need for large canal preparations (ie, greater than #60) to enable a 28-G needle to reach the canal terminus and reduce the microbial load significantly (23). Inherent in large apical size preparations is the potential for canal transportation while canal ramifications remain untouched by any instrument (24). Only energized irrigation sources have been shown to permit fluid interchange throughout the root canal system and the disruption of tissue and the significant removal of debris (25). This study used 30-G needles to ensure canal penetration to within 1 mm of the working length during SNI.

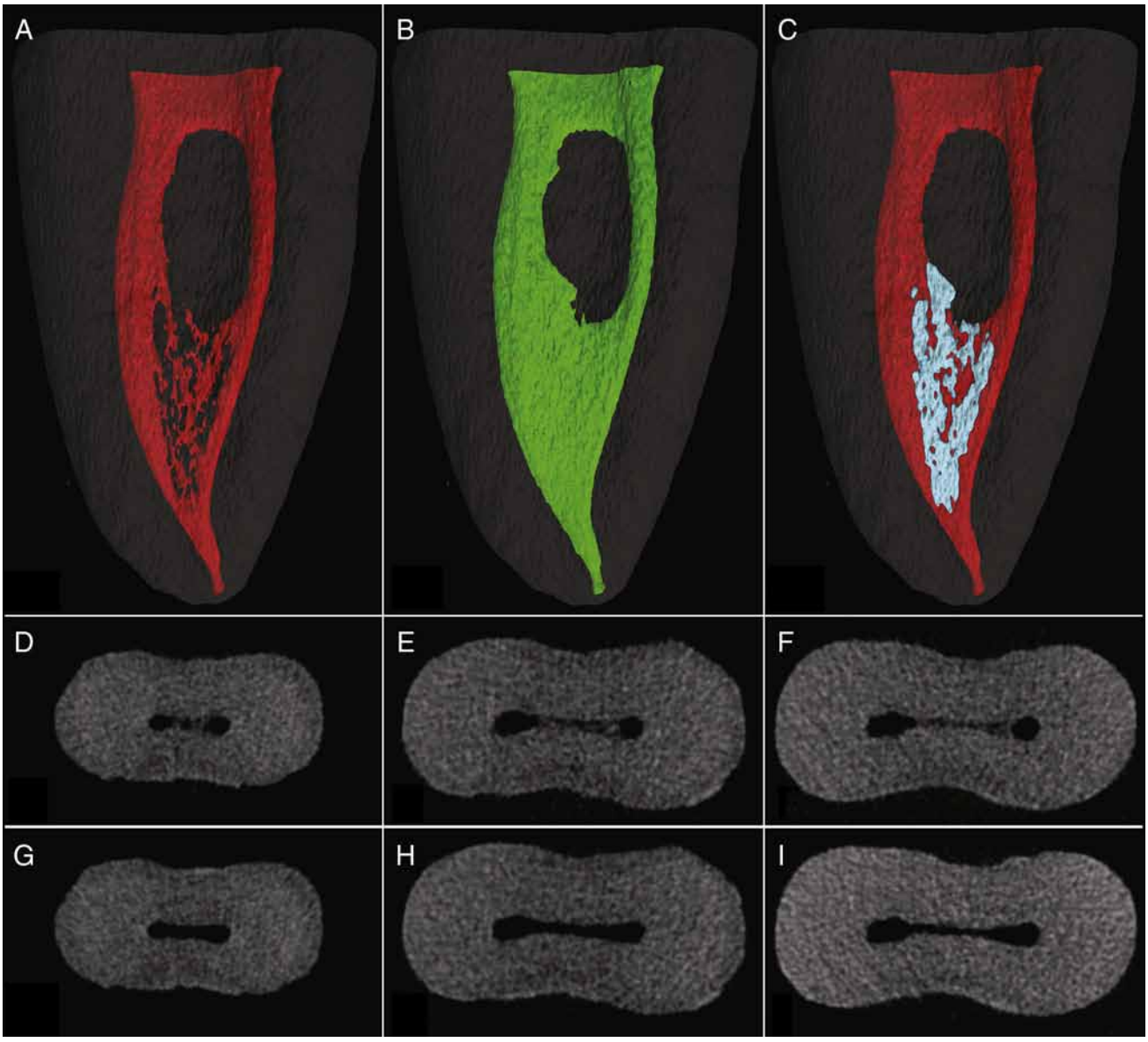

Figure 1. A 3-dimensional volumetric representation of a sample from the PIPS group. (*A*) Canal morphology following standardized instrumentation showing a complex isthmus system occupied with islands of soft tissue with surrounding calcifications. *Red* represents the canal system before laser-activated irrigation. (*B*) The *green* 3-dimensional representation signifies a change in volume after irrigation with PIPS and the removal of inorganic and organic debris from the isthmus. (*C*) Superimposition showing the original canal before irrigation and the altered canal after laser-activated irrigation. The difference between the 2 states is shown in *light blue*. (*D–F*) The axial slice from the same specimen at 2, 4, and 6 mm from the canal terminus before irrigation. Note the accumulation of hard tissue debris with voids in the isthmus area. (*G–I*) The corresponding axial slices after PIPS irrigation showing complete elimination of tissue from complex canal spaces.

Er:YAG lasers have shown the ability to create turbulent flow by virtue of creating a gaseous bubble at the laser tip as the irrigant is vaporized, resulting in expansion of a bubble as the laser continues to emit energy and evaporates the irrigant at the leading edge. At the end of the cycle, the vapor cools, causing the bubble to implode and separate from the firing tip (26). The alternating bubble expansion and implosion create a shear stress along the canal wall that is capable of removing biofilm and the smear layer. This nonablative system has been commercialized as PIPS. The stripped radial firing tip of the PIPS handpiece is placed at the orifice level, relying on pulsed energy to transmit an acoustic shockwave throughout the root canal system to disrupt debris and allow fluid exchange. PIPS has been shown to decrease the bacterial load significantly in teeth that had established biofilms (20), which is necessary for long-term clinical success and the disinfection of the root canal system. Instrumentation provides the convenience form that permits delivery of irrigants that kill bacteria. The delivery of irrigants to the canal terminus occurs with the PIPS tip firing at a significant distance from the apex, but the question of optimal canal size and taper has yet to be addressed. The convenience form of a 30/.06 rotary instrument was used in this *in vitro* study to closely mimic what the absolute minimal shape is to permit a 30-G needle close to the working length. The ability to create turbulent flow in unprepared areas of the root canal system is a significant benefit of irrigation with PIPS. In doing so, smaller convenience forms may be possible, sparing canal

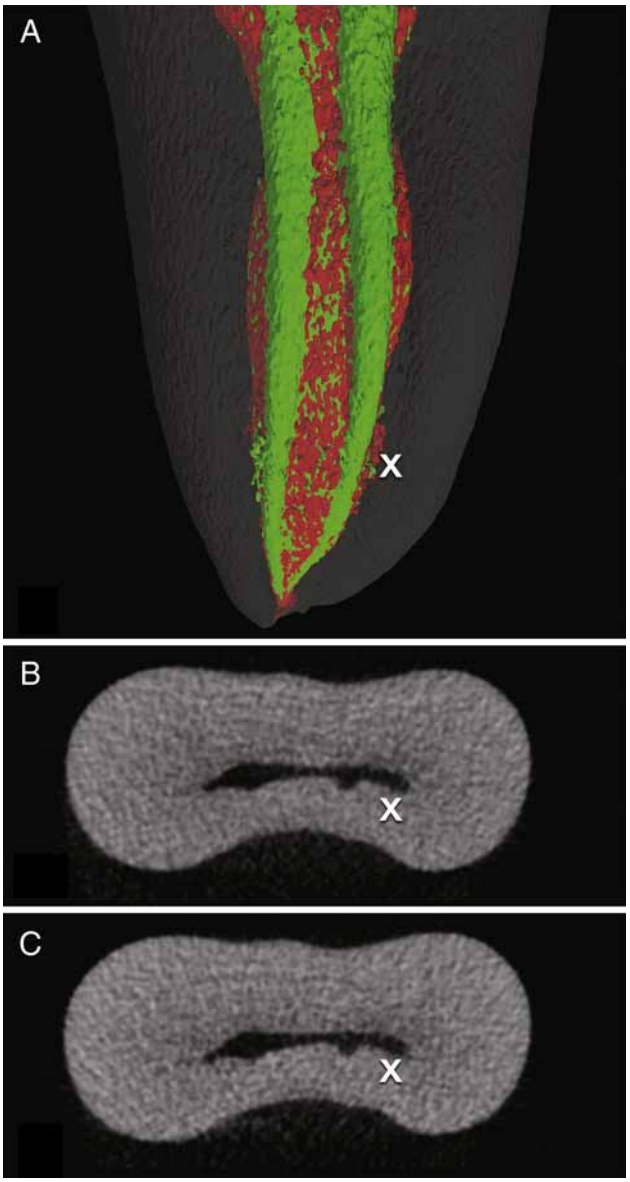

Figure 2. (*A*) The 3-dimensional volume showing canal preparation and disinfection (*green*) via SNI surrounded by complex canal spaces filled with debris. (*B*) The axial slice before SNI at a level represented by "x" showing the fin buccal to the prepared canal space occupied with debris. (*C*) After SNI, debris removal appears virtually unchanged. Also, note that the debris, from canal instrumentation, has packed laterally from the prepared canal.

enlargement to accommodate the placement of irrigation needles to within 1 mm of the canal terminus. In light of this, ongoing research should be performed on the elimination of debris and bacteria in canals prepared to considerably smaller sizes.

Conclusion

Debris removal from the clinically challenging mesial roots of complex root canal systems from vital mandibular molars was assessed. Under the conditions of this study, PIPS laser-activated irrigation was able to significantly improve the debris clearing 2.6 times greater than for SNI.

Acknowledgments

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

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Bioactivity Potential of EndoSequence BC RRM Putty

Amir T. Moinzadeh, DDS, MSc,[*†] *Carlos Aznar Portoles, DDS, MSc,*[‡] *Pierre Schembri Wismayer, MD, PhD,*[§] *and Josette Camilleri, BCHD, MPhil, PhD, FADM, FIMMM*[||]

Abstract

Introduction: The aim of this study was to characterize and assess the interaction of EndoSequence BC RRM putty (Brasseler USA, Savannah, GA) in contact with blood and simulated body fluid. Tricalcium silicate–based materials are in contact with blood and tissue fluids during and after their setting. These materials are hydraulic; thus, their properties improve in moist conditions. However, specific environmental conditions may modify the material setting. **Methods:** EndoSequence BC RRM putty was characterized by scanning electron microscopy, energy dispersive spectroscopy, and X-ray diffraction analysis. This was done before setting and after contact with water, Hank's balanced salt solution, and heparinized whole blood. Furthermore, characterization of an explanted material from a failed root-end surgery was performed. **Results:** The EndoSequence BC RRM putty was composed of tricalcium silicate, tantalum oxide, and zirconium oxide. The tricalcium silicate reaction led to the formation of calcium hydroxide, and this was evident over the putty in contact with water and Hank's balanced salt solution. In the latter case, there was also the formation of globular crystals synonymous with hydroxyapatite formation. The material in contact with blood exhibited a poorly crystalline surface with additional peaks for calcium, phosphorus, and chlorine, whereas the material retrieved from the failed root-end surgery had deposition of calcium carbonate on its surface. **Conclusions:** The environmental conditions affect the hydration of the EndoSequence RMM putty and consequentially the material interaction with the environment. However, *in vitro* material assessment may not be representative of the clinical situation because carbon dioxide present *in vivo* leads to the formation of calcium carbonate rather than the hydroxyapatite reported in *in vitro* studies. *(J Endod 2016;42:615–621)*

Key Words

Bioactivity, characterization, root repair material, tissue interaction

Materials based on tricalcium silicate (TCS) are hydraulic because they have the ability to set in the presence of moisture (1). This property is very well recognized for Portland cement used in the construction industry and was thus behind the inception of mineral trioxide aggregate (MTA), which was introduced as a root-end filling material for this purpose (2). Recently developed materials are based on TCS cement. One particularity of TCS-based materials is their potential to express bioactivity, which is considered as a surrogate for bone-bonding ability (3). Several studies have evaluated the ability of different TCS-based materials used in dentistry to precipitate apatite onto their surface when immersed in simulated body fluid (SBF) (4–6). However, immersion in SBF represents a situation that may be far from the clinical reality in which the material comes concomitantly into contact with fluids such as blood plasma, irrigation, and body fluids. Therefore, it seems fair to question whether such an environment could effectively promote the hydration of the material and ultimately its bioactivity.

EndoSequence BC RRM putty (ERRM) (Brasseler USA, Savannah, GA) is a TCS-based material that is commercialized as a root repair material that can be used for perforation repair, resorption repair, root-end closure procedures, pulp capping, and retrograde filling material during surgical procedures (7). It is available in paste or putty consistency, and the manufacturer claims that it has osteogenic potential.

The aim of this study was to characterize and assess the interaction of ERRM in contact with heparinized blood and SBF and thus evaluate whether the particular environments affect the hydration and bioactivity of the material.

Materials and Methods

ERRM regular set was investigated. One sample of the material was retrieved from a failed root-end restoration after having been in function for 6 months. The other groups tested included unset putty and putty immersed in water, Hank's balanced salt solution (HBSS) (H6648; Sigma-Aldrich, St Louis, MO), and whole heparinized blood for a period of 28 days at 37°C. The solutions were refreshed daily.

Retrieval of Material from Failed Root-end Restoration

A 33-year-old woman was seen in consultation with the chief complaint of pain with chewing and a buccal sinus tract of tooth #8 (Fig. 1*A*). The tooth was painful on percussion and palpation. The mobility and probing depths recorded were within normal limits. A periapical radiograph revealed a radiolucency at the apical third of tooth #8 (Fig. 1*B*) that had been microsurgically treated 6 months earlier because of persistent apical periodontitis after conventional root canal treatment. Briefly, the root tip had been resected, 3 mm retroprepared, and retrofilled with ERRM putty. According to the patient, a sinus tract appeared shortly after the surgical treatment. At this point and after discussing all alternative treatment options, the patient opted for a new surgical retreatment.

From the *Department of Endodontology, Academic Center for Dentistry Amsterdam, VU University and †University of Amsterdam, Amsterdam, The Netherlands; ‡Private Practice Limited to Endodontics, Santpoort-Zuid, The Netherlands; and §Department of Anatomy, Faculty of Medicine and Surgery and ||Department of Restorative Dentistry, Faculty of Dental Surgery, University of Malta, Malta.

Address requests for reprints to Prof Josette Camilleri, Department of Restorative Dentistry, Faculty of Dental Surgery, University of Malta, Medical School, Mater Dei Hospital, Msida MSD 20090, Malta. E-mail address: josette.camilleri@um.edu.mt

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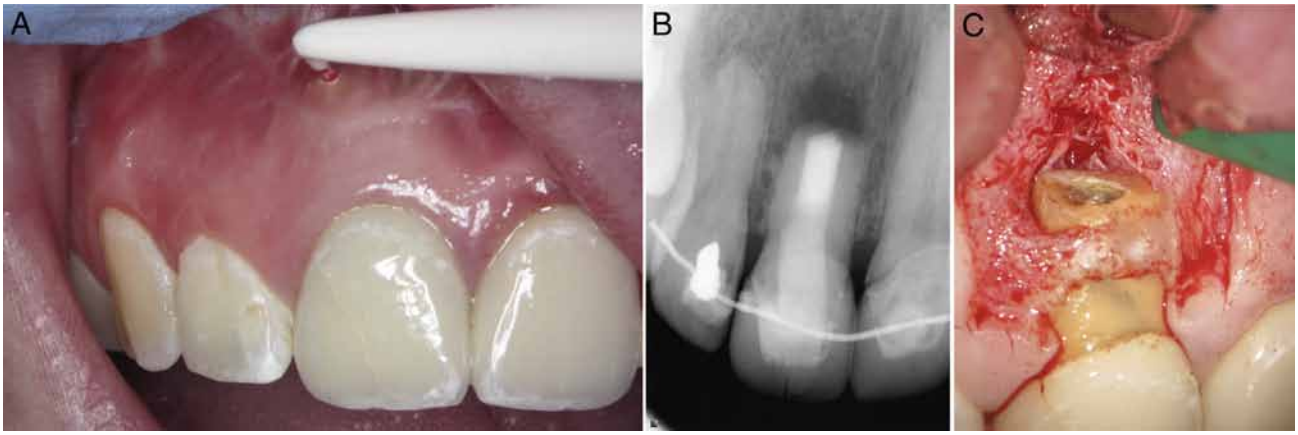


Figure 1. Clinical and radiographic findings of the failed root-end filling case. (*A*) Persistent sinus tract, (*B*) preoperative radiograph showing the presence of a periapical radiolucency, and (*C*) view of the previously treated apical third after flap reflection.

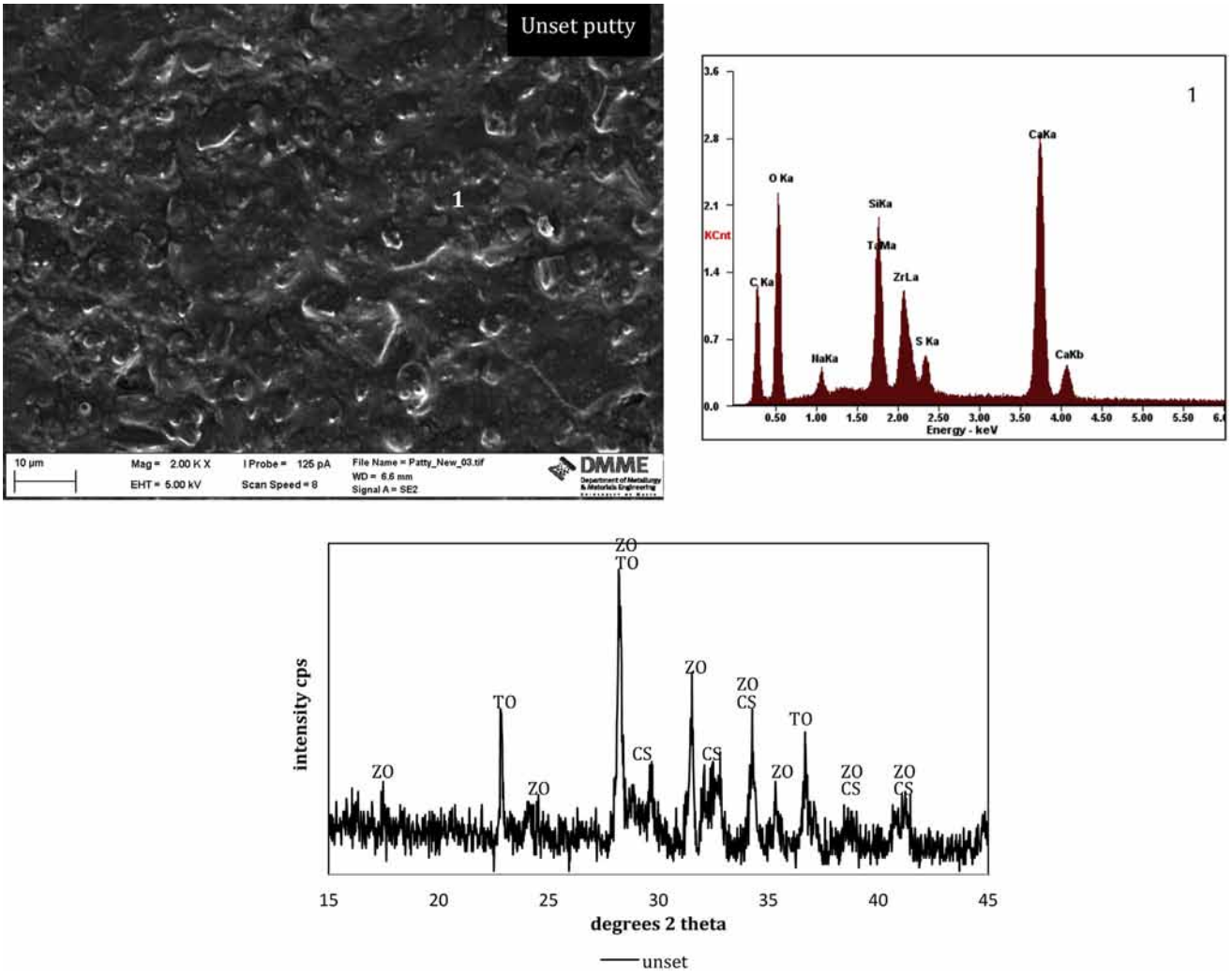
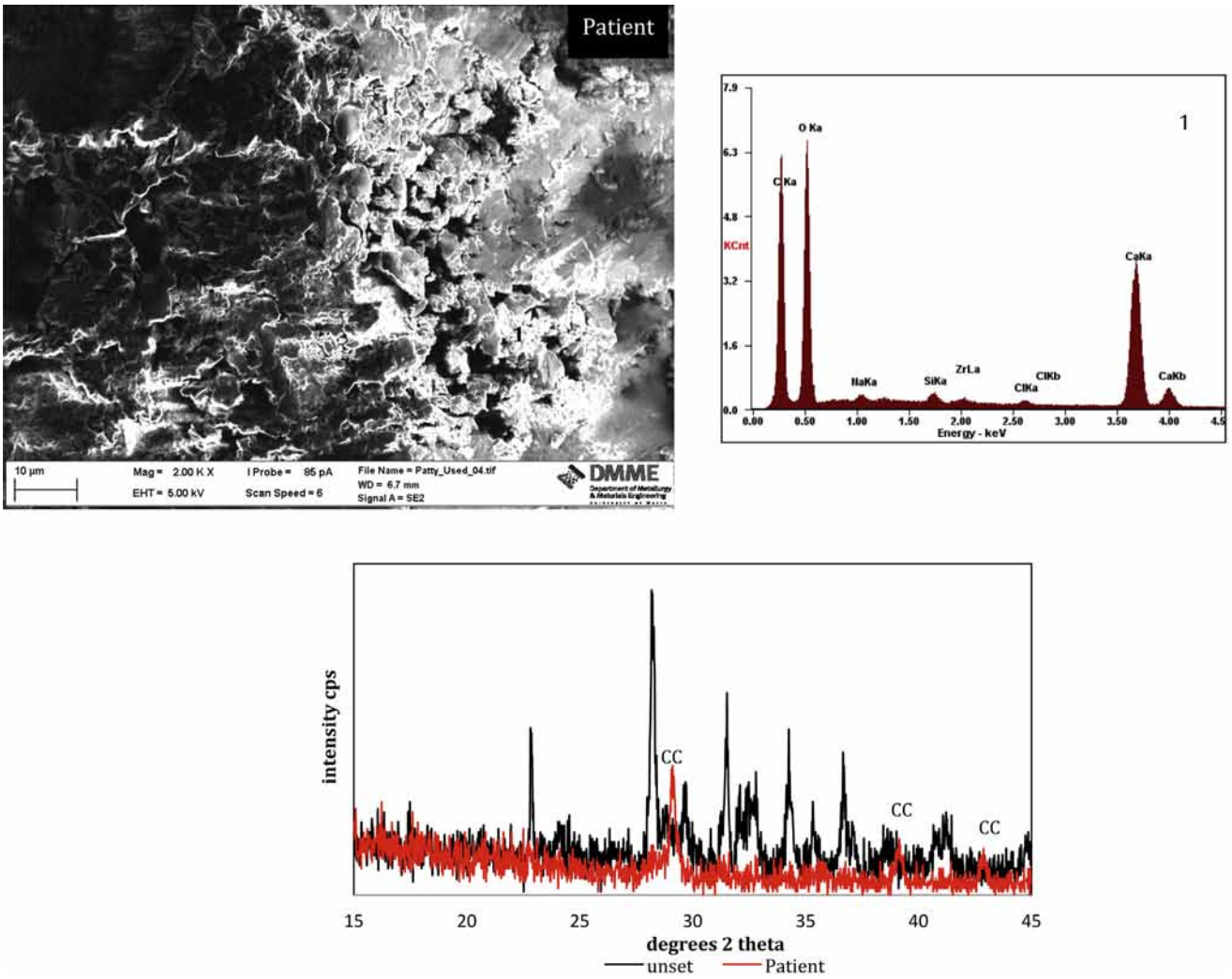


Figure 2. Scanning electron micrographs, EDS analysis, and XRD scans of Endosequence BC RRM putty exposed to various environmental conditions showing the deposition of crystalline structures on the materials surface. CC, calcium carbonate; CS, calcium silicate; CH, calcium hydroxide; TO, tantalum oxide; ZO, zirconium oxide.



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The root-end filling material was retrieved after a submarginal incision and raising a mucoperiosteal flap to expose the previously resected root tip (Fig. 1*C*). The previous apical retrofilling was carefully removed using an ultrasonic tip (Apical Surgery Set; Satelec, Mérignac, France), sealed in a container, and placed in a carbon dioxide–free environment. The tooth was retrofilled again and the flap sutured in place.

Material Characterization

The ERRM putty was characterized by X-ray diffraction (XRD), scanning electron microscopic (SEM), and energy-dispersive spectroscopic (EDS) analyses. The fresh putty was shaped into diskettes 5 mm in diameter with a 1-mm height. One group was tested without any further preparation. The materials stored in water, HBSS, and heparinized blood were first removed from the soaking solution. All the subgroups with set materials including the explanted putty were dried in a vacuum desiccator in a carbon dioxide–free environment. XRD analysis was performed first because this was a nondestructive technique. This was followed by SEM and EDS analyses.

XRD. Phase analysis of materials was performed using a Bruker D8 diffractometer (Bruker Corp, Billerica, MA) with Co Kα radiation (1.79 Å). The X-ray patterns were acquired in 2θ (15°–45°) with a step of 0.02° and 0.6 seconds per step. Phase identification was accomplished using search match software using the ICDD database (International Centre for Diffraction Data, Newtown Square, PA).

SEM and EDS Analyses. The samples were mounted on aluminum stubs, carbon coated, and viewed with a scanning electron microscope (Zeiss MERLIN Field Emission SEM; Carl Zeiss NTS GmbH, Oberkochen, Germany). Scanning electron micrographs of the material microstructural components at different magnifications in the secondary electron mode were captured, and EDS analysis was performed.

Results

The scanning electron micrographs and EDS and XRD analyses of the unset putty and the putty exposed to different environmental conditions are shown in Figure 2. The unset putty exhibited a smooth surface topography. EDS analysis showed peaks for calcium, silicon, oxygen, tantalum, and zirconium with minor peaks for sodium and sulfur. XRD analysis showed the phases present, namely monoclinic tricalcium silicate (ICDD: 00-049-0442) with the main peaks at 29.30, 32.13, 32.46, and 34.28°2θ. Zirconium oxide (ICDD: 00-037-1484) with the main peaks at 28.17 and 31.47°2θ and tantalum oxide (ICDD: 01-081-8067) at 22.83, 28.26, and 36.64°2θ were also present.

Specimens in contact with water exhibited a smooth surface topography with clusters of elongated crystals, which exhibited peaks for

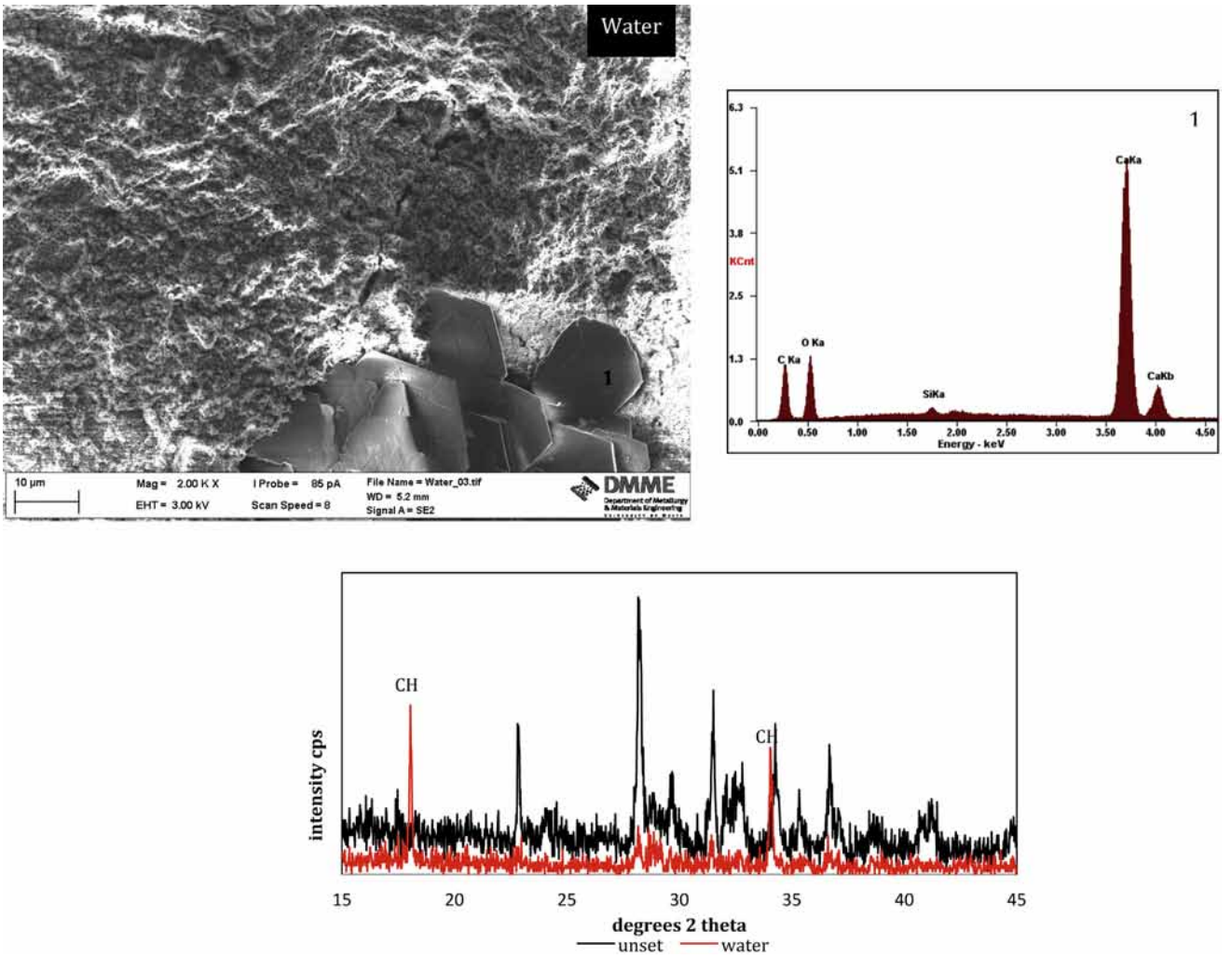

Figure 2. (*Continued*).

calcium and oxygen and were identified as calcium hydroxide (ICDD: 00-004-0733) with the main peaks at 18.09 and 34.09°2θ. The material soaked in HBSS exhibited similar calcium hydroxide deposits in addition to globular deposits over the material surface shown in the high-power micrograph of the material surface. The globular deposits are typical of hydroxyapatite.

The specimens in contact with whole blood were discolored. The surface had a dark brown/black color after removal from the blood. Globular surface deposits with peaks of calcium, phosphorus, and oxygen and minor peaks of chlorine, sodium, magnesium, and potassium were present. The material surface deposit was amorphous as seen by the lack of specific peaks present on the XRD scan.

The specimen retrieved from the patient with the failed root-end surgery exhibited surface deposits. EDS analysis of the material's surface exhibited peaks predominantly for calcium, oxygen, and carbon with the presence of silicon, chlorine, sodium, and zirconium. The XRD plot exhibited peaks that did not coincide with the main peaks exhibited by the unset material. The peaks were identified as calcium carbonate (ICDD: 01-080-2809).

Discussion

The unset ERRM was composed of tricalcium silicate, tantalum oxide, and zirconium oxide. This was verified in the EDS analysis showing the elemental composition and XRD plots, which gave the crystalline phases. The manufacturer indicates that the material is composed of both calcium silicates and calcium phosphate monobasic, which makes the material a biphasic cement (7). The presence of phosphate was not shown on the EDS analysis and XRD scans. The phosphorus peak coincides with the zirconium peak in EDS analysis; thus, it is difficult to distinguish between the 2 elements using this testing methodology. This limitation of testing methodology has already been reported for EndoSequence BC Sealer (8) and other materials containing both phosphate and zirconia additives (9). The phosphate phase could not be identified by XRD, thus indicating that it is either present in low amounts or it is amorphous and thus not detected by XRD.

The setting of the material in water led to the release of calcium hydroxide, which is reported for all TCS-based materials (10). The setting in HBSS and whole blood also showed the precipitation of amorphous deposits corresponding to calcium phosphate. Amorphous calcium phosphate is known to be a precursor of apatite. Depending on whether CO_3^{-} groups replace the OH^{-} or PO_4^{3-} groups, the resulting apatite will be either α- or β-type apatite, the β-type carbonated apatite corresponding to that found in osseous tissues (11). A material capable of precipitating carbonated apatite on its surface would be considered as potentially bioactive, having the potential to create a bond with bone. The precipitation of apatite crystals or even calcium phosphate on the surface of biomaterials immersed in SBF has indeed often been

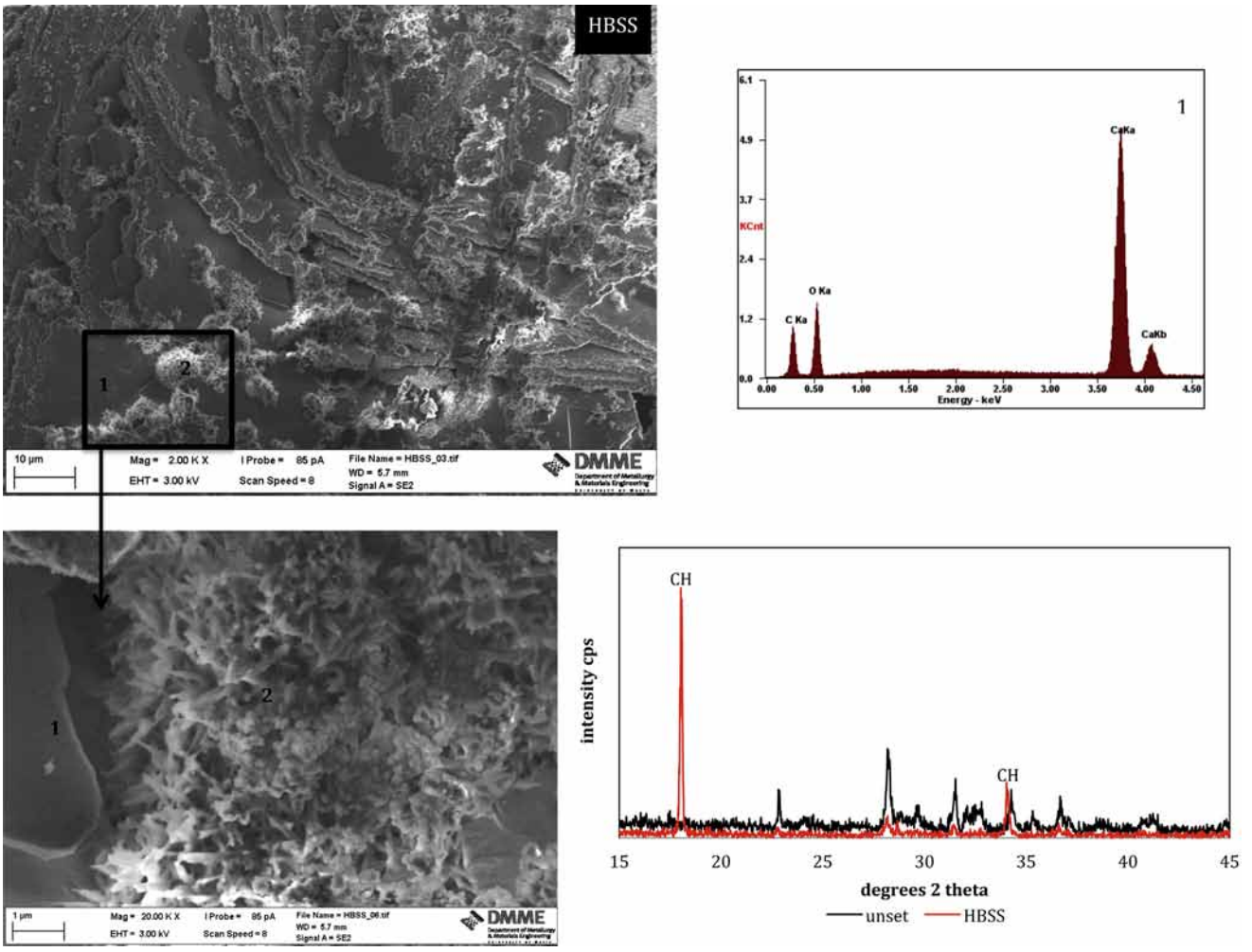

Figure 2. (*Continued*).

considered as a proof of their bioactivity (4, 5, 12). Current methods for investigating the so-called bioactivity of endodontic materials consist in immersing them in SBF for a relatively long period of time and observing the precipitation of these crystals on the material surface and/or at its interface with bone or dentin, as was done in the present study. However, this is setting aside the fact that SBF are metastable systems that precipitate apatite crystals to reach thermodynamic stability (13). The detection of these crystals in these *in vitro* models as a surrogate for bioactivity is questionable, and the role of environmental factors, such as temperature, pH, carbon dioxide partial pressure, and agitation, has often been overlooked as reported by several authors (11, 14, 15). Also, the ratio of the specimen surface area to the volume of the SBF solution could affect the tendency to form apatite and/or calcite. This ratio will influence the availability of the phosphorus and therefore the formation of apatite (16).

The concentration of carbonate ions in the solution is correlated to the environmental carbon dioxide and will influence the carbonation of the apatite by replacing phosphate or hydroxyl ions (14). Also, the increase in pH resulting from the hydroxyl ions release could provoke a supersaturation (which will favor apatite nucleation). This is typically observed when the pH level is $\geq$7.65 for SBF with an ion concentration close to that of human blood plasma (11). Therefore, it is important to realize that the local increase reached *in vitro* with samples immersed in SBF solutions in static conditions could result in hydroxyl ion saturation, provoking a local increase in pH and subsequent apatite nucleation.

The specimen retrieved from the surgical site was covered by crystals rich in calcium, which were identified as calcium carbonate by XRD analysis. Calcium carbonate is the result of the reaction between released calcium hydroxide and environmental carbon dioxide (14). Calcium carbonate is frequently observed on the surface of phosphorus-free glasses immersed in SBF, has the ability to make direct contact with bone, and will influence the ability of the material to form apatite (17). Calcium carbonate can also coexist with an Si-, Ca-, and P-rich layer (18). Whether calcium carbonate is formed or not is dependent on the HCO_3^-/CO_2 equilibrium *in vitro* (14). In the present study, only the *in vitro* models revealed the formation of an apatite precursor, namely calcium phosphate. This could be explained by the availability of the phosphate ions and their stagnation for the samples tested *in vitro* as well as the HCO_3^- concentration of the testing media. The interactions with the body environment *in vivo* are overlooked in the current *in vitro* models. Such interactions are the contact with the blood in physiological context, the adsorption of biological molecules onto the surface of the material, and the migration of mesenchymal cells at the wound site and their potential attachment to the material via protein mediation (19). Further interactions occurring *in vivo* when grafting the material on tooth structure are the loading of the material, its fatigue, and changes in the surrounding tissues (eg, bacterially induced periapical inflammation) (20). All these interactions are not evaluated in the settings of the current *in vitro* models

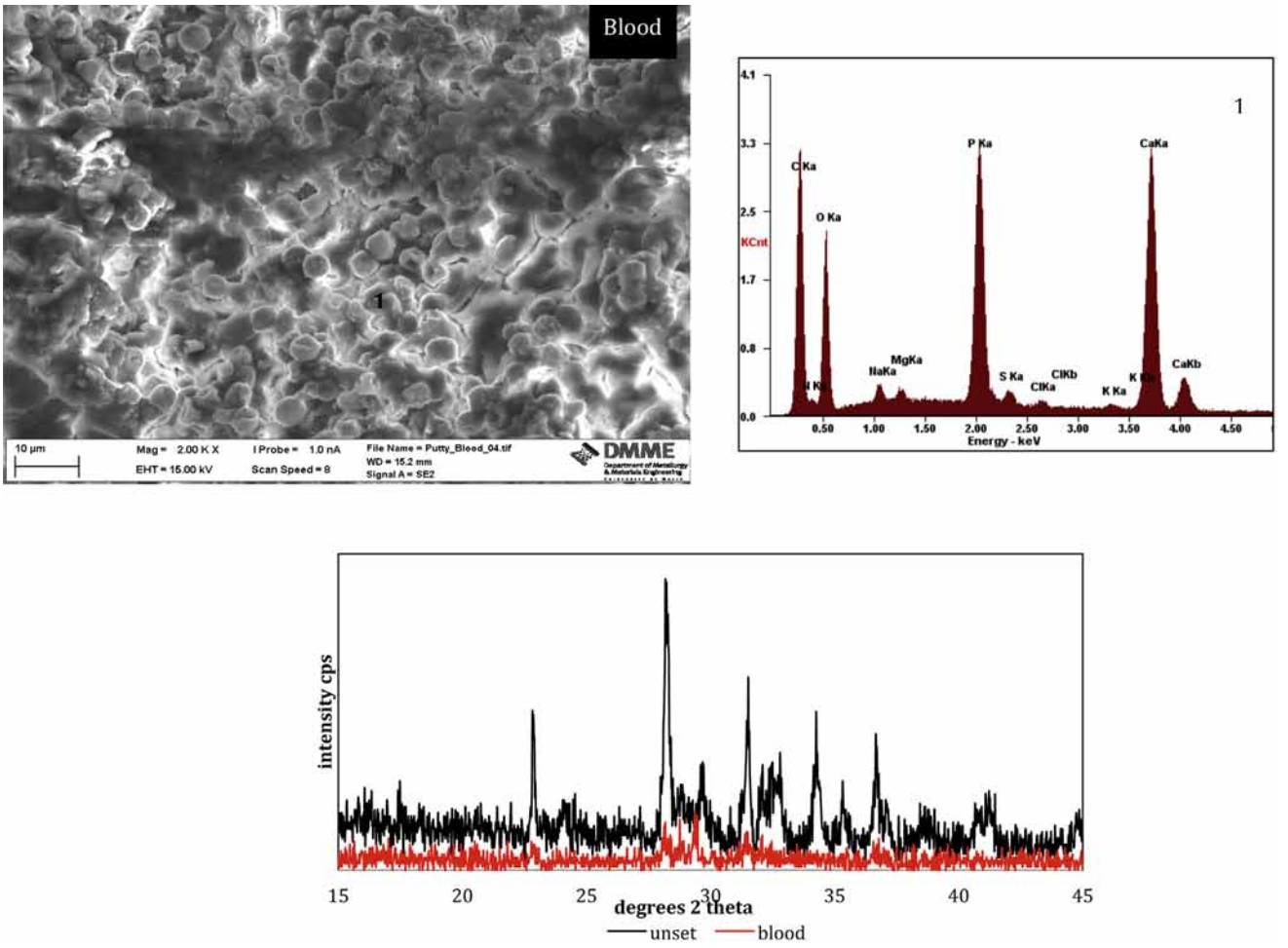

Figure 2. (*Continued*).

and could account for the differences observed between the *in vitro* results with the samples immersed in the different media and the sample retrieved from the patient.

The current study also investigated the effect of blood on material hydration. No calcium hydroxide peak was detected on the material in contact with blood as opposed to that observed with the putty in contact with water and HBSS. This is in accordance with previous studies investigating the hydration of MTA in contact with blood in which blood contamination was shown to result in the absence of calcium hydroxide crystals and a reduction in the formation of ettringite, both of which are by-products of hydration of Portland cement (21). In the latter study, the absence of calcium hydroxide was interpreted to indicate a lack of hydration. However, the calcium hydroxide may have reacted to form other phases, which may not necessarily be easily identified on XRD analysis because of their amorphous nature as is indicated in the current study. Investigation of the hydration of tricalcium silicate cement in water and HBSS showed that hydration was similar to that of Portland cement (10) and corroborates the present findings that the presence of blood both *in vitro* and *in vivo* (clinically) modified the setting and reaction kinetics of the materials.

Based on the present findings, the clinically placed ERRM neither exhibited any bioactivity nor led to a successful outcome. However, no conclusion can be drawn on a single case, and more research is necessary to evaluate and understand the observed difference between *in vitro* and *in vivo* tests for this material.

Conclusions

TCS-based materials are hydraulic, and the material properties improve in the presence of water. The environmental conditions affect the material hydration and consequentially the material interaction with the environment. Calcium phosphate–type deposits were observed on the EndoSequence RMM putty in blood and HBSS *in vitro*, but the formation of calcium carbonate was detected in the *in vivo* case. *In vitro* material assessment may not be representative of the clinical situation.

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

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Biofilm removal by 6% sodium hypochlorite activated by different irrigation techniques

R. Ordinola-Zapata[1], C. M. Bramante[1], R. M. Aprecio[2], R. Handysides[2] & D. E. Jaramillo[2]

[1]Department of Endodontics, Dental School of Bauru, University of São Paulo, Bauru, Brazil; and [2]Department of Endodontics, School of Dentistry, Loma Linda University, Loma Linda, CA, USA

Abstract

Ordinola-Zapata R, Bramante CM, Aprecio RM, Handysides R, Jaramillo DE. Biofilm removal by 6% sodium hypochlorite activated by different irrigation techniques. *International Endodontic Journal*, **47**, 659–666, 2014.

Aim To compare the removal of biofilm utilizing four irrigation techniques on a bovine root canal model.

Methodology Fifty dentine specimens (2 × 2 mm) were infected with biofilm. The samples were then adapted to previously created cavities in the bovine model. The root canals were irrigated twice with 2 mL of 6% sodium hypochlorite for 2 min (4 min total). Following initial irrigation, the different treatment modalities were introduced for 60 s (3 × 20 s intervals). The evaluated techniques were needle irrigation, Endoactivator (Dentsply Tulsa Dental, Tulsa, OK, USA), passive ultrasonic irrigation and laser-activated irrigation (photon-induced photoacoustic streaming). The controls were irrigated with distilled water and conventional needle irrigation. Subsequently, the dentine samples were separated from the model and analysed using a scanning electron microscope (SEM). Fifteen operative fields were scanned per block, and SEM pictures were captured. Two calibrated evaluators examined the images and collected data using a four-degree scale. Nonparametric tests were used to evaluate for statistical significance amongst the groups.

Results The group undergoing laser-activated irrigation using photon-induced photoacoustic streaming exhibited the most favourable results in the removal of biofilm. Passive ultrasonic irrigation scores were significantly lower than both the Endoactivator and needle irrigation scores. Sonic and needle irrigation were not significantly different. The least favourable results were found in the control group.

Conclusions Laser activation of 6% sodium hypochlorite significantly improved the cleaning of biofilm-infected dentine followed by passive ultrasonic irrigation.

Keywords: biofilms, irrigant solutions, laser-activated irrigation, photoacoustic streaming.

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Introduction

The aim of irrigation in root canal treatment is to improve the cleaning and disinfection process within the root canal system (Siqueira & Rocas 2008). Irrigants play multiple roles in endodontic therapy. They are necessary from an antimicrobial aspect as the mechanical instrumentation process is insufficient on its own to remove the microbial load (Byström & Sundqvist 1983). Sodium hypochlorite (NaOCl) is considered the main root canal irrigant because of its tissue dissolution and antimicrobial properties. Whilst some microscopic studies have shown that complete dissolution of biofilms by sodium hypochlorite is possible using the direct contact test (del Carpio-Perochena *et al.* 2011), incomplete dissolution and residual biofilm appears to be common under clinical conditions following full-strength NaOCl irrigation (Vera *et al.* 2012). Residual biofilm may contain viable bacteria and may decrease the interfacial adaptation of root filling materials (Vera *et al.* 2012).

Significant information regarding the physical effect of fluids in root canals has been previously reported

Correspondence: Ronald Ordinola-Zapata, Faculdade de Odontologia de Bauru, University of São Paulo, Al. Octávio Pinheiro Brisolla, 9-75, CEP 17012-901, Bauru, SP, Brazil (e-mail: ronaldordinola@usp.br).

(Chow 1983, Ahmad *et al.* 1987, de Gregorio *et al.* 2010, Jiang *et al.* 2010). These studies have shown that positive or negative apical pressure can affect the diffusion of the irrigant solutions into the root canal system, improving their cleaning ability (Chow 1983, de Gregorio *et al.* 2010). In addition, the use of ultrasonic irrigation has been shown to improve the cleaning efficacy of irrigants showing in many cases superiority in comparison to common positive apical pressure techniques (Burleson *et al.* 2007).

Lasers have been used to produce cavitation of liquids, thereby increasing the cleaning ability of the liquid (Lauterborn & Ohl 1997, Blanken 2007, Peel *et al.* 2011). When laser pulses are focused into a limited volume of fluid, plasma is generated. Plasma formation can lead to rapid heating of the material followed by an explosive expansion and the emission of a shock wave. This is possible by the high absorption of the Er:YAG wavelength in water (DiVito *et al.* 2012). These lasers have been evaluated for the elimination of the smear layer and dentinal debris (George *et al.* 2008) with promising results (de Groot *et al.* 2009). These techniques, referred to as laser-activated irrigation (de Groot *et al.* 2009), have been evaluated for endodontic irrigation applications basically using Erbium YAG (Er:YAG) or Er, CrYSGG lasers, with energy levels that vary from 25 to 300 mJ (Blanken 2007, George *et al.* 2008, Blanken *et al.* 2009, Peters *et al.* 2011).

In this work, a novel tapered and stripped tip of a laser-activated irrigation technique called photon-induced photoacoustic streaming (PIPS) at energy levels below those previously cited in the literature (20 mJ) was used. It has been demonstrated that the transition of the laser light from the tip to the fluid creates a photoacoustic pressure wave throughout the liquid with no thermal effects on the dentine surface (DiVito *et al.* 2012). The efficacy of laser-activated irrigation to clean biofilm-infected dentine has not been fully evaluated. This study compared the cleaning ability of passive ultrasonic irrigation, Endoactivator (Dentsply Tulsa Dental, Tulsa, OK, USA), needle irrigation and laser-activated irrigation in conjunction with 6% NaOCl to clean *in situ* biofilm-infected bovine dentine.

Materials and methods

Biofilm development

Fifty sterile bovine dentine sections (2 × 2 mm) were used. The samples were treated with 17% EDTA for 3 min to eliminate the smear layer produced during the sectioning process. To induce dentine infection, an *in situ* model was selected using a Hawleys orthodontic device. The dentine surface exposed to the oral cavity was fixed 1 mm above the surface to allow the accumulation of plaque. One volunteer used the device continuously for 72 h, except during oral hygiene procedures, to generate biofilm (Human committee and ethic research approval, CEP134/2010). Daily food diet was maintained. After the intraoral contamination process, each sample was incubated in 2 mL of BHI at 37 °C for 48 h in aerobic conditions. Then, each sample was rinsed with 1 mL of distilled water to remove culture medium and nonadherent cells (Fig. 1).

The 50 specimens were randomly divided into five groups according to the final irrigation protocol used. G1: conventional needle irrigation, G2: Endoactivator (Dentsply Tulsa Dental), G3: passive ultrasonic irrigation, G4 Laser-activated irrigation (PIPS; Fotona, Ljubljana, Slovenia) and G5: control (distilled water).

Root canal irrigation model

A root canal irrigation model was developed using decoronated bovine incisors. The root canals of 10 roots, 12 mm in length, were prepared to an apical size of 1.30 mm using Gates Glidden burs (Dentsply Maillefer, Ballaigues, Switzerland). Thereafter, a perforation (2.5 × 2.5 mm) was made 3 mm from the apical foramen to adjust the infected dentine block to the perforation (Fig. 1).

The infected dentine sections were fixed into the perforation site with the infected side placed facing the root canal. The apical foramen was sealed with silicone (Aquasil Monophase, Dentsply, Milford, DE, USA) to provide a closed system. This device allows the adaption of the infected area of the intraorally infected dentine block at the same level of the apical area of the root canal of a bovine incisor tooth. Ten bovine roots were used during the experiments. Each root was used a maximum of five times. The irrigation protocol was divided in two steps:

All canals were irrigated with 2 mL of 6% NaOCl (Clorox, Oakland, CA, USA) delivered by positive apical pressure using a 10-mL syringe and a double side-vented needle (SybronEndo, Glendare, CA, USA) inserted until 2 mm from the apex. A flow rate of 1 mL per 10 s was used, and the NaOCl solution was left in the canal space for 2 min. After aspiration of the solution, this procedure was repeated once more.

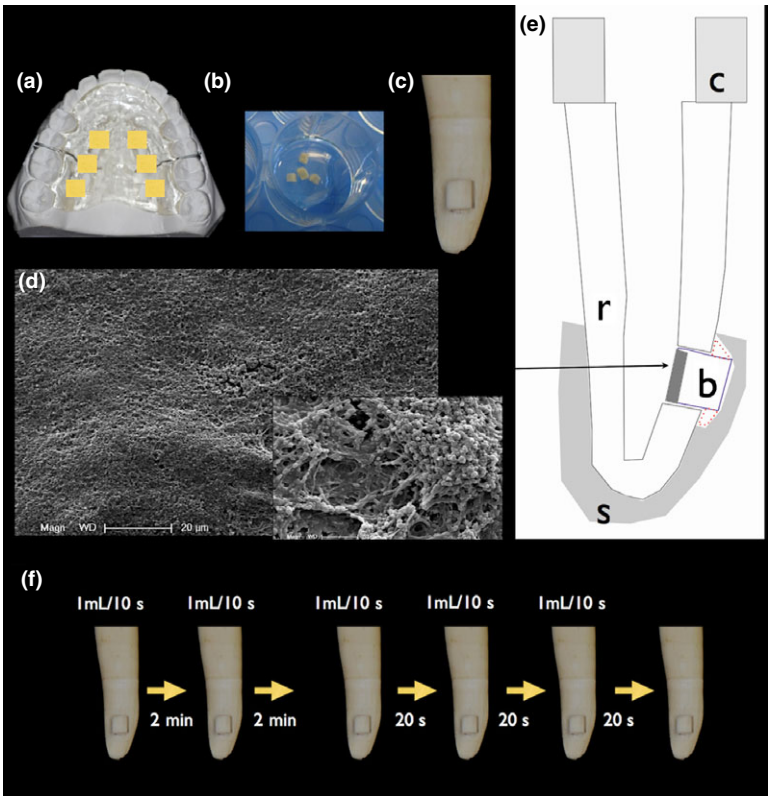

Figure 1 A removable orthodontic device was used to induce the contamination of dentine (a). Then, the blocks were incubated for 48 h in BHI (b). Image and schematic representation of roots modified for the experiment (c, e). The pulp chamber walls were reconstructed using composite resin (c). A perforation was made at the apical portion to adapt the infected dentine (b). The infected dentine sections were fixed into the perforation site facing the root canal (arrow). The dentin block was set with fluid silicone (s). Representative scanning electron microscope of a biofilm-infected dentin (d). The steps of the irrigation procedure are represented in (f). In the first step, NaOCl was applied for 4 min. Then, the tested irrigation techniques were performed for 20 s and repeated two more times.

The total time in this step (without taking into consideration the 20 s of NaOCl application) was 4 min, and 4 mL of 6% NaOCl was used for all the experimental groups (Fig. 1f).

Experimental procedures

Sodium hypochlorite solution was applied at a rate of 1 mL per 10 s, and the irrigation technique tests were performed for 20 s. Each procedure was repeated twice more. In all the experimental groups, the final amount of NaOCl used was 3 mL in the last minute (Fig. 1f). The evaluated irrigation techniques were as follows:

Group 1: Conventional needle irrigation using double side-vented needles. In this technique, the needle was inserted until 2 mm from the apex. Then, 1 mL of NaOCl was applied using a flow rate of 1 mL per 10 s and was left in root canal for 20 s, this procedure was repeated two more times for a total period of 1 min of treatment.

Group 2: Endoactivator: 1 mL of NaOCl was applied at the apical third followed by the sonic activation of the irrigant using a yellow Endoactivator (15.02) tip for 20 s. This procedure (irrigation/sonic activation) was repeated two more times for a total period of 1 min of sonic treatment. The Endoactivator tip was inserted until 2 mm from the apex.

Group 3: Passive ultrasonic irrigation (PUI): In this technique, a similar procedure was applied in the same manner described for the Endoactivator group, but an Irrisafe file 20.00 (Satelec Acteongroup, Merignac, France) was used in conjunction with a Satelec P5 suprasson ultrasonic unit (Suprasson P5; Satelec Acteongroup) at a power setting of 4.

Group 4: Laser-activated irrigation (LAI). An Er:YAG laser with a wavelength of 2940 nm (Fidelis; Fotona) was used to irradiate the root canals using a 12-mm 400-μm quartz tip. The laser operating parameters were: 20 mJ per pulse, 0.30 W, 15 Hz and 50 μs pulse duration. An endodontic fibre tip (PIPS; Fotona) was placed into the coronal access opening of the access cavity. One millilitre of NaOCl was applied and activated for 20 s. This procedure was repeated two more times.

Group 5: Control, the initial irrigation procedures were similar to group 1, except that distilled water was used for the initial and final irrigation procedures. In this technique, 4 mL of distilled water was initially used for 4 min. For the final irrigation purposes, 1 mL of distilled water was applied using a flow rate of 1 mL per 10 s and was left in the root canal for 20 s. This procedure was repeated two more times for a total period of 1 min of treatment.

Following irrigation, the dentine blocks were detached from the root, treated for 1 min with 1 mL of 5% sodium thiosulfate and then fixed in formalin for 24 h. The samples were dehydrated with alcohol, mounted on stubs sputter coated with platinum and observed using a scanning electron microscope (XL30 FEG; Phillips, Eindhoven, the Netherlands). Fifteen images from random areas were obtained from each sample at 2400× magnification. One hundred and fifty SEM pictures were evaluated for each group. For quantification purposes, a modified four-score scale system was used based on Bhuva *et al.* (2010).

Score 1: Clean dentine or residual isolated microbial cells that cover <5% of the dentine. Absence of residual biofilm layers.

Score 2: Residual isolated microbial cells cover 5–33% of the dentine. There is absence of residual biofilm layers.

Score 3: Biofilm structures and microbial cells can be identified covering 34–66% of the dentine.

Score 4: Biofilm structures and microbial cells can be identified covering 67–100% of the dentine.

Two evaluators with SEM experience evaluated the pictures in a blinded manner. The evaluations were performed in two occasions with interval of 4-weeks. In cases of disagreement between the evaluators, the higher score was selected.

Statistical analysis was performed using the non-parametric Kruskal–Wallis and Dunn tests ($P < 0.05$). Kappa test was used to measure intra- and inter-rater agreement. Prisma 5.0 (GraphPad Software Inc, La Jolla, CA, USA) was used as the analytical tool.

Results

Control specimens (distilled water irrigation) were characterized by the presence of a thick biofilm layer covering the dentine structure. The presence of several morphotypes as cocci and rods could be identified. From the 50 dentine blocks, 750 SEM images were examined (15 images for each sample).

The variability between examiners as measured by kappa coefficient was 0.78 (strong). The intraobserver agreement was 0.82 and 0.85 for the first and second evaluator, respectively. The mean and median scores of the different groups are shown in Table 1. Distilled water irrigation score was classified as 4 in all the SEM pictures evaluated. Kruskal–Wallis and Dunn's tests showed significant differences amongst the groups. LAI had the lowest scores compared with PUI, Endoactivator and needle irrigation ($P < 0.05$). PUI scores were lower than both Endoactivator and needle irrigation scores ($P < 0.05$). There was no difference between Endoactivator and needle irrigation ($P > 0.05$). The worst result was found in control group that do not show any significant effect against biofilm ($P < 0.05$). Representative SEM pictures and distribution of the scores in the evaluated groups are shown in Fig. 2.

Discussion

This study revealed that the disruption of biofilm by 6% NaOCl can be enhanced using LAI and PUI techniques. Most of the research available about cleaning ability of these techniques has compared the efficacy to eliminate dentine debris (de Groot *et al.* 2009, Jiang *et al.* 2010). However, there is a lack of evidence comparing the ability of PUI and LAI to improve the cleaning of biofilm-infected dentine (de Moor *et al.* 2009, Peters *et al.* 2011).

Several models of biofilms are used in endodontic research, and the efficacy of NaOCl depends on variables such as the method of biofilm growth (Bhuva *et al.* 2010), NaOCl concentration (Ordinola-Zapata *et al.* 2013) and exposure time (del Carpio-Perochena *et al.* 2011). It could also be considered that oral mixed biofilms can be more resistant and have a greater adhesion to dentine in comparison with biofilms developed under laboratory conditions (Stojicic *et al.* 2012). This detail can possibly explain

Table 1 Score distribution in the evaluated groups. Mean and median are also presented

	Score 1	Score 2	Score 3	Score 4	Mean	Median*	Total
Control	0	0	0	150	4	4[a]	150
Needle	23	40	56	31	2.63	3[b]	150
Endoactivator	31	35	52	32	2.56	3[b]	150
PUI	72	33	25	20	1.95	2[c]	150
LAI	107	21	10	12	1.52	1[d]	150

LAI, laser-activated irrigation; PUI, passive ultrasonic irrigation.
*Letters shows statistically significant differences between groups (Kruskal–Wallis test,- Dunn's test).

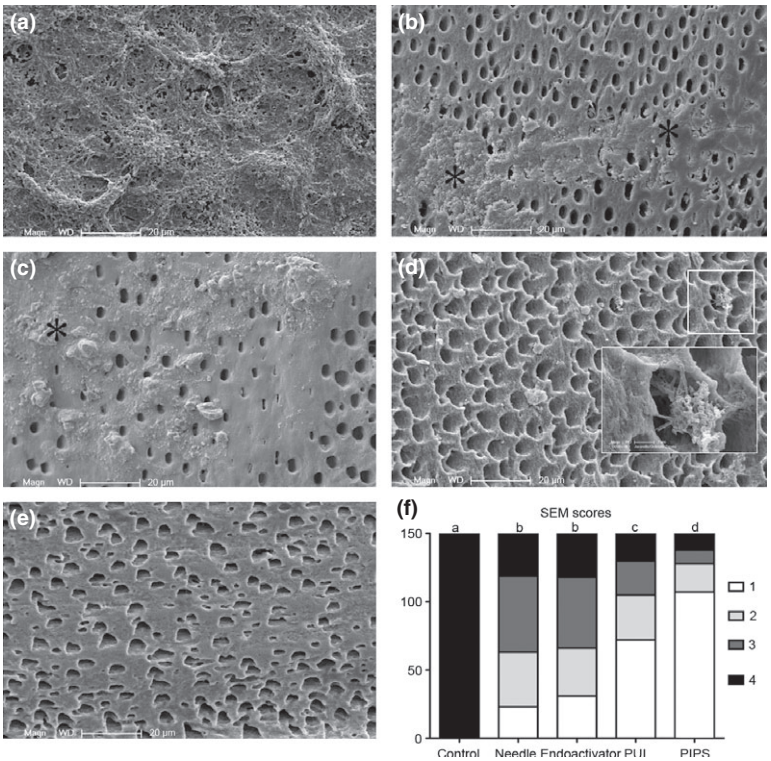

Figure 2 Representative images of the evaluated groups: Control (distilled water) showing extensive biofilm colonization (a), needle irrigation (b), showing biofilm residual layers (*). Dentine treated with the Endoactivator (c) and passive ultrasonic irrigation (d) showing residual biofilms and bacteria. Clean dentine and open dentinal tubules can be seen in the laser-activated irrigation technique (e). Distribution of scores after the scanning electron microscope (SEM) evaluation (f).

why a previous study that used *in vitro* monospecies biofilms found no difference between conventional and PUI irrigation (Bhuva *et al.* 2010). The authors found that an *Enterococcus faecalis* biofilm can be completely dissolved using 6 mL of 1% NaOCl for 2 min (Bhuva *et al.* 2010). In another direct contact test, Stojicic *et al.* (2012) found that 1–2% NaOCl destroyed *E. faecalis* biofilms in 3 min.

In the present study, similar to previous studies (Barthel *et al.* 2002, Peters *et al.* 2011), intraorally developed dental plaque was used. The results showed that conventional needle irrigation in combination with 6% sodium hypochlorite failed to completely dissolve the biofilm. This result is similar to studies performed *in vivo* (Vera *et al.* 2012). Even though this research was not performed in a complex anatomy, the method used allows comparisons between different NaOCl irrigation protocols using a standardized area of infected dentine at the apical level. One limitation to take into account in the present study is the lack of actual anaerobic conditions such as those present in the root canal, so the

amount of residual biofilm may vary under those conditions.

Scanning electron microscopy is commonly used as an evaluative tool to observe infected dentine (George *et al.* 2005). Although this technique allows only bidimensional and semi-quantitative analysis, it provides the advantage of higher resolution and details of the dentine surface in comparison to confocal microscopy or stereomicroscopy used in previous studies (de Groot *et al.* 2009, del Carpio-Perochena *et al.* 2011). To minimize bias, randomization and a considerable numbers of images were taken of a small-predefined infected area placed at the apical third.

Similar to previous reports, the cleaning efficacy of the Endoactivator or sonic devices was similar to needle irrigation (Brito *et al.* 2009, Uroz-Torres *et al.* 2010, Johnson *et al.* 2012, Seet *et al.* 2012). This observation has been made by studies using scanning electron microscopy (Uroz-Torres *et al.* 2010, Seet *et al.* 2012), microbiological culture (Brito *et al.* 2009) or by histological methods (Johnson *et al.* 2012). In general, it is accepted that ultrasonic irrigation provides higher frequency, and this improves the acoustic microstreaming of NaOCl in comparison with the Endoactivator device (Jiang *et al.* 2010). According to Jiang *et al.* (2010), the Endoactivator device did not improve canal cleanliness regardless of frequency or tip size. These authors found that the amplitude of the Endoactivator tip was 1 mm, which implies a high probability of contact between the tip and the root canal wall, decreasing its efficacy in comparison with the ultrasonic movements that is in the range of 75 μm (Jiang *et al.* 2010).

In the present study, minimal or negative canal wall contact of the Endoactivator and ultrasonic device was expected due to the diameter of the root canal (1.3 mm). Enlarging the canal also allowed the Endoactivator and ultrasonic file tips to be placed at the same level of the infected dentine to maximize their effectiveness. Conversely, the tip of the laser technique was located in the access chamber and activated several millimetres coronal or distant from the target point.

The use of shockwaves has gained the attention of some medical areas to treat biofilm-related diseases. Local deposition of energy as heat or light is necessary to induce cavitation (Lauterborn & Ohl 1997), and photoacoustic streaming appears to be the mechanism of cleaning at the liquid/dentine interface (Blanken 2007, Blanken *et al.* 2009, de Groot *et al.* 2009). A previous study has shown that this technique was effective in disrupting *Pseudomona aureginosas* and plaque-derived biofilm in the absence of antimicrobials (Krespi *et al.* 2008, 2011, Muller *et al.* 2011). Biofilm disruption can change the bacteria to their planktonic form, making them more susceptible to antimicrobial agents (Kizhner *et al.* 2011).

One associated effect of the application of acoustic or photoacoustic waves on chemicals systems is sonochemistry. Previous studies in the industrial area have shown that ultrasonics can enhance the effectiveness of NaOCl disinfection (Duckhouse *et al.* 2004, Zifu Li *et al.* 2012). A previous study showed that ultrasonic and laser activation increase significantly the reactivity of NaOCl (Macedo *et al.* 2010). Temperature is also a variable that can influence the effectiveness of NaOCl (Al-Jadaa *et al.* 2009). Two previous reports described a rise in root canal temperature after the use of passive ultrasonic activation (Cameron 1988, Al-Jadaa *et al.* 2009), which could increase the ability of sodium hypochlorite to remove biofilm. Parameters used in the laser-induced irrigation include subablative power settings (20 mJ), and the use of the PIPS tip at the coronal level avoids the undesired effects of the thermal energy on the dentinal walls (DiVito *et al.* 2012), thus, the cleaning ability of the laser could not be necessarily associated to a rise in the temperature of the irrigant solution. The significant difference with this laser-induced irrigation technique (PIPS) in comparison with PUI may be attributed to the high peak powers created with minimal energy (20 mJ or less) with low pulse durations (50 μs) leading to pressure waves that move irrigants in three dimensions distant to the tip position. The better cleaning ability of laser-activated irrigation is in agreement with a previous study (Peters *et al.* 2011). Because the cleaning effect of NaOCl is a time-dependent phenomenon (del Carpio-Perochena *et al.* 2011), the results can also reflect that there is acceleration in the dissolution and cleaning effect of NaOCl when laser-activated irrigation is used.

Due to the limited comparisons between acoustic- and photoacoustic-induced shockwaves, future studies are necessary for the understanding of laser-activated irrigation, including the effect of activation time, the ability to avoid the accumulation of hard tissue debris and the cleaning ability in the presence of pulp tissue in complex anatomies.

Conclusions

Under the conditions of the current study, laser-activated irrigation using the photon-induced photoacoustic streaming technique of 6% sodium hypochlorite significantly improved the cleaning of biofilm-infected dentine compared with passive ultrasonic, sonic or mere needle irrigation.

Acknowledgement

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A Comparative Investigation of Cone-beam Computed Tomography and Periapical Radiography in the Diagnosis of a Healthy Periapex

Oliver Pope, BDSc, DCD, Chankhrit Sathorn, DCD, PhD, and Peter Parashos, MDSc, PhD

Abstract

Introduction: This research aimed to compare the appearance of healthy periapical tissues on cone-beam computed tomography (CBCT) with periapical radiography and to measure the periodontal ligament (PDL) space on CBCT for teeth with healthy and necrotic pulps. **Methods:** Patient records from specialist endodontic practices were examined for teeth that had a high-resolution (0.08-mm voxel) and small field-of-view CBCT scan, a periapical radiograph, and clinical pulp tests (CO_2 and electric pulp testing). The periapical regions of the CBCT scans and radiographs were scored individually by 2 calibrated, blinded examiners by using a modified CBCT-periapical index (CBCT-PAI) for both and represented as CBCT-PAI and PAI, respectively. The Fisher exact and χ^2 statistics tested the relationships between CBCT-PAI, PAI, and pulp status. **Results:** Of 200 teeth included in the study, 166 showed clinical signs of pulpal health, and the CBCT-PAI score was greater than the PAI in 72% (119 of 166), with a vital pulp likely to have a radiographic PDL space widening of 0–1 mm ($P < .001$). Although 2 healthy teeth showed radiolucencies 2–4 mm on CBCT scan when the periapical radiograph showed none, a PDL space of greater than 1–2 mm was indicative of a necrotic pulp ($P < .001$). **Conclusions:** Teeth with necrotic pulps were more likely to have PDL widening, but the PDL space of a healthy tooth demonstrated significant variation when examined by CBCT. The radiographic interpretation of health and disease on CBCT must be further investigated before usage in outcome or epidemiologic investigations. This research questions the traditional radiographic interpretation of the PDL space. *(J Endod 2014;40:360–365)*

Key Words

Cone-beam computed tomography, diagnosis, intraoral radiographs, periapical lesions

From the Melbourne Dental School, University of Melbourne, Melbourne, Victoria, Australia.

Address requests for reprints to Prof Peter Parashos, Melbourne Dental School, University of Melbourne, 720 Swanston Street, Melbourne, Victoria 3010, Australia. E-mail address: parashos@unimelb.edu.au

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Radiographic imaging is essential in all stages of endodontics from the diagnosis through long-term assessment of healing outcomes. In conjunction with symptoms, outcome is assessed by comparison of preoperative and/or immediate postoperative radiographs, with subsequent radiographs taken at review appointments. The appearance of the periapical tissues on a radiograph is influenced by the superimposition of anatomic structures and the variable nature of the overlying bone density and texture; interpretation can have a high degree of both interexaminer and intraexaminer variability (1, 2).

The diagnostic value of pretreatment radiographs depends on how well they reflect the histology of apical periodontitis. Studies that have investigated the correlation between histologic appearance and radiographic manifestations (3–6) have found that the absence of radiographic signs does not preclude apical inflammation, and the radiographic appearance is always smaller than the histologic extent of the lesion. Radiographic signs pathognomonic of apical periodontitis include radiolucent changes in periradicular trabecular pattern and altered shape and width of the periodontal ligament (PDL) space (3–6).

The limitations of periapical radiography have led to significant interest in cone-beam computed tomography (CBCT) and specific endodontic applications. Although there are no published data available, anecdotally it seems that the number of CBCT scans taken every year is increasing as awareness increases, resolution increases, and costs reduce. A similar parallel may be drawn with the use of medical CT scans. Since the 1990s, the per-capita use of head CT scans has doubled, and chest CT scans have increased 5-fold (7), and it is likely that the use of CBCT may increase similarly. Although an attractive imaging modality, including as a research tool (8), the radiation safety principles of as low as reasonably achievable require adherence, and an increase in clinical use is not yet supported by the development of evidence-based protocols to show an improved outcome for the patient. Along these lines, although CBCT may be a useful tool for observing the bone accumulation in periapical lesions, Kaya et al (9) confirmed there is a need to reduce the radiation dosage, confirming the American Association of Endodontists and the American Academy of Oral and Maxillofacial Radiology Joint Position Statement recommending against routine use but limited to assessment and treatment of complex endodontic conditions (10).

Many clinical and laboratory studies have compared the diagnostic accuracy of CBCT and traditional periapical radiography (11–19). Although these conclude that CBCT is more readily able to detect simulated defects, they lack clinical applicability because of failure to use an appropriate reference standard to delineate the true presence of disease, and there are still limitations to the accuracy of CBCT that should be considered during interpretation (18). In 2 studies that reported CBCT was a more accurate diagnostic method, the CBCT itself was used as the reference standard, with no mention of what was defined as apical periodontitis and whether such an appearance correlated to true presence of disease (11, 12). Another study (14) used histopathology as the reference standard to correlate with radiographic appearance and concluded CBCT was a significantly more accurate diagnostic tool than periapical radiographs.

Although the sensitivity is, or approaches, 100% in *in vitro* studies, CBCT has limitations. Comparing the diagnostic accuracy of CBCT complete 360° versus partial 180° scans in the detection of artificial periapical lesions reveals similar accuracy, sensitivity, and specificity (20). The sensitivity is the proportion of apical periodontitis that has been correctly diagnosed, whereas the specificity is the proportion of healthy

apical tissues correctly diagnosed by CBCT. The sensitivity for both approached 1.0, whereas mean specificity values were relatively low at 0.73 (ie, 27% of teeth had diagnoses of apical periodontitis when there was no disease present) (20). Thus, although CBCT is accurate at diagnosing the presence of disease, it has a relatively low specificity and a significant potential for false positives. The clinical implication is that apical periodontitis may be incorrectly diagnosed for a healthy periapex. Some authors suggest that outcome/epidemiologic studies should be revised because of increased diagnosis of apical periodontitis with CBCT (21). However, to accurately diagnose radiographic presence of disease, the normal variations of a healthy periapex must first be understood.

The aims of this study were to compare the appearance of healthy periapical tissues on CBCT with periapical radiography and to investigate the size of the PDL space on CBCT for teeth with healthy and necrotic pulps.

Materials and Methods

Data Acquisition

This study was a retrospective analysis of imaging and clinical data acquired by 4 endodontic practices in Melbourne, Victoria, Australia involving 10 endodontists between January 2010 and December 2011. The study design was approved by the University of Melbourne Human Research Ethics Committee.

After exclusions, 68 patient records were identified from examinations that had teeth with a CBCT scan, a periapical radiograph, percussion test, CO_2 pulp test, and/or electric pulp test (EPT) undertaken within 1 week of examination. The CBCT scans had previously been requested as an adjunct to traditional radiography for the management of various complex endodontic conditions and were not taken as a routine investigation according to the principles of as low as reasonably achievable (10). CBCT scans were obtained with a 3D Accuitomo 80 (J. Morita, Corporation, Kyoto, Japan), small (40 × 40 × 40 mm) field of view, and voxel size of 0.08 × 0.08 × 0.08 mm. The periapical radiographs were taken according to the paralleling radiographic technique. Sixty of the radiographs were digitally acquired, and 8 were processed manually and scanned by using Epson Perfection V700 Transparency Photo (Epson America, Long Beach, CA) scanner at 800 dpi. The condition of the pulp was ascertained primarily by the CO_2 pulp test, with lack of response for 10 seconds considered a negative result (22). If the coronal pulp chamber was calcified, an EPT (Analytic Technology, Redmond, WA) was performed to further ascertain pulpal response. Tenderness to percussion and mobility were evaluated by percussion with a mirror handle and finger pressure.

Of the 68 patient records available, teeth were included in the study if at least a 3-mm border of the periapical region was visible on both the periapical radiograph and the CBCT scan. Teeth with ankylosis or abnormal mobility (grade I, II, or III) were excluded from the study. After exclusions, 200 teeth were available for investigation and comprised maxillary molars (n = 37), maxillary premolars (n = 43), maxillary canines (n = 19), maxillary incisors (n = 34), mandibular molars (n = 23), mandibular premolars (n = 20), mandibular canines (n = 10), and mandibular incisors (n = 14). Six teeth was the most from a single patient, but there were insufficient numbers to assess whether there was a variance within each patient.

Image Analysis

On a PC workstation running Microsoft Windows 7 Professional (Microsoft Corp, Redmond, WA) using i-Dixel OneVolumeViewer (J. Morita Corp) software, for each tooth the CBCT scan was deconstructed into 0.5-mm slices in 3 dimensions: buccopalatal, mesiodistal, and axial. For each cross section, the set of individual images were placed onto a Microsoft Office PowerPoint 2011 (Microsoft Corp) slide with only 1 cross-sectional series per slide. Other teeth and anatomic structures were blocked out, leaving only the tooth in question in view (Fig. 1). Periapical radiographs were displayed as 1 per slide with attached millimeter scale. All CBCT cross sections and periapical radiographs were randomized before evaluation.

Two calibrated, blinded examiners (1 specialist endodontist and 1 senior postgraduate endodontic student) separately performed visual analysis of all the images on an Asus VH222D LCD (Asus Inc, Taipei, Taiwan) monitor with resolution of 1920 × 1080 at 60 Hz. The periapical status of the CBCT and periapical radiograph images was determined by using a modified CBCT-periapical index (PAI) as proposed by Estrela et al (23). The examiners were unaware of the pulpal status of the teeth and were under the assumption there was an even distribution of healthy, treated, and diseased teeth. The examiners were trained by using the reference images included by Estrela et al, with the only difference being exclusion of categories E (cortical expansion) and D (bone destruction) because such characteristics were not part of the present study's objectives (Table 1). The modified CBCT-PAI score for the periapical radiographs is represented as PAI and the CBCT scan as CBCT-PAI. The measurements for the CBCT-PAI were made by using the millimeter scale that was present when cross sections were extracted from i-Dixel OneVolumeViewer software (Fig. 1). Measurements for the PAI were made by using a millimeter scale added to the PowerPoint slide scaled to the size of the film edge.

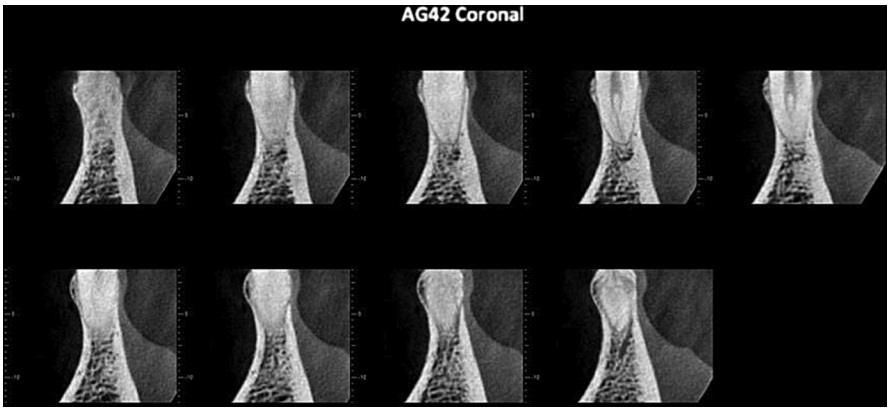

Figure 1. Example of a series of coronal cross sections for a single lower incisor tooth with healthy pulp status.

TABLE 1. Modification of CBCT-PAI

Score	Quantitative bone alterations in mineral structures
0	Intact periapical bone structures
1	Diameter of periapical radiolucency > 0.5–1 mm
2	Diameter of periapical radiolucency > 1–2 mm
3	Diameter of periapical radiolucency > 2–4 mm
4	Diameter of periapical radiolucency > 4–8 mm
5	Diameter of periapical radiolucency > 8 mm

Adapted from Estrela C, Bueno M, Azevedo J, Pecora J. A new periapical index based on cone beam computed tomography. J Endod 2008;34:1325–31.

Although examiners were asked to evaluate all teeth, previously treated teeth were present only as a blinding factor (ie, to ensure examiners believed an even distribution of pulp statuses) and not included in the analysis. This was because there was no way to validate the true status of their periapical tissues, as opposed to untreated teeth that were assessed through the response to pulp and percussion tests.

In cases of disagreement, consensus was reached by discussion between the examiners. The level of intraobserver agreement was assessed by weighted kappa statistics in 20% of the sample repeated 1 month after initial evaluation, and the level of interobserver agreement was calculated over the entire data set. Exploratory data analysis was undertaken by using simple proportions and cross-tabulations of CBCT-PAI score, PAI score, and pulp test response. Comparisons of CBCT-PAI and PAI scores were made between tooth type (incisor/canine, premolar, and molar), location (maxilla and mandible), and number of roots (single-rooted and multirooted teeth). The Fisher's exact and χ^2 tests were carried out to examine the null hypothesis that the distribution of CBCT-PAI scores does not vary with pulp status. SPSS v15.0 (SPSS Inc, Chicago, IL) and Minitab v16.0 (Minitab Inc, State College PA) were used for data analysis.

This methodology was validated by a pilot study that compared the CBCT-PAI scores of 2 viewing techniques; "live" scrolling of the CBCT scan was compared with individual cross-sectional slices, as done in this study. The pilot study involved the first 40 teeth that met the inclusion criteria. These teeth were evaluated by first scoring with the modified CBCT-PAI by live scrolling of the scan with i-Dixel OneVolumeViewer and then with the method outlined above. The time taken and CBCT-PAI scores from each method were compared.

Results

Of the 200 teeth, 166 yielded positive clinical signs of having healthy vital pulps, 14 had pulpal necrosis, and 20 had previous endodontic treatment. The CBCT-PAI scores for teeth that had a positive or negative response to the clinical pulp test are summarized in Table 2. Cross-tabulations indicated that the data were not consistent with the null hypothesis that the distribution of CBCT-PAI did not vary according to pulp status ($P < .001$). Pairwise comparisons between CBCT-PAI 0 versus 1, 0 versus 2–4, and 1 versus 2–4 indicated significant differences ($P < .01$) between the latter two but not the first ($P = .458$), which justified combining CBCT-PAI 0 and 1 and comparing with 2–5. Teeth with necrotic pulps were more likely to have PDL spaces of greater than 1–2 mm. There was a highly significant ($P < .001$) difference between healthy and necrotic teeth when CBCT-PAI 0–1 was compared with 2–5 (Table 3). There was no statistical difference between tooth type, location, or number of roots. Table 4 presents the interexaminer and intraexaminer kappa scores. The calculated estimates of interexaminer reliability were fair, and the intraobserver reliability was very high.

TABLE 2. Relationship of CBCT-PAI Score to Pulp Status

Pulp status		CBCT-PAI 0	1	2	3	4	5	Total
Vital	Count	43	90	27	3	3	0	166
	%	26	54	16	2	2	0	100
Necrotic	Count	2	2	4	1	5	0	14
	%	14	14	29	7	36	0	100

TABLE 3. Relationship of CBCT-PAI Score to Pulp Status (pairwise comparisons)

Pulp status		CBCT-PAI 0-1	2-5	Total
Healthy	Count	133	33	166
	%	80	20	100
Necrotic	Count	4	10	14
	%	29	71	100

A significant difference was found between groups when CBCT-PAI scores 0–1 and 2–5 were combined because of low cell frequencies ($\chi^2 = 18.870$, $df = 1$, $P < .001$; Fisher's exact test: $P < .001$).

For teeth with a vital pulp, when viewed on the periapical radiograph, a score of 0 was most common (Fig. 2), but when viewed on CBCT scan, more than 60% were scored 1 or larger. Among the teeth with vital pulps, the CBCT-PAI score was greater than the PAI in 72% of teeth (119 of 166) (Fig. 3). Almost 20% of vital teeth had a CBCT-PAI of 2+, and 2 had radiolucencies 2–4 mm when the periapical radiograph showed none (Figs. 2 and 4). Only 26% of teeth with healthy vital pulps had no PDL space widening, 54% had widening of 0.5–1 mm, and 19% had widening of greater than 1–2 mm, which was greater than on the periapical radiograph. Two examples of pulpally healthy teeth with no mobility or percussion tenderness but with CBCT-defined radiolucencies are shown in Figure 4.

Discussion

Distribution of pulp status was weighted toward teeth that responded positively to the pulp test because within the prescribed region of interest, there were generally more adjacent healthy teeth included in the field of view. This was beneficial because the objective of the current study was to investigate the normal variations of the apical tissues of teeth on a CBCT scan, which could potentially lead to false-positive diagnoses of apical periodontitis.

The results of this study showed that CBCT showed greater size PDL space than indicated by the periapical radiograph (Fig. 3). This is in agreement with previous studies (11, 12, 17, 19). The statistical results (Table 3) can be interpreted as indicating that when the pulp is vital, the radiographic apical dimension is significantly more likely to be 0–1 mm rather than greater than 1 mm. Conversely, when the pulp is necrotic, the radiographic apical dimension is significantly more likely to be greater than 1 mm. The lack of any significant findings comparing CBCT-PAI 0 and 1, and within range CBCT-PAI 2–5, could indicate difficulty in differentiating the different widths or simply lack of numbers.

There was no specific tooth type or location showing a larger periapical space on CBCT compared with traditional radiography. Although the difference was not significant, the only vital teeth with larger PDL spaces on periapical radiography than on CBCT were single-rooted teeth in the maxilla and mandible.

In this study, the CBCT scan was displayed in separate orthogonal slices to reduce variations that would occur if viewed and scrolled from different perspectives. This methodology was validated by a pilot study of

TABLE 4. Weighted Kappa Scores

	Interexaminer weighted kappa			Intraexaminer (Examiner 1/Examiner 2) weighted kappa		
	Observed	95% Confidence interval	Possible maximum	Observed	95% Confidence interval	Possible maximum
PAI	0.3622	0.1982–0.5262	0.5748	0.885/1.00	0.641–1.000	1
CBCT	0.3298	0.2321–0.4275	0.6402	0.8107/0.8028	0.6088–1	0.88–0.9369

Possible maximum is calculated to compensate for clustering of data around CBCT-PAI/PAI scores of 0, 1, and 2.

40 teeth that compared the CBCT-PAI scores of 2 viewing techniques. The live scrolling of the CBCT scan was compared with individual cross-sectional slices as done in this study. Results showed 93% correlation of the CBCT-PAI scores, with significantly shorter examination time, justifying use of the current methodology.

Some potential confounding factors exist in this study. Subtle apical radiographic changes may be the result of neurogenic inflammation in the presence of undiagnosed caries. A vital pulp may exist in a state of inflammation, with radiographic and clinical signs of periapical involvement seen as radiolucency (24, 25). Usually, radiographic evidence of apical periodontitis is associated with total pulp necrosis; however, histologic studies have shown that such periapical changes may not necessarily be associated with necrosis (26, 27), and that the radiographic apical changes around an inflamed pulp are due to neurogenic inflammation (28). Abella et al (19) reported that 13.7% of teeth diagnosed with irreversible pulpitis had periapical lesions visible on CBCT as opposed to only 3.3% on periapical radiograph. Although it is possible that such periapical changes have occurred in the presence of a positive pulp test response, within this study, the teeth displaying the radiographic changes noted were unrestored and caries-free (Fig. 4), so neurogenic inflammation is unlikely to affect the findings. Occlusal trauma was eliminated as a cause for the periapical changes by only including those pulp response positive teeth with a negative percussion result and normal mobility. Although this excludes overt occlusal trauma, the influence of the occlusal scheme on the appearance of the PDL space on CBCT cannot be excluded, and this warrants investigation in future studies.

The "fair" interexaminer variability (29) can be attributed to the clustering of data around CBCT-PAI of 0, 1, and 2, as would be expected because of the higher proportion of teeth with healthy pulps. An explanation for the relatively high variability is the disparity in examiner experience combined with the clinical difficulty in delineating the difference between "no radiolucency, 0.5–1 and 1–2 mm radiolucencies". Use of the live scrolling method and the digital measurement tool in i-Dixel One-VolumeViewer software may have helped to reduce this interexaminer variability. The "maximum-possible weighted kappa" considers the observed marginal frequencies, and the calculation allows for the minimal number of categories and such clustering of data. When the weighted maximum kappa coefficients are considered (Table 4), the estimated agreements are similar to a number of reported CBCT studies and can be considered substantial (29). Lennon et al (20) reported a widely varying correlation coefficient of between 26%–100%, depending on examiner.

The evaluation of any diagnostic test requires an appropriate reference standard by which to measure the test's accuracy. The closer the reference standard is to the true diagnosis, the greater is the accuracy of the evaluation, and if the reference standard is imperfect, results will be biased or invalidated (30). Unfortunately, endodontic investigations of radiographic diagnostic accuracy are complicated by the availability of an appropriate reference standard (31). A majority of CBCT studies show that CBCT is able to detect apical radiolucencies more readily than periapical radiography (11, 12, 17), but many of these have failed to use a clinically relevant reference standard. This study overcame this by using the clinical pulp test (CO_2 or EPT) as the reference standard against which CBCT and periapical radiography were assessed. The best available evidence suggests the specificity (percentage correctly diagnosed as vital) of the CO_2 and EPT pulp tests to be between 92% and 99%, justifying the present study's methodology (32–34). Furthermore, in this study the pulp tests were performed by specialist practitioners or senior postgraduates with experience in the execution and interpretation of the test.

De Paula-Silva et al (14) overcame the limitations of previous studies by using histopathology as the reference standard to correlate with radiographic appearance. The study reported that all cases of 1-mm^3 radiolucencies diagnosed as apical periodontitis on CBCT scan were shown to have inflammatory cells around the apex, implying a positive predictive value of 1 and an incidence of false positives of 0. However, the present study shows that large numbers of healthy teeth

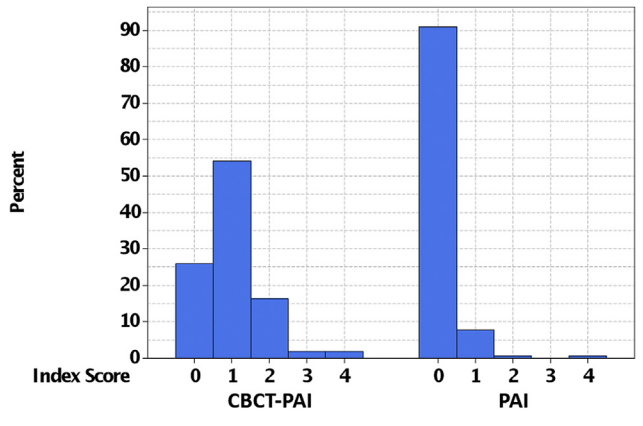

Figure 2. Distribution of PAI and CBCT-PAI scores for teeth with normal pulp response.

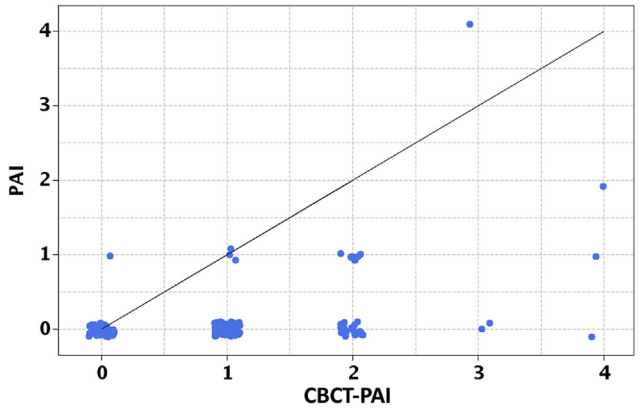

Figure 3. Relationship between PAI and CBCT-PAI scores for teeth that responded positively to clinical pulp test. CBCT-PAI score was greater than PAI in 72% of teeth (119 of 166).

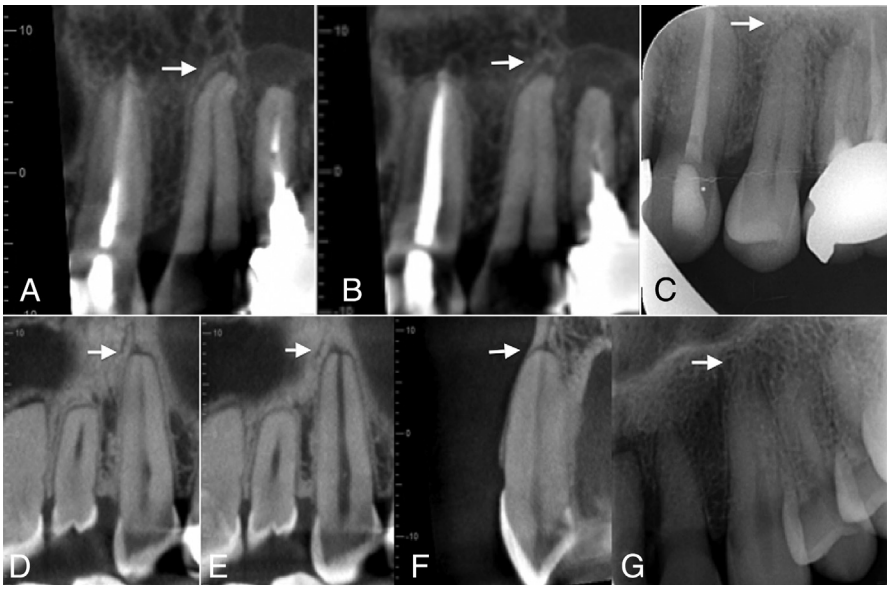

Figure 4. Periapical radiolucency associated with maxillary left second premolar with normal pulp test response and no percussion tenderness or mobility on sagittal CBCT cross sections (*arrows* in *A* and *B*) not evident on periapical radiograph (*arrow* in *C*). Periapical changes in maxillary left canine tooth evident on CBCT cross sections (*arrows* in *D–F*) but not periapical radiograph (*arrow* in *G*) in tooth with normal pulp test response and no percussion tenderness.

have PDL spaces of >1–2 mm (Table 2). In addition, de Paula-Silva et al (14) reported the incidence of false negatives (radiographically intact apex) with large numbers of inflammatory cells apically was 21% and 9% for periapical radiography and CBCT, respectively. They concluded that CBCT was more sensitive at detecting apical periodontitis than periapical radiography, which was more likely to miss apical periodontitis when present. Importantly, reported results did not include the healthy controls in calculation of sensitivity, specificity, and positive and negative predictive values. A study of diagnostic accuracy should include an even number of "true" healthy and diseased (apical periodontitis) conditions (35); however, the reference standard (ie, "true" diagnoses) of histopathology showed 92% of roots (77 of 83) to be inflamed. The effect of this is to overestimate the positive predictive value (proportion of test results that are true by histopathology) of CBCT in the diagnosis of apical periodontitis.

Although radiographic periapical changes appear larger on a CBCT scan compared with a periapical radiograph, so does the normal appearance of the PDL. This study found the CBCT-PAI to be significantly higher than the PAI for most teeth, as clearly shown in Figure 3. The presence of apical periodontitis on traditional radiography is often considered to be an apical radiolucency exceeding twice the width of the normal PDL space (15). The present study showed that if a CBCT-PAI of 2 (>1–2 mm radiolucency) is used to delineate the presence of apical periodontitis, a significant number of teeth (19%) may be incorrectly diagnosed as having apical periodontitis when they are in fact healthy, leading to an overestimation of disease. The ramifications of this are in the potential for overdiagnosis (36), but also that our historical interpretation of the appearance of the PDL space in health and in disease, which is based on traditional radiography, is flawed and that morphologic variations are biologically realistic. The latter requires further investigation.

Overdiagnosis may be trivial or profound in varying circumstances (36) and result in significant biological (37) and financial (38) costs. Our current understanding of the dynamics of healing on CBCT is lacking, and it is flawed logic to directly apply our knowledge of periapical radiography disease diagnosis to that of CBCT.

Conclusions

The direct application of traditional interpretation of periapical radiography to CBCT interpretation may be flawed because the normal 3-dimensional anatomy of the PDL space appears to entail greater variation than previously thought. The findings of this study indicate that with CBCT, the majority of vital teeth show some degree of PDL widening. Additional research is required to develop our understanding of the appearance of healthy periapex and the manifestations of apical periodontitis on CBCT before use in outcome studies.

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

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Photomedicine and Laser Surgery
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Comparison of the Antibacterial Effect and Smear Layer Removal Using Photon-Initiated Photoacoustic Streaming Aided Irrigation Versus a Conventional Irrigation in Single-Rooted Canals: An *In Vitro* Study

Xiaofei Zhu, DDS,[1,2] Xingzhe Yin, DDS,[1] Jeffrey W.W. Chang, DDS, PhD,[2] Yu Wang, DDS,[3] Gary S.P. Cheung, DDS, PhD,[2] and Chengfei Zhang, DDS, PhD[2]

Abstract

Objective: The Er:YAG laser with photon-induced photoacoustic streaming (PIPS) technique was reported to be effective in root canal disinfection. This study attempted to further investigate the antibacterial efficacy and smear layer removal ability of PIPS in comparison with conventional syringe irrigation *in vitro*. ***Methods:*** For antibacterial analysis, 48 single-rooted human teeth were prepared and inoculated with *Enterococcus faecalis*, and then divided into six groups of eight roots each. The colony-forming units (CFUs) per milliliter were determined after infection as the baseline. Then, the teeth were subjected to either PIPS plus 3% sodium hypochlorite (PIPS + NaOCl) or conventional syringe irrigation with 0.9% saline, 3% NaOCl, 17% ethylenediaminetetraacetic acid (EDTA), 0.2% chlorhexidine gluconate (CHX), or 3% NaOCl alternating with 17% EDTA. The reduction of CFUs in the individual group was determined. Additionally, scanning electron microscopy (SEM) examination of the canal walls for *E. faecalis* colonization was performed. For comparing the smear removal efficacy, another 48 single-rooted teeth, assigned to different groups as mentioned, were irrigated after mechanical instrumentation. The presence of a smear layer at different levels of the root canal was scored by SEM examination. ***Results:*** No significant differences were found in CFU reduction. No bacteria could be observed by SEM in the NaOCl, NaOCl + EDTA, and PIPS + NaOCl groups. The scores of smear layer of the NaOCl + EDTA and PIPS + NaOCl groups were significantly lower than those of the other groups in the coronal and middle third of the root canal. None of the methods can effectively remove smear layer in the apical third. ***Conclusions:*** PIPS system supplied with NaOCl and conventional syringe irrigation with NaOCl + EDTA are comparable in their ability to remove *E. faecalis* and smear layer in single-rooted canals.

Introduction

Studies have demonstrated that a large proportion of root canal walls remain untouched after mechanical preparation,[1,2] emphasizing the essential role of irrigation in endodontic procedures. Irrigation can improve the removal of bacteria, necrotic pulp tissue, debris, and smear layer in combination with mechanical root canal instrumentation.[3] Traditionally, irrigation is performed using a needle and syringe. Nevertheless, the mechanical flushing action of irrigants created via various conventional syringe needles is considered insufficient to thoroughly clean the root canal walls.[4] The penetration depth of the irrigant and its capacity to disinfect dentinal tubules are limited, especially in narrow or curved canals.

Several techniques and devices have been proposed to improve the efficacy of irrigation, including sonic or ultrasonic devices and different types of lasers.[4–6] Lasers can be used to activate photosensitizers that have been taken up by bacteria: a mechanism called light- or photoactivated disinfection,[7] or through activating the irrigation solution by the transfer of pulsed energy.[8,9] The Er:YAG laser of 2940 nm wavelength has the highest absorption in water and a high affinity for hydroxyapatite. It works on the principle of

[1]Special Department, School and Hospital of Stomatology, Beijing University, Beijing, China.
[2]Comprehensive Dental Care Endodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong, China.
[3]Department of Paediatric Dentistry, School and Hospital of Stomatology, Beijing University, Beijing, China.

transferring the pulsed energy to activate the irrigation solutions, which makes it suitable for use in root canal disinfection and cleaning.[10] Recently, a photoacoustic technique called photon-induced photoacoustic streaming (PIPS), was reported to result in effective debris and smear layer removal with a newly designed radial and stripped tip[11] or a 21 mm long, 400 μm diameter endodontic fiber.[12] With this PIPS technique, the laser tip was placed into the pulp chamber only, with no need to advance into the orifice of the canal; therefore, more cleaning of the root canal walls and a higher quantity of open tubules was achieved in comparison with traditional irrigation.[11,12] Therefore, we hypothesize that Er:YAG laser with PIPS technique may have greater efficacy in promoting root canal irrigation and disinfection.

However, to the best of our knowledge, neither the antibacterial effect nor the debridement ability in the apical third of root canals has been well established for PIPS. This study aimed to investigate the antibacterial effect and smear layer removal of PIPS, compared with conventional syringe irrigation in the apical area of root canals.

Materials and Methods

Ninety-six single-rooted human teeth extracted for periodontal reasons were used. Approval for conducting the study was granted by the Beijing University Institutional Review Board.

Experiment 1: Antibacterial effect of conventional versus PIPS-aided irrigation on Enterococcus faecalis

Root canal preparation, inoculation and disinfection. The external root surfaces of 48 single-rooted human teeth were cleaned with a curette to remove calculus and periodontal tissues. Presence of a single canal was determined by radiographs. Conventional access cavities were prepared. After the patency was established, the canals were enlarged to an apical size of #40 using stainless steel K-files (Dentsply Maillefer, Ballaigues, Switzerland) and rotary nickel titanium BioRace instruments (BR5, 4% taper, FKG Dentaire, La Chaux-de-Fonds, Switzerland). Copious irrigation with 3% sodium hypochlorite (NaOCl) was used throughout the root canal instrumentation. The smear layer was removed by using 17% ethylenediaminetetraacetic acid (EDTA) for 3 min. A final flush with sterile 10% sodium thiosulfate solution was performed to inactivate any residual NaOCl. A flowable composite resin (3M Dental Products, St. Paul, MN) was used to block the apical foramen by applying it over the root apex. After that, the teeth were immersed in brain heart infusion broth (BHI, Difco, Detroit, MI), ultrasonicated for 1 min, and then sterilized by autoclaving for 15 min at 121°.

E. faecalis strain (ATCC 29212) was used to infect the root canals. The flasks with infected teeth were incubated aerobically for 4 weeks at 37° under gentle shaking, and the culture media were replenished every week.

Experimental groups and root canal management. The infected teeth were distributed into six groups of eight roots each as follows. Control group, conventional irrigation with 10 mL 0.9% sterile saline (NS); NaOCl group, irrigation with 10 mL 3% NaOCl; CHX group, irrigation with 10 mL 0.2% chlorhexidine gluconate (CHX); EDTA group, irrigation with 10 mL 17% EDTA; NaOCl + EDTA group, irrigation with 5 mL 3% NaOCl and 5 mL 17% EDTA alternatively;,PIPS + NaOCl group, canal and pulp chamber bathed in 3% NaOCl and irradiated with Er:YAG laser in the pulp chamber for 1 min. All manual irrigation was performed with a 5 mL syringe and NaviTip 30-gauge safety needles (Ultradent, South Utah, USA). The tip of needle was at 1 mm short of the working length. For the PIPS + NaOCl group, an Er:YAG laser with a wavelength of 2940 nm (Fidelis, Fotona, Ljubljana, Slovenia) was used with a 12 mm long 400 μm diameter quartz tip. The laser operating parameters were 20 mJ per pulse, 15 Hz, and 50 μs pulse duration.[11] The coaxial water spray feature of the handpiece was set to "off." The tip was placed into the coronal access opening of the pulp chamber only, and kept stationary and activated for 1 min. During the laser irradiation, additional solution (3% NaOCl) was not deposited except when it was noted that the pulp chamber was depleted of any irrigant. In such cases, care was taken to replenish the irrigant in the pulp chamber only. Approximately 3 mL NaOCl was needed for one canal in the PIPS + NaOCl group.

Microbial analysis and scanning electron microscopy (SEM) examination. Root canals were sampled bacteriologically before (S1) and after (S2) irrigation/irradiation with a "paper point method."[13] Briefly, the root canal was gently rinsed with 1 mL of NS to remove nonadherent cells, and an initial sample was taken by the sequential use of three paper points placed to the working length. All paper points for the same tooth were transferred to 1 mL NS and immediately processed. This sample was labeled as S1. After irrigation/irradiation, the same procedure was conducted to obtain postoperative sample S2.

In the laboratory, sample was first vortexed for 1 min, followed by 10-fold serial dilutions in saline. Then, aliquots of 50 μL were plated onto BHI agar plates (Difco, Detroit, MI) and incubated at 37° for 48 h. The colony-forming units (CFUs) were counted and then transformed into actual counts on the basis of the known dilution factors. Each count was performed in duplicate on two occasions.

SEM observation of root canal walls after irrigation/irradiation. The roots were split longitudinally after S2 sampling. Each specimen was fixed in 4% gluteraldehyde at room temperature for 24 h, washed with phosphate-buffered saline (PBS) for 15 min, and post-fixed for 12 h in 1% osmium tetroxide. After a final wash with PBS, serial dehydration was performed with increasing concentrations of ethanol. The specimens were finally dried by using a SAMDRI PVT-3 critical point dryer apparatus (Tousimis Research Corp., Rockville, MD), coated with a 200 Å layer of gold palladium, and examined by using a Hitachi S3400N scanning electron microscope (Hitachi, Tokyo, Japan) at 12 kV.

Experiment 2: Removal of smear layer by conventional and PIPS aided irrigations

Another 48 single-rooted human teeth were recruited and similarly prepared as in Experiment 1, except that NS irrigation was used throughout instrumentation. Experimental group distribution and root canal management were conducted as in Experiment 1. After different irrigation/irradiation procedures, the roots were split longitudinally for SEM observation.

The coronal (3 mm from orifice), middle, and apical third (3mm from apex) of the root canal were examined individually in each specimen. More than 200 photographs per specimen were taken at various magnifications ranging from ×300 to ×5000 by the same operator. The SEM photographs were evaluated by two blinded observers using a scoring method for evaluating smear layer removal described by Hülsmann in 1997.[14] Briefly, those SEM images at ×1000 magnifications were used for this quantitative assessment. A mean smear layer score was calculated for each specimen. The inter-observer agreement was very good as indicated by a Fleiss' κ of 0.84. A scoring index of 1–5 was used as described:[14]

Score 1: No smear layer; dentinal tubules open
Score 2: Small amount of smear layer; many dentinal tubules open
Score 3: Homogeneous smear layer covering the root canal walls; only a few dentinal tubules open
Score 4: Complete root canal wall covered by a homogeneous smear layer; no dentinal tubules open
Score 5: Heavy, nonhomogeneous smear layer completely covering root canal walls

Data analysis was performed using the Kruskal–Wallis and the Mann–Whitney Wilcoxon *U* tests. A level of $p < 0.05$ was considered statistically significant.

Results

Antibacterial effect

E. faecalis reduction. The initial levels of colonization of *E. faecalis* (S1) were high in all groups ranging from $7.38 \times 10^6 \pm 8.56 \times 10^5$ CFU/mL (Table 1). The post-irrigation samples (S2) for the corresponding groups (Table 1) showed a significantly lower value than pretreatment (S1) samples ($p < 0.05$, Friedman test). Tukey honestly significant difference (HSD) test was used for intra-group analysis comparing the reduction rate in the number of CFU counts from S1 to S2. Among the groups, significant differences were revealed: the NS group had significantly lower reduction than any other group ($p < 0.001$); the reduction in the EDTA group was significantly lower than in the NaOCl, NaOCl + EDTA, CHX, and PIPS + NaOCl groups, but higher than in the NS group ($p < 0.001$). No significant differences were detected among the NaOCl, NaOCl + EDTA, CHX, and PIPS + NaOCl groups ($p > 0.05$).

SEM observations. The surfaces of root canal walls in all specimens were evaluated by SEM examination after S2 sampling. No bacteria were found in the specimens from the NaOCl, NaOCl + EDTA, and PIPS + NaOCl groups (Fig. 1B, C, and F). A mass of bacterial cells residing around and into the dentin tubules was observed in all samples from the NS group (Fig. 1A). Some bacterial cells were seen in six and three samples from the EDTA and CHX groups, respectively (Fig. 1D and E).

Smear layer removal

SEM study. Specimens from the NS group (negative control) showed a homogeneous smear layer in every part of the root canals. No open dentinal tubules could be found (Fig. 2A). For other groups, debris, defined as dentin chips and pulp remnants loosely attached to the internal surface of the root canals, was eliminated in coronal third of root canals (Fig. 2B). Specimens from the NaOCl + EDTA group seemed to have less debris than other groups in middle third of the root canals (Fig. 2C,D). Decontamination was incomplete in nearly all specimens at the apical third of the canals. Occasionally, an area of open dentin tubules was observed at the apical third of some specimens in the EDTA, NaOCl + EDTA, and PIPS + NaOCl groups (Fig. 2E,F).

Quantitative evaluation. Results of the smear layer score are summarized in Table 2. The NS group gave a significantly higher score than that of all other groups ($p < 0.05$, Fig. 2).

In the coronal third of the canal, both the NaOCl + EDTA group and the PIPS + NaOCl group scored significantly lower when compared with the other groups (Table 2, $p < 0.05$, Tukey HSD test). In the middle third, the NaOCl + EDTA group gained the lowest score, which was significantly different from that of the NS, NaOCl, EDTA, and CHX groups (Table 2, $p < 0.05$, Tukey HSD test), but not the PIPS + NaOCl group ($p = 0.109$, Tukey HSD test) (Fig. 2). In

Table 1. *Enterococcus faecalis* Reduction in Viable Counts After Treatment

Group	n	SEM (-)	S1 (CFU/mL) Mean	S1 (CFU/mL) SD	S2 (CFU/mL) Mean	S2 (CFU/mL) SD	Red.	Sig.
NS	8	0	7.25E+06	1.08E+06	1.70E+06	8.43E+05	77.41%	[a]
NaOCl	8	8	6.75E+06	8.86E+05	205	91.18	>99.99%	
NaOCl + EDTA	8	8	7.88E+06	7.55E+05	257	90.99	>99.99%	
EDTA	8	2	6.95E+06	6.12E+05	7.19E+05	1.35E+05	89.67%	[b]
CHX	8	5	7.45E+06	4.75E+05	1.29E+05	5.94E+04	98.25%	
PIPS + NaOCl	8	8	8.00E+06	6.05E+05	417.5	288.13	99.99%	
Total	48		7.38E+06	8.56E+05				

SEM(-), the number of samples that no bacteria could be found by scanning electron microscopic observation; CFU, colony forming units; Red., *E. faecalis* reduction rate before and after treatment (1-S2/S1)×100%; Sig., significant differences ($p < 0.05$).

NS, sterile saline; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; CHX, chlorhexidine gluconate; PIPS, photon-induced photoacoustic streaming.

[a]NS group had significantly lower reduction than other five groups.

[b]The reduction in the EDTA group was significantly lower than in the NaOCl, NaOCl + EDTA, CHX, and PIPS + NaOCl groups, but higher than in the NS group.

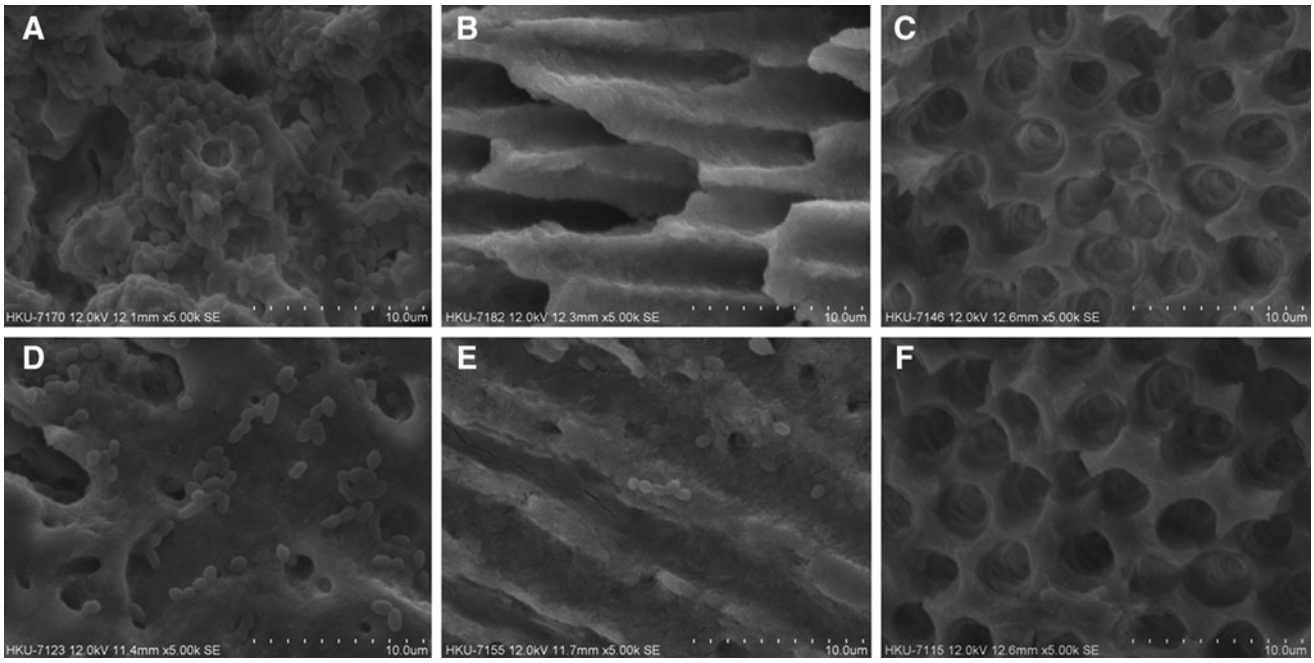

FIG. 1. Scanning electron microscopic (SEM) observation on *Enterococcus faecalis* infected root canals after treatment. **(A)** From the sterile saline (NS) group, a significant number of bacteria surround and reside in the dentinal tubules. **(B)** From the sodium hypochlorite (NaOCl) group and **(C)** from the NaOCl + ethylenediaminetetraacetic acid (EDTA) group, no bacteria can be found. **(D)** From the EDTA group and **(E)** from the chlorhexidine gluconate (CHX) group, several cells can be seen. **(F)** From the photon-induced photoacoustic streaming (PIPS) + NaOCl group, no bacteria can be found.

the apical third, no significant differences were found among all treatment groups.

The decontamination efficacy decreased from the coronal to the apical portion of the canal in all groups (Table 2 and Fig. 3). In the PIPS + NaOCl group, the reduction of smear layer score was significantly different from the coronal to the middle, then to the apical part (Table 2, $p < 0.05$, Tukey HSD test). For the NaOCl + EDTA group, however, the scores in the coronal and middle third showed no significant difference, both being kept at a low level. A significantly higher score was found for the apical third (Table 2, $p < 0.05$, Tukey HSD test). In the NaOCl, EDTA, and CHX groups, significant differences could only be found between the coronal third and the apical third (Table 2).

Discussion

Sodium hypochlorite is the most widely used irrigating solution. It kills bacteria rapidly even at low concentrations,[15] however, it has been criticized for its unpleasant taste, relative toxicity, and its inability to remove smear layer.[16,17] EDTA is an effective chelating agent, which is widely used in endodontic preparation for smear layer removal.[18] Therefore, alternating the irrigation regimen of NaOCl and EDTA has been recommended to be a more efficient protocol than NaOCl alone, in reducing the bacterial load in the root canal system.[19] CHX has been in use for a long time in dentistry because of its antimicrobial properties, its substantivity, and its relatively low toxicity.[20] Although many products containing 2% CHX are available on the market, a lower concentration (0.2%) of CHX can also kill *E. faecalis* within 30 sec.[21] In the present study, 0.2% CHX showed similar antibacterial effect with the NaOCl group and the NaOCl + EDTA group.

In the present study, the amount of bacterial reduction of the EDTA group was significantly less than that of the NaOCl, NaOCl + EDTA, CHX, and PIPS + NaOCl groups, which corroborates the fact that EDTA is an ineffective bactericidal irrigant. Furthermore, we showed that needle and syringe irrigation with NaOCl plus EDTA was as effective as Er:YAG laser irradiation at low energy parameters (PIPS + NaOCl group) in *E. faecalis* elimination. The Er:YAG laser light has the highest absorption in water, and its wavelength correlates closely with the absorption maximum of hydroxyapatite, compared with any other laser used for dental applications.[22] Highly absorbed laser energy produces reactive oxygen species to disrupt bacterial membrane, causing rapid death of microorganisms.[6] Theoretically, laser energy may not only kill bacteria directly, but also activate the irrigant to enhance its bactericidal actions.[8,23] However, no difference in bacterial reduction was found between the PIPS + NaOCl, NaOCl, and NaOCl + EDTA groups in the present study. This is probably because of the lower volume of NaOCl being used in the PIPS + NaOCl group as compared with the other groups. Also, the placement of the laser tip in the pulp chamber only may be too far to activate the fluid flow in the apical part of the canal, affecting its bactericidal effects. Although the most remarkable feature of Er:YAG application in root canal treatment has been attributed to its remote effectiveness in killing the microorganisms,[24] the highly variable anatomy of the root canals may limit this type of remote action. It was claimed that one of the benefits of the PIPS system is the minimal root canal preparation required. As the tip is only placed within the pulp

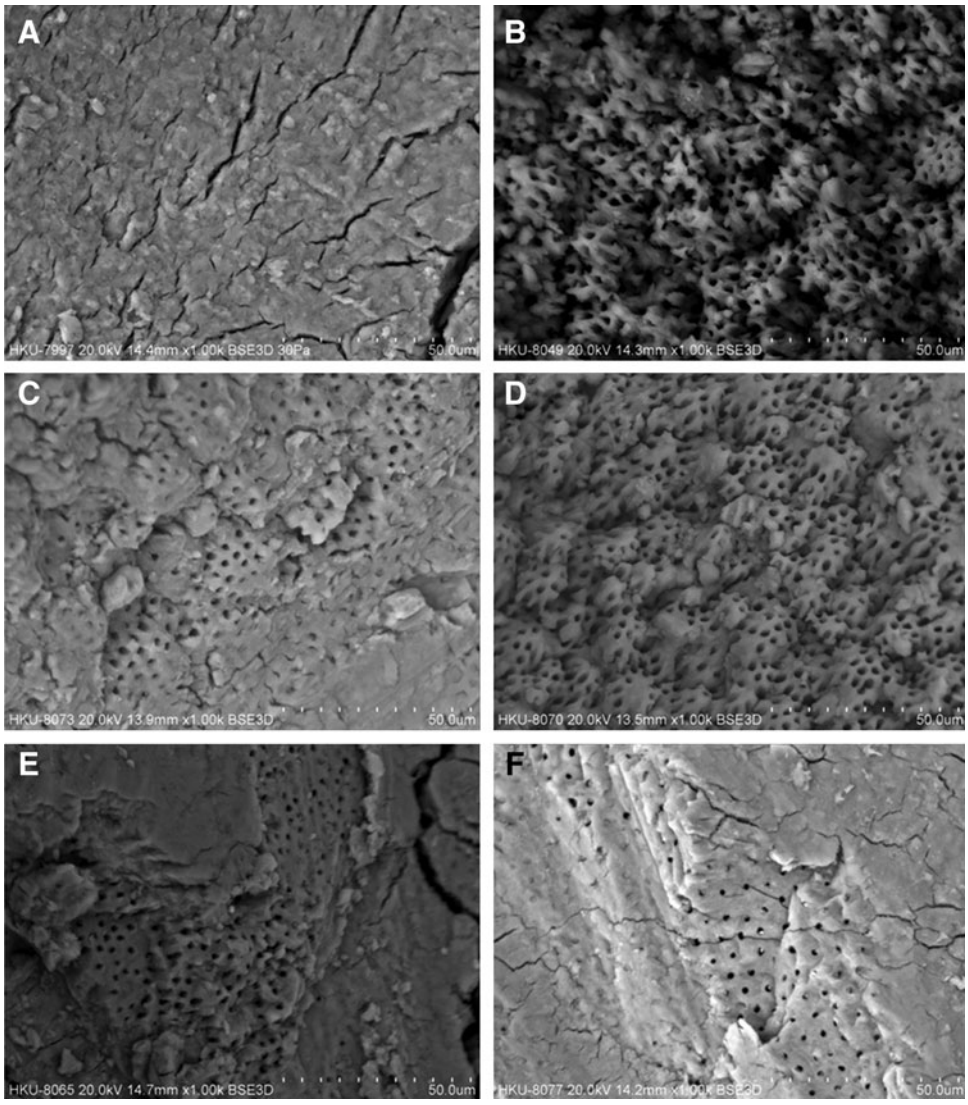

FIG. 2. Scanning electron microscopic (SEM) observation of smear layer in each group. **(A)** From the sterile saline (NS) group, a homogeneous smear layer covers the entire root canal, with no open dentinal tubules. **(B)** From the sodium hypochlorite (NaOCl) group, smear layer in the coronal third has been eliminated. In the middle third of the root canals (**D**, from the ethylenediaminetetraacetic acid [EDTA] group), seemed to have less debris than **C** (from the chlorhexidine gluconate [CHX] group). An area of open dentinal tubules in the apical third could be found in some specimens from the NaOCl + EDTA **(E)** and photon-induced photoacoustic streaming (PIPS) + NaOCl **(F)** groups.

chamber, enlargement of the canal to assist irrigation is not as necessary as in conventional needle irrigation.[11] In the aforementioned study,[11] the PIPS was activated for 20 sec, whereas in the current study, the activation time was extended to 1 min. Despite the longer activation time in the present study, we found no significant enhancement of the antibacterial efficacy, compared with the use of hypochlorite. This may be because the canals in this study had been enlarged to a size #40 K-file and 4% taper, which would facilitate the placement of the irrigation needle for enhancing its cleaning capacity. The canal size may also play a role in the cleaning efficacy of the PIPS, as it would either reduce the efficacy of the PIPS because of the dispersion of the pulsed energy or the fact that as long as the conventional needle is

Table 2. Quantitative Evaluation of Smear Layer Removal

Group	*Coronal*	*Middle*	*Apical*	*Overall*
NS	3.75 ± 0.46 a	4.00 ± 0.54 a	4.38 ± 0.52 a	4.04 ± 0.55 a
NaOCl	2.75 ± 0.46	3.25 ± 0.46	3.88 ± 0.83	3.29 ± 0.75
NaOCl + EDTA	1.75 ± 0.46 b	2.13 ± 0.35 c	3.63 ± 0.52 B	2.50 ± 0.93
EDTA	2.63 ± 0.52	3.25 ± 0.46	3.63 ± 0.52	3.17 ± 0.64
CHX	3.13 ± 0.35	3.50 ± 0.54	4.00 ± 0.76	3.54 ± 0.66
PIPS + NaOCl	1.88 ± 0.35 bA	2.75 ± 0.46 A	3.88 ± 0.64 A	2.83 ± 0.96

Different lower case letters indicate statistically significant difference ($p < 0.05$) in the same column. Different upper case letters indicate statistically significant difference ($p < 0.05$) in the same row.

NS, sterile saline; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; CHX, chlorhexidine gluconate; PIPS, photon-induced photoacoustic streaming.

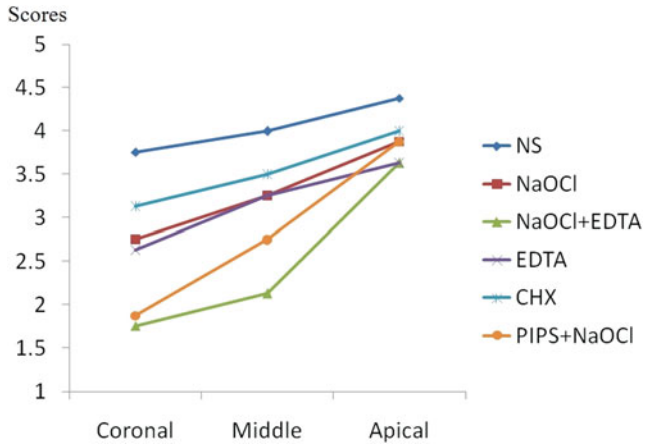

FIG. 3. A quantitative evaluation of smear layer at different positions within the root canals. All debridement properties were less effective in removing smear layer in the apical third.

able to reach the apical third, there would be no difference in their disinfecting ability. It is, therefore, essential to further investigate an appropriate canal size and penetration depth of the PIPS to achieve maximum levels of bactericidal action while at the same time not extruding any irrigant beyond the apical foramen.

Neither conventional nor PIPS-aided irrigation could effectively remove the smear layer in the apical third of the canal, even for single-canal teeth in this study. It is well known that needle irrigation is relatively ineffective in the apical portion of the root canal, because traditional needle irrigation delivers solutions no further than 1 mm past the tip of the needle.[25,26] Furthermore, hand needle irrigation alone was not able to create sufficient volume and flow of the irrigant in closed canal systems.[27] In the closed canal system, irrigant extrusion beyond 1–1.5 mm of the needle could have generated a liquid film along the air bubble–canal wall interface. Adequate irrigant replacement cannot be achieved in this area, resulting in gross debris retention.[27]

It has been suggested that Er:YAG laser activated in a limited volume of fluid, the high absorption of the laser wavelength in water, combined with the high peak power derived from the short pulse duration, would have resulted in a photomechanical phenomenon.[11] This action may remove bacteria and smear layer in the root canal. However, the present results indicate that this kind of effectiveness occurred only in the coronal and middle thirds of the root canals. The cleanliness of the intra-canal surface declined from the coronal to the apical portion. Interestingly, with EDTA irrigation, Er:YAG laser irradiation showed more effective removal of smear layer than with non-chelating irrigants.[11] To achieve higher efficacy of apical smear layer removal, PIPS technique with EDTA irrigant could be a rational combination.

Penetration of the laser tip is another critical factor for apical smear removal. It is still unknown as to what extent the rapid flow and the action of cavitation bubbles can contribute to root canal cleaning. When the laser tip was placed 2 mm short of the bottom of the root canal model and the laser was emitted at 50 mJ and 20 pps, an effective fluid flow could be created within 4 mm from the apex.[12] The literature is still obscure on how far the laser tip should be kept away from the apex to allow adequate cleaning and disinfection without injury to periapical tissues from the increase in the temperature or the extrusion of the irrigant. It has been suggested that the fiber tips (200 and 320 mm in diameter) be kept 2 or 3 mm away from the anatomical apex for better apical cleaning;[28] however, in a dye penetration study, a distance of 5 mm from the apical stop has been reported to be better than 4 mm in terms of extrusion of the irrigant.[29] The efficacy for cleaning versus apical extrusion of the irrigant would require further evaluations to postulate the ideal balance between cleaning and safety. This forms the theme of our next study. It is obvious from the present results, that laser irradiation from the pulp chamber was not able to clean the apical third, as the tips were placed within the pulp chamber. Various depths of penetration of the laser tip into the canal would need to be investigated for their cleaning efficacy in relation to the apical extrusion.

There are potential advantages of PIPS over chemical disinfectants with hand irrigation techniques. The bactericidal effect of the pulsed Er:YAG laser is non-thermal, which can avoid the undesired effects of thermal energy.[30] The rapid fluid motion caused by expansion and implosion of laser-induced bubbles can assist in cleaning the apical region, indicating that it is not always necessary to insert the laser tip up to the apex.[31] It implies that PIPS technique allows easy access for the photomechanical effects to occur within the root canal, and improves the success of root canal treatment, especially in narrow curved canals where the irrigation needle and ultrasonic tip might be restricted by the canal walls.

Conclusions

PIPS-aided irrigation and conventional syringe irrigation with NaOCl plus EDTA can significantly reduce *E. faecalis* colonization and remove smear layer in the coronal and middle thirds of single-rooted teeth, but cannot effectively remove the smear layer in the apical third of the root canal.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Chengfei Zhang
Comprehensive Dental Care Endodontics
Faculty of Dentistry
The University of Hong Kong
Hong Kong
China

E-mail: zhangcf@hku.hk