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Praktijk voor Integrale Tandheelkunde
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endodontologie

[redacted]

068

Aan: Nederlandse Zorg Autoriteit

Aanvraag Tarief ten behoeve van het gebruik van Laser Technologie in de Endodontie.

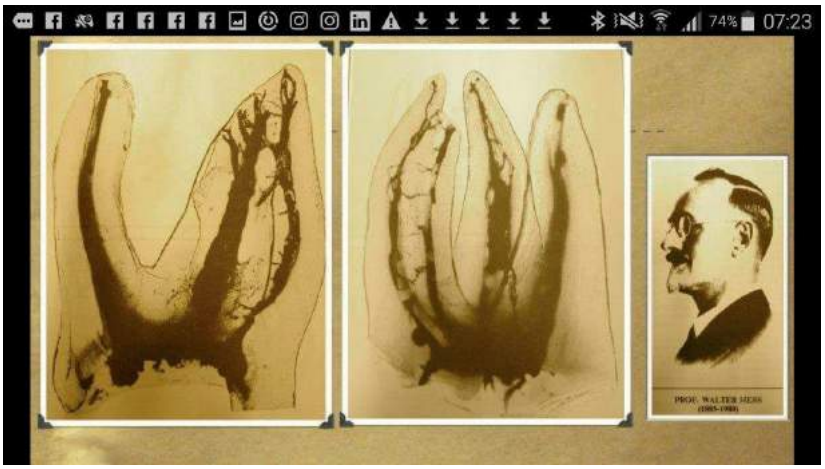
Gedurende de 35+ jaar dat ik heb gepraktiseerd in de klinische Endodontie en cursussen voor collega's heb ontwikkeld en gegeven zijn er veel vernieuwingen voorbij gekomen.
Ik noem de operatiemicroscoop die zijn introductie in Nederland in de tandheelkunde mocht beleven in mijn praktijk. De komst van roterend Nikkel Titanium instrumentarium, met name het Protaper gamma van de firma Dentsply. Ultrasoon geactiveerd instrumentarium. Mineraal Trioxy Aggregaat oftewel MTA. Om er een paar op een rijtje te zetten.
Recenter is de opkomst van CBCT scans, het 3-dimensinaal desinfecteren en onderzoek naar de mogelijkheden van regeneratieve methoden.

In deze aanvraag willen wij graag uw aandacht voor de PIPS Laser, oftewel de Photon Induced Photo-acoustic Streaming Laser. Een revolutionaire manier om geprepareerde wortelkanalen nog beter te reinigen en om daarna die wortelkanalen nog beter te kunnen vullen.

HET PROBLEEM

Het probleem is de complexiteit van wortelkanaalstelsels of ook wel kanaalsytemen genoemd. Hele generaties tandartsen en specialisten zijn opgeleid met het idee dat er een enkelvoudige verbinding bestaat tussen pulpakamer en omliggend bot. Dat enkelvoudige kanaal zou hoogstens in de laatste paar millimeter wat meer of minder gekromd zijn.

Sinds 1925 toen professor Walter Hess zijn boek uitgaf met daarin 10000 afbeeldingen van wortelkanaalsytemen zouden we beter moeten weten. Na Hess zijn er nog veel tandartsen geweest die zich bezig hebben gehouden met anatomie. Ik noem Craig Barrington, Frank Paque, Herbranson met zijn Tooth Atlas.



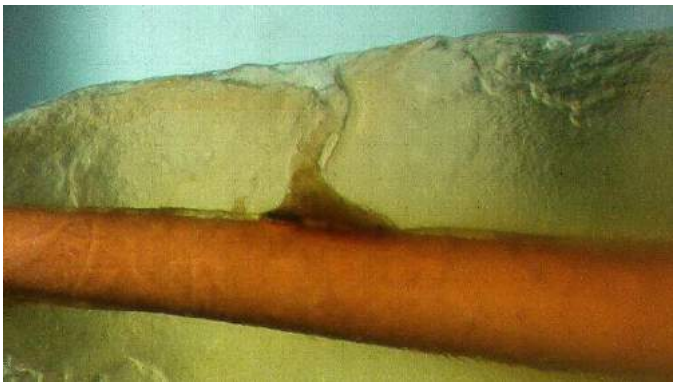
Afbeelding 1: Illustratie van een door Walter Hess doorzichtig gemaakte kies in de jaren 20 van de vorige eeuw! Hij liet al zien hoe ingewikkeld die anatomie is.

HUIDIGE STAND VAN ZAKEN

Met de huidige stand van zaken is het praktisch en klinisch gezien niet mogelijk om wortelkanaalsytemen te ontdoen van hun inhoud, hetzij weefselresten, hetzij bacteriën en bacteriële bijproducten. Er is voldoende onderzoek naar succespercentages van kanaalbehandelingen. Het onderzoek van b.v. L.Peters komt op een succespercentage van 60 a 70 %, wat uiterst teleurstellend genoemd mag worden.

Dat we desondanks toch redelijk succesvol zijn, zeker op korte termijn in het doen van kanaalbehandelingen ligt onder meer aan het feit dat we verhoudingsgewijs ruime kanaal vormen preparen in elementen ten einde er zeker van te zijn dat onze reinigingsmiddelen hun werk kunnen doen, en dat we ook ruim de tijd nemen om spoelmiddelen hun werk te laten doen.

Dat ruim prepareren leidt in een te groot aantal gevallen tot latere fracturen van de behandelde elementen. Laser kan een belangrijke rol spelen bij het reinigen van systemen.

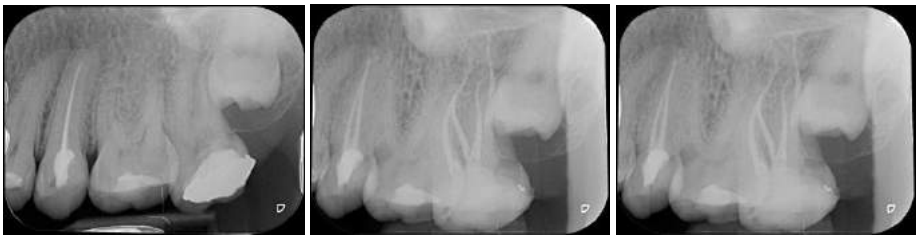


Afbeelding 2: Illustratie van de complicaties bij de huidige manier van prepareren.

OVER SHAPE

Hoe wordt er nu gewerkt?

De systemen die ons nu ter beschikking staan om wortelkanaalsystemen te reinigen zijn niet perfect. Ultrasone activatie met metalen tips zou het moeten kunnen doen maar helaas, die kunnen niet gebruikt worden voorbij de bocht aangezien ze veel te agressief zijn op de wanden van de geprepareerde kanalen. Subsonische activatie met de Endoactivator is dan een betere keus. Het apparaat kost zelf niet zoveel maar de energie waarmee de vloeistoffen geactiveerd worden is gering en de range van werking strekt zich derhalve uit tot de onmiddellijke omgeving van de tip.
Kortom, er valt nog veel winst te halen bij de reinigingsfase.



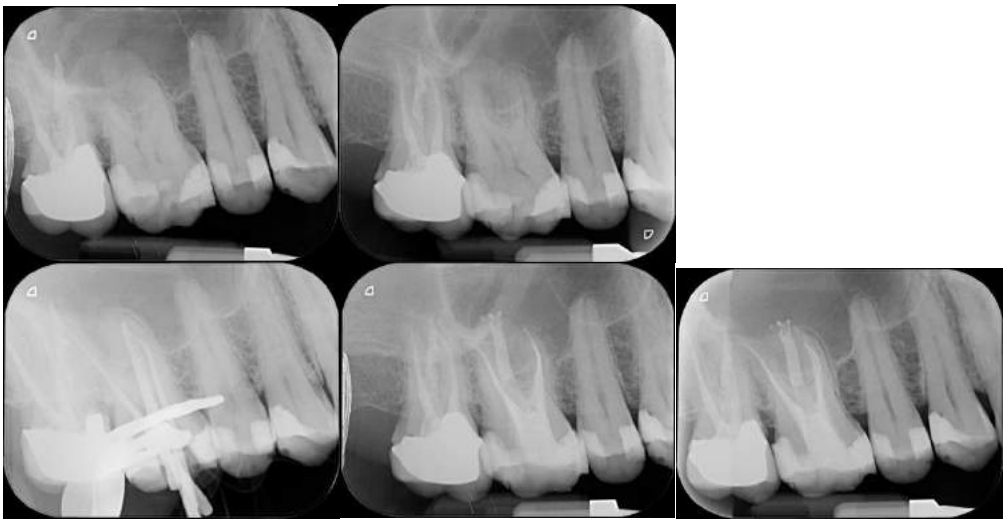
Afbeelding 3: Röntgenopnames

De afbeelding hierboven betreft een kies, een zeven boven, waar de interne anatomie zo lastig was dat ik geen van de kanalen tot op de volle lengte kon instrumenteren.
Na laseren is duidelijk te zien dat de obturatie, ofwel de kanaalvulling tot de terminus van de kanalen reikt.

Hoe werkt PIPS?

PIPS is ontwikkeld door tandarts Enrico Divito uit de USA. Hij verkocht het patent aan de firma Sonendo in California. Fotona, een Sloveens bedrijf en een grote speler op de laser markt heeft nu een apparaat waarmee PIPS beschikbaar komt voor de Europese tandartsen en specialisten.

Het werkt aldus, via een speciaal ontwikkelde tip wordt een laserpuls afgegeven in de waterige spoelmiddelen. De golflengte van de puls luistert heel nauw aangezien er gestreefd wordt naar volledige absorptie van de energie van de puls door de watermoleculen. Dat steekt nauw en daarom zijn de apparaten ook zo kostbaar. De puls veroorzaakt een vacuümbubbel in het spoelmiddel en de bubbel implodeert waarna er een schokgof door de hele kies gaat tot aan de terminus van de radix.
De puls wordt frequent herhaald.



Afbeelding4: Röntgenopnames

HOEVEEL KOSTIE?

Veel. De aanschafkosten van de laser geschikt voor dit doeleinde bedragen 65000 euro.
Een bedrag waarbij de tandartsen niet in de rij gaan staan zolang er niet een redelijke vergoeding tegenover staat.

DEZE AANVRAAG.

Vandaar deze aanvraag. Er zijn aanzienlijke extra kosten te verwachten voordat een gecompliceerd apparaat als dit daadwerkelijk efficiënt ingezet kan worden in een praktijk.
Dan heb ik het over instructiekosten, cursus en reiskosten voor het bezoeken van seminars etc.
Het is geen sinecure om een apparaat als dit te leren bedienen en er de patiënten het voordeel van te laten hebben zonder de patiënt, of de behandelaar schade te berokkenen.
Een laser is gevaarlijk in onoordeelkundige handen.

HET TARIEF.

Mijn insteek is om het tarief van het gebruik van dit apparaat te koppelen aan de endodontische behandeling net als het gebruik van de operatie microscoop.
De Zeiss microscoop die ikzelf heb aangeschaft kostte 45000 euro.
Het tarief voor microscopie is 64 euro. aangezien de laser een stuk duurder is en er meer bijkomende kosten zijn zou ik willen voorstellen om het tarief 95 te maken.
Dat zal vooral de tandartsen specialisten kunnen verleiden om het toch maar aan te schaffen.

DE ONDERBOUWING.

Voor de wetenschappelijke onderbouwing verwijs ik graag naar de litteratuurlijst met daarachter de complete publicaties. De voordelen blijken daaruit evident.

EndoTriad_May2015.pdf FocusOn-Lasers_Mar2017.pdf PIPS_Summary_Sept2016.pdf

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

BIJLAGEN

International Endodontic Journal

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

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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.*

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

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(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 ultra sonic irrigation has been shown to improve the clean ing 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 lim ited volume of fluid, plasma is generated. Plasma for mation 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 absorp tion of the Er:YAG wavelength in water (DiVito *et al.* 2012). These lasers have been evaluated for the elimi nation 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 ortho dontic 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 com mittee 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 irriga tion, 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 perfo ration (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 irriga tion protocol was divided in two steps:

All canals were irrigated with 2 mL of 6% NaOCl (Clorox, Oakland, CA, USA) delivered by positive api cal 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.

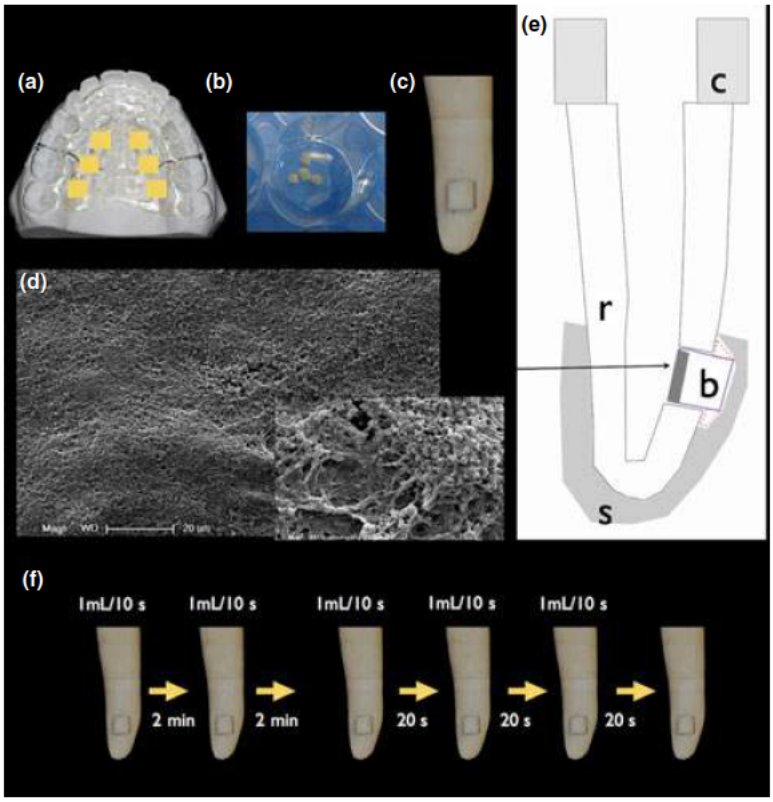

Figure 1 A removable orthodontic device was used to induce the contamination of dentine (a). Then, the blocks were incu bated for 48 h in BHI (b). Image and schematic representation of roots modified for the experiment (c, e). The pulp chamber walls were reconstructed using composite resin (c). A perforation 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 per formed for 20 s and repeated two more times.

The total time in this step (without taking into con sideration the 20 s of NaOCl application) was 4 min, and 4 mL of 6% NaOCl was used for all the experi mental 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 dou ble side vented needles. In this technique, the nee dle 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 Endoacti vator (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, Mer ignac, France) was used in conjunction with a Sat elec 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).

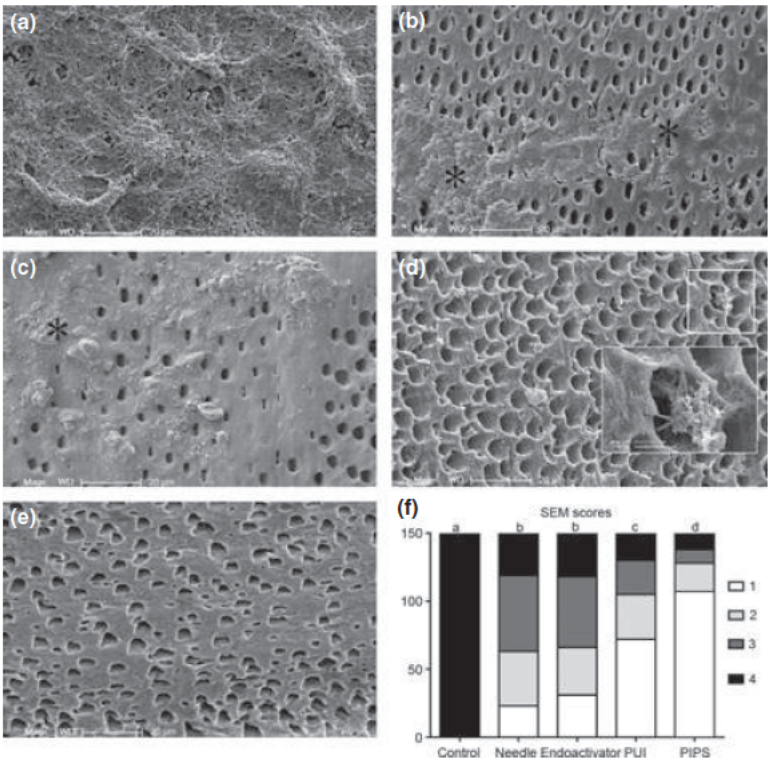

Figure 2 Representative images of the evaluated groups: Control (distilled water) showing extensive biofilm colonization (a), needle irrigation (b), showing biofilm residual layers (*). Dentine treated with the Endoactivator (c) and passive ultrasonic irrigation (d) showing residual biofilms and bacteria. 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 pro vides 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 Endoacti vator 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 move ments 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 effec tiveness of NaOCl disinfection (Duckhouse *et al.* 2004, Zifu Li *et al.* 2012). A previous study showed that ultrasonic and laser activation increase signifi cantly 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 tem perature 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 irriga tion 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 irri gation 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 dura tions (50 μs) leading to pressure waves that move irrigants in three dimensions distant to the tip posi tion. 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 irriga tion 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.

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Disinfection efficacy of photon-induced photoacoustic streaming on root canals infected with Enterococcus faecalis: An ex vivo study

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ADA CE Program ARTICLE 3

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

An ex vivo study

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

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

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

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

ABSTRACT

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

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

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

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

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

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

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

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

METHODS

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

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

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

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

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

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

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

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

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

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

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

between cycles. We irrigated the teeth with distilled water for 30 seconds to avoid any chemical reaction between different irrigant solutions. We then flushed the teeth with 3 mL of 17 percent EDTA for 30 seconds.

In both groups, we performed a final flush with 3 mL sodium thiosulfate for 30 seconds to inactivate the chemical microbicidal and tissue-dissolving action of the irrigants. An expert operator (G.O.) who was familiar with this laser-activated technique deposited the 3 mL of irrigant during the 30-second periods. Using a syringe with a 25-gauge needle positioned above the laser tip in the coronal access opening, he provided continuous irrigation with 3 mL of fluid during the laser-activation cycles to maintain fluid levels (Figure 1B).

We sealed the specimens with parafilm and vortexed them for 10 seconds (MSE Clinomixer, MSE Scientific Instruments, Milan, Italy). We performed this step to ensure maximum sampling efficacy. We introduced a sterile ringer solution into the root canal and obtained aliquots of 10 µL of solution from the main root canal of each specimen and performed an immediate bacterial count.

Assessment of bacterial recontamination. After we performed irrigation and immediate bacterial count, we incubated the specimens in groups A and B for 48 hours at 37°C in an atmosphere with 5 percent carbon dioxide. We added fresh tryptic soy broth every 12 hours. After the incubation period, we obtained 10-mL aliquots of solution as above and performed a new bacterial count.

Scanning electron microscopy (SEM). We categorized all of the teeth in groups A and B into the positive (*E. faecalis* infection) and negative (sterile) control groups by using SEM to determine the presence of bacterially infected matter in canal cross-sections and dentin tubule penetration. We dried the tooth roots for 24 hours at 21°C, sectioned them longitudinally by using a diamond disk with water spray and split them into two halves. The samples were dried for an additional 24 hours at 21°C, and we sputter-coated them with gold and examined them by using a scanning electron microscope (Philips XL30/CP, Philips, Eindhoven, Netherlands) at 20 kilovolts. We examined the entire root canal area (1-8 mm from the apex) in each sample.

Statistical analysis. We measured bacterial counts in CFUs by using log calculations to conform to normal distribution for bacterial counts. We then performed statistical analyses by using statistical software (JMP 10, SAS, Cary, N.C.). We used one-way repeated-measure analysis-of-variance testing for the statistical calculations. We presented data as mean, median and range of absolute bacterial counts or as percentages. We considered a *P* value (two sided) of less than .05 to be statistically significant.

RESULTS

SEM images of the split root sections indicated that bacterial biofilm was present in all of the positive control specimens (Figure 2, A1-A3). Overall, the SEM images

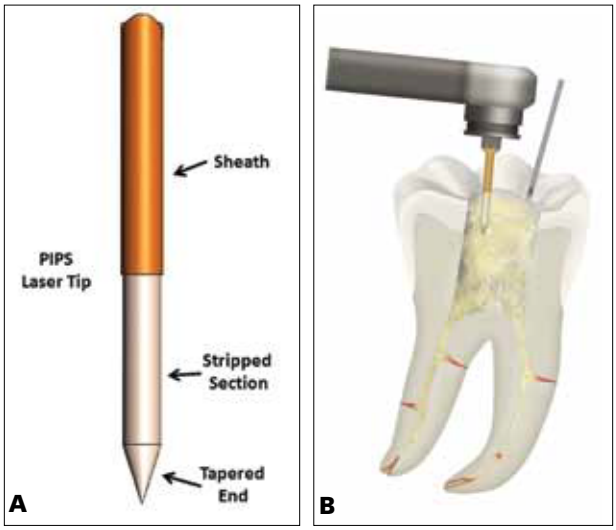

Figure 1. A. Image of typical photon-induced photoacoustic streaming tip (PIPS) with a tapered end and 4-millimeter stripped section. **B.** Correct positioning of the PIPS tip in the coronal access opening only with the irrigation tip supplying continuous solution during irrigation.

were suggestive of a qualitative reduction of bacteria in group A (Figure 2, B1-B3) and group B (Figure 2, C1-C3) compared with the positive control specimens. Also evident in group B were the lack of a smear layer, bacteria and open dentin tubules in addition to the absence of laser-induced thermal damage.

To provide a quantitative assessment, we enumerated CFUs of bacterial suspensions. We performed subsequent analyses of variance for repeated measures (before treatment, immediately after treatment and 48 hours after treatment) to evaluate the level of disinfection attained by means of each of the treatment methods. Both irrigation regimens significantly reduced bacterial growth ($P < .0001$). Immediately after the treatment, LAI in group A resulted in no detectable growth in all 10 samples, whereas there was no detectable growth in six of the 10 samples in group B. When we compared bacterial counts, this difference was statistically significant ($P < .05$).

After 48 hours, we detected no bacterial growth in any of the incubated samples in group A (Table, page 847) compared with the 10 of 10 samples in group B that had bacterial growth. The results of statistical analysis revealed a significant overall difference ($P < .0001$) between group A (PIPS laser activation and irrigation) and group B (conventional irrigation with NaOCl) after 48 hours. The negative control samples had no detectable bacterial growth.

DISCUSSION

In this ex vivo study, we compared the effectiveness of laser-activated root canal disinfection by using PIPS with the conventional hand irrigation method.

We used a vancomycin-resistant strain of *E. faecalis* to infect the root canals of extracted teeth. This micro-

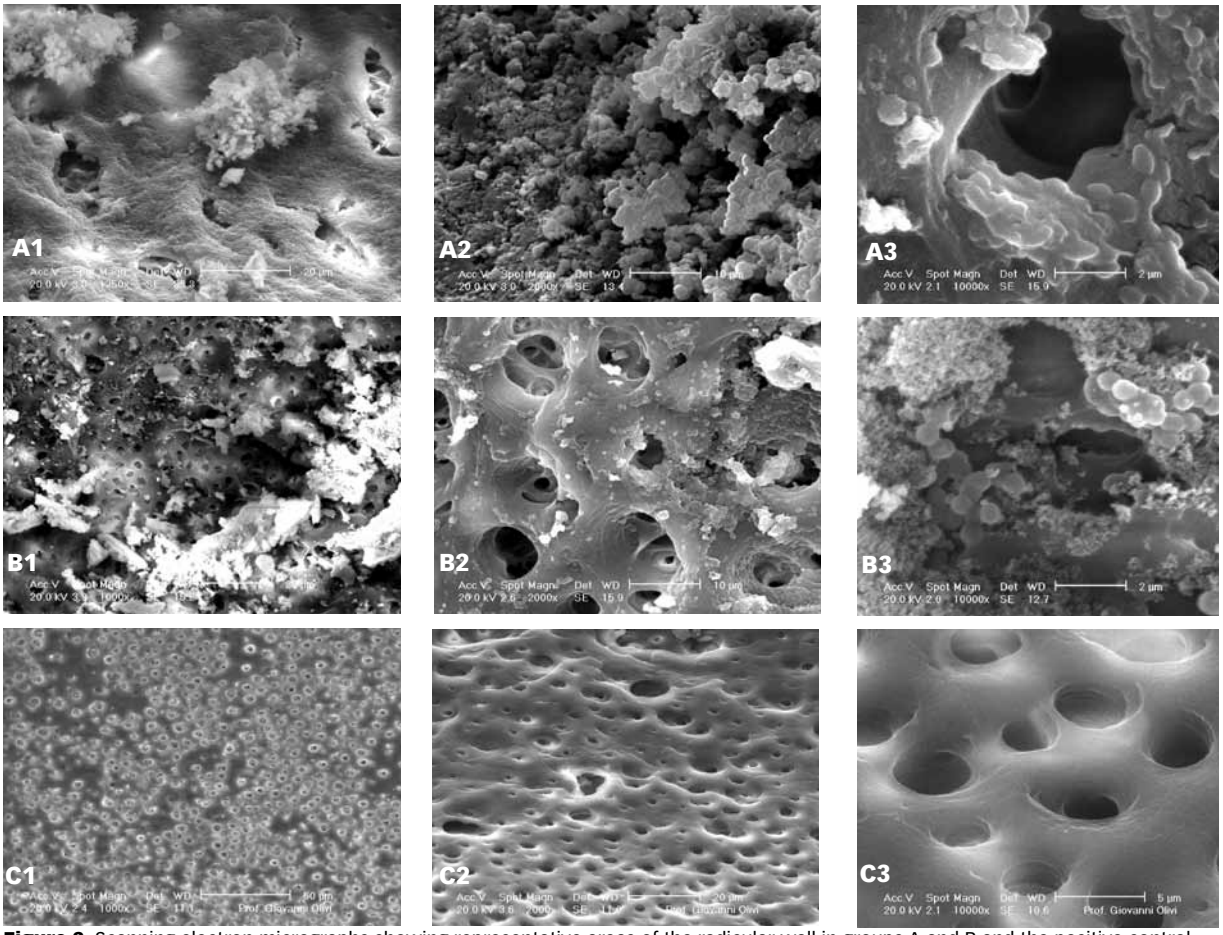

Figure 2. Scanning electron micrographs showing representative areas of the radicular wall in groups A and B and the positive control samples. Positive control samples (**A1-A3**) images (at ×1,250, ×2,000 and ×10,000 magnification, respectively) showing that bacterial biofilm growth is present in all of the control specimens before treatment. Group A (**B1-B3**) images (at ×1,000, ×2,000 and ×10,000 magnification, respectively) showing the remaining smear layer and bacteria at the 4-millimeter level after needle irrigation. Group B (**C1-C3**) images (at ×1,000, ×2,000 and ×10,000 magnification, respectively) showing no bacteria or smear layer after the use of photon-induced photoacoustic streaming (PIPS) at the 4-mm level. The collagen fibers and organic structure of dentin walls appear preserved, the dentin tubules are clean after the use of laser activation with PIPS and there is no evidence of thermal damage.

organism is a commonly isolated bacterium found in teeth in which root canals have failed, and it is difficult to eradicate it from the canals.[22] We used an incubation period of four weeks to enhance bacterial penetration into dentin tubules and promote bacterial biofilm formation. After irradiation, irrigation or both and before sampling, we vortexed the specimens to move any remaining bacteria to the main root canal, where we collected and counted them.

We used NaOCl, the most commonly used root canal irrigation solution, activated by an Er:YAG laser with a quartz radial and stripped PIPS tip.[19,20] When we activated 5 percent NaOCl via the Er:YAG laser for two cycles of 30 seconds each, we found a greater reduction in bacterial infection compared with using conventional hand irrigation.

This LAI protocol introduced several modifications to currently used laser-assisted techniques and protocols that involve higher laser energy, longer laser pulse duration, different tip designs and different positioning of the tip or fiber within the canal.[16-18,23-26] The 2,940-nm wavelength of the Er:YAG laser was chosen for its high absorption in water. We used a subablative pulsed energy (20 mJ at 15 Hz, average power 0.3 W) to produce an effective activation and streaming of fluids within the canal while reducing the thermal side effects of laser irradiation on the dentin walls.[19,27] Investigators in a previous study measured continuously during the irradiation period the temperature change on the external root surface of the tested samples by using a modified thermocouple measurement sensor to determine the absence of thermal effect.[19] They observed minimal average temperature increases (1.2°C and 1.5°C for the 20-second and 40-second irradiation time groups, respectively) at the root surfaces during laser irradiation.

The use of a short pulse duration of 50 μs produced

TABLE

Means, medians and ranges of absolute bacterial counts.*

GROUP	CFU† COUNT BEFORE PIPS‡ TREATMENT			CFU COUNT IMMEDIATELY AFTER PIPS TREATMENT			CFU COUNT 48 HOURS AFTER PIPS TREATMENT			REDUCTION IMMEDIATELY AFTER PIPS TREATMENT, %	REDUCTION 48 HOURS AFTER PIPS TREATMENT, %
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range		
A	1.15×10^9	7.8×10^8	1.00×10^8 to -4.20×10^9	NG§	NA¶	NA	NG	NA	NA	99.99	99.99
B	4.83×10^8	4.10×10^8	1.60×10^8 to 1.10×10^9	8.12×10^5	9.9×10^6	NG to 8.10×10^6	3.86×10^8	1.58×10^8	2.50×10^4 to 1×10^9	99.83	0.00

* There was a significant difference between groups A and B regarding bacterial reduction at 48 hours ($P < .0001$). N = 10 for all time points.
† CFU: Colony-forming unit.
‡ PIPS: Photon-induced photoacoustic streaming.
§ NG: No growth detected (zero CFUs).
¶ Not applicable.

a high peak power of 400 W at only 20 mJ, generating shock wave phenomenon (photoacoustic/mechanical effect) and secondary and tertiary cavitation in the fluids.[19,20] We used a quartz radial and stripped PIPS tip, which had a newly designed size and length (600-µm diameter and 9-mm length with the final 4 mm stripped) to allow for an improved lateral emission of energy, as reported by George and colleagues (Figure 1A).[17] During activation, we positioned the tip in the coronal pulp chamber filled with irrigant and did not insert it into the canal system as did investigators in other studies (Figure 1B).[16-18,23-26] This action facilitated the irrigation process and allowed for a more photoacoustic interaction with the irrigants in the pulp chamber (the main chromophore for Er:YAG laser absorption is water) than a direct photonic interaction with the root dentin walls. The mechanism of action of the Er:YAG lasers within the liquid-filled root canal might depend on rapid fluid motion caused by expansion and implosion of laser-induced bubbles.[23,24] In the root canal model, the vapor bubble expands in a vertical direction along the canal wall.[24] Some investigators studied the effect of LAI on root canal disinfection.[20-28] The results of our study suggest a positive effect of LAI and are in line with those of other ex vivo studies.[20-30]

The use of NaOCl alone was not able to kill bacteria and prevent new bacterial growth completely. However, after we used PIPS laser–activated NaOCl for a total of 60 s, we observed complete eradication of bacteria and biofilm. The results of subsequent testing showed that there was no bacteria growth in the incubated samples after 48 hours. This aspect can be explained with the lateral emission of photonic energy from the tip that created a strong three-dimensional streaming of irrigants and forceful shock waves within the endodontic system. The effectiveness of this laser technique also might be due to the increased consumption of available chlorine ions during the resting interval that occurred after the activation of the irrigant by means of an Er:YAG laser.[25] Another explanation might be related to the lysing and mechanical breaking up of the bacterial biofilm due to the shock wave–like phenomenon. Because the volume of the liquid in the root canal is small, this effect amplifies and improves the removal of bacteria, smear layer and residual tissue tags, which has been confirmed with the results of other studies.[19,20]

CONCLUSION

Under the ex vivo conditions in our pilot study, PIPS appears to be effective in enhancing the efficacy of irrigation solutions that are used commonly in endodontics. The use of a combination of two cycles of 5 percent NaOCl for 30 seconds each and Er:YAG laser activation with PIPS was effective in eradicating *E. faecalis* and in inhibiting new bacterial growth. Additional clinical studies are needed to clarify the effect on endodontic treatment outcomes. ■

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In-Vitro Evaluation of the Efficacy of PIPS Irrigation System on Disinfection of Type 2 Canal Systems in Molars

Abstract

Objective: This in vitro study was designed to compare the disinfection potential of an Er:YAG photon-initiated photoacousitc (PIPS) irrigation mechanism to standard needle irrigation (SNI) in eliminating biofilms from complex root canal systems.

Method and materials: Twenty-four extracted mandibular molar teeth exhibiting a Vertucci Type II canal system were selected and underwent canal instrumentation to create either a minimal (20/.04) or fully-flared (25/.06) preparation. Teeth were inoculated with bacterial plaque and incubated for 3 weeks creating a mature biofilm. Both instrumentation groups were sub-divided according to mode of canal disinfection: sterile saline or sodium hypochlorite (NaOCl) with PIPS laser-activation or same irrigants delivered with SNI. Each irrigation protocol used similar quantities of NaOCl or saline over the same time periods. Viable intracanal bacteria were quantified by MTT bacterial viability assay. Sub-groups were analyzed for statistical significance by ANOVA, followed by Scheffe's F-test (P = .05).

Results: All saline groups showed significantly more viable bacteria after irrigation than the corresponding NaOCl sub-groups. The #20/.04 sub-group that underwent PIPS and NaOCl irrigation had greater disinfection than the SNI counterpart, which was not significant (P > 0.05). The #25/.06 PIPS/NaOCl sub-group showed greater disinfection than SNI (P > 0.0008). All other groups showed no significant differences in disinfection of root canals.

Conclusion: In this in vitro study PIPS laser-activated irrigation achieved higher disinfection in #25/.06 fully-flared preparations than SNI. Although not significantly different, greater disinfection was found in the #20/.04 minimal canal preparations that were treated with PIPS irrigation compared to SNI.

Keywords: Photon-initiated photoacousitc (PIPS) irrigation; Biofilm; Type 2 canal system; Mixed bacterial oral plaque; MTT assay

Research Article

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Introduction

Apical periodontitis results from cultivable bacteria found within an infected root canal system that elicits host defences [1]. Elimination of the intracanal microbiome, or reduction to a subcritical level, is essential for periapical healing to occur [2]. Chemomechanical preparation that removes organic tissue, infected dentin and dislodges biofilms has been shown to be clinically effective in elimination of patient symptoms with concomitant radiographic healing [3,4]. Bacterial contamination related to non-healing apical lesions healing is the primary etiology of post-treatment disease requiring retreatment, periapical surgery or extraction [5]. A significant challenge for instrumentation and disinfection is the inability of contemporary techniques to address all the complexities of root canal systems [6]. A determinant factor for endodontic success is creating convenience form and canal shape that permits canal disinfection, specifically addressing areas of the root canal system that remain untouched by instruments. A recent change in the study of bacterial colonization of root canals has moved the focus from single organism laboratory-based planktonic cultures to the biofilm mode of growth [7]. Within the confines of the root canal system, residual biofilm is common even following irrigation with 5% sodium hypochlorite [8]. Furthermore, oral mixed biofilms are more resistant to removal having a greater adhesion to dentin [9]. Many alternative irrigation techniques intended to replace or serve as adjuncts to standard needle irrigation have been investigated including: passive or active ultrasonics [10,11]; sonic agitation [12]; photo-activated dyes (PAD) [13]; negative apical pressure devices [14]; and most recently photon-initiated photoacoustic streaming (PIPS) [15]. Laser activation of intra-canal irrigant creates large elliptical vapor bubbles that expand and subsequently implode, causing a cavitation and shock wave effect that promotes debridement and smear layer removal and the release of energy further down the canal [16]. Previous studies have shown PIPS to be effective in biofilm elimination [17], smear layer removal [16], and debris removal from complex nonseparated systems [18]. Apical size and taper size have been studied in the past by multiple studies, with varying conclusions reflecting the needs of obturation and irrigation techniques

[19,20]. The combination of varying tip size and taper continues to be studied, aimed at improving canal disinfection while conserving dentin. As apical preparation size increases, irrigant replacement was enhanced, providing space existed between the needle and the canal wall [21]. Increased canal taper also improved fluid interchange and reduced the risk of extrusion; minimal taper with large apical preparations also improved irrigant replacement [22]. *Ex vivo* investigation of the optimal tip size taper combination for laser activated irrigation have yet to be determined. The objective of this study was to examine the disinfection of intentionally contaminated Type II canals in the mesial root of mandibular molars by comparing needle flushing versus PIPS activation during endodontic irrigation of complex Vertucci Type II canal systems of two different sizes and taper. The null hypothesis was that no difference exists between bacterial disinfection present in #20/.04 taper and #25/.06 flared taper preparations using either PIPS or needle irrigation.

Methods and Materials

Tooth collection conformed to the protocols approved by the Institutional Review Board of the University of Tennessee Health Science Center with no identifiable data associated with samples obtained (IRB 10-00832-XP). Digital radiographs were used to identify mandibular molars with similar root lengths and fully-formed apices, with two distinctly separate canals from orifice to apex, with a common apical segment [23]. This canal system is notable for having cul-de-sacs and communicating isthmuses. A coronal reservoir with sufficient volume of irrigant is essential to photoacoustic wave propagation by the Er: YAG laser and PIPS tip. Samples that had broken down walls that would allow irrigant to flow out of the chamber were repaired with composite (ParaCore, Coltène Whaledent, Altstätten, Switzerland) such that all chambers were of similar height and anatomical dimension. Polyalkenoate cement (GC Fuji IX, GC America, Alsip, Ill.) was used to seal the orifice of each distal canal to prevent establishing a biofilm in the distal system. Mixed bacterial plaque samples were collected from laboratory personnel and grown in Todd Hewitt Broth (BD Diagnostics, Sparks, Md.) at 37° C for 24 hrs in the presence of 5% CO_2 [24]. A standard bacterial suspension (1×10^8 cells/ml) was prepared by measuring the optical density at 600 nm and confirmed by the plate dilution method. All teeth were kept in saline throughout the experiment and sterilized between uses by steam autoclave. Once sterilized, samples were transferred to sterile glass vials containing 15 ml of sterile Todd Hewitt Broth (THB) and incubated at 37º C for 24 hours. No bacterial growth confirmed sample sterility. Each sample was repeatedly used in the different subgroups, outlined below, as sterility was achievable with the teeth serving as a control. Each sub-group was repeated twice.

Canal instrumentation technique

Twenty-four mandibular molars were divided into either a minimal canal (20/.04) or fully-flared (25/.06) preparation. Irrigation protocols further subdivided the samples into four subgroups per preparation technique, each comprised of an irrigation delivery mechanism and irrigant (Table 1). The mesial canals were negotiated to their apical terminus and a minimum pathway developed with size 10 K-files (Roydent, Johnson City, Tenn.). Canals were enlarged to an apical matrix of 0.20 mm at a length 0.05 mm short of observing a file at the canal terminus. Crown-down preparation was completed using a complete series of rotary nickel-titanium (NiTi) instruments (ProFile Vortex, Dentsply Tulsa Dental Specialties, Tulsa, Okla.) in either .04 taper (#40/.04 to 20/.04) or .06 taper (40/.06 to 25/.06). Additionally, Vortex Orifice Openers (Dentsply Tulsa Dental Specialties) were used to create coronal flare in the fully-flared group. Each canal had to demonstrate resistance with the last NiTi instrument in the apical third to guarantee correct group allocation.

Table 1: Irrigation protocol followed in disinfection of infected root canals, *in vitro*.

Saline Protocol	Sodium Hypochlorite Protocol
6ml Saline over 30 sec	6ml Hypochlorite over 30 sec
Wait 30 seconds	Wait 30 seconds
6ml Saline over 30 sec	6ml Hypochlorite over 30 sec
Wait 30 seconds	Wait 30 seconds
6ml Saline over 30 sec	6ml Hypochlorite over 30 sec
Wait 30 seconds	Wait 30 seconds
6ml Saline over 30 sec	6ml Saline over 30 seconds
6ml 17% EDTA over 30 sec	6ml 17% EDTA over 30 seconds
6ml Saline over 30 sec	6ml Saline over 30 seconds
6ml Saline over 30 sec	6ml Saline over 30 seconds
6ml Saline over 30 sec	6ml Saline over 30 seconds

Creating mature intracanal biofilms

All prepared teeth were infected by injecting 40 µl of the standard bacterial suspension (1×10^8 cells/ml) equally into both mesiobuccal and mesiolingual canals. An established protocol for the creation of a 3 week biofilm was followed, with the addition of 0.5 ml fresh THB to each sample every 2 days during the incubation cycle [24]. Following incubation, but prior to irrigation, the external apical third of each mesial root was coated with sticky wax (Kerr Dental Laboratory Products, Orange, Calif.).

Irrigation

Sterile saline (Baxter International Inc., Deerfield, Ill.) or 6.0% sodium hypochlorite (diluted from 8.25% stock solution; The Clorox Company, Oakland, Calif.) were delivered using PIPS or SNI, followed by 17% EDTA (Roth International Ltd., Elgin, Ill.) as irrigating solutions. The irrigation regimen required 18 ml of irrigant (saline or 6% NaOCl), 6 ml of sterile saline, 6 ml of 17% EDTA and 18 ml of additional saline delivered over a period of 6 minutes (Table 2), a protocol documented previously [18]. The 9 mm long, 600 µm diameter PIPS tip was moved in a constant circular motion within the chamber while irrigants were delivered by syringe. The PIPS tip did not touch the chamber walls or enter

the canal orifices. Energy was supplied by a 2940 nm wavelength Er: YAG laser (LightWalker DT, Fotona, Ljubljana, Slovenia), at 15 Hz and 20 mJ at 30 second intervals. SNI was performed using a 30 Ga. side-vented needle (ProRinse irrigation probes, Dentsply Tulsa Dental Specialties) by placing the tip as apical as possible without binding, at least 1 mm short of working length. Irrigants were delivered with an up and down motion within the canal system.

Table 2: Viable bacteria recovered from root canals following irrigation methods used in the study.

Root Canal Treatment	Mean Bacteria ± Se			
PIPS Saline .04	193.4 ± 19.10	A		
PIPS NaOCl .04	19.43 ± 6.13		B	
SNI Saline .04	245.2 ± 13.75	A		
SNI NaOCl .04	32.71 ± 6.01		B	
PIPS Saline .06	251.14 ± 18.60	A		
PIPS NaOCl .06	10.75 ± 4.35			C
Needle Saline .06	279 ± 10.99	A		
Needle NaOCl .06	34.33 ± 4.07		B	

Columns with similar letters are not statistically significant according to anova (P > .05).

Quantifying intra-canal bacterial viability following irrigation

Following the final saline rinse, a new sterile 30 Ga. needle was used to withdraw10 µl of fluid from the canal system into a sterile tuberculin syringes. The solution was placed in a sterile 96-well microtitre plate along with 90 µl of sterile saline. The number of viable bacteria was determined by the MTT bacterial viability assay (Roche Diagnostics Corp., Indianapolis, Ind.). The assay is based upon the reduction of yellow tetrazolium salt (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) by metabolically active bacterial cells by the action of dehydrogenase enzymes. The resulting purple formazan crystals formed after a 4 hour incubation period with MTT label were further incubated with the provided solubilizing solution overnight at 37°C and the absorbance measured at 560 nm using a spectrophotometer (SPECTROstar, BMG LABTECH Inc., Cary, N.C.). The number of viable bacteria were calculated from the MTT absorbance values of a standard curve prepared with a known number of bacteria. Data were analyzed for statistical significance by ANOVA, followed by Scheffe's F-test, with $P < 0.05$ considered significant.

Results

The number of viable bacteria obtained from root canals was found to be different depending upon the irrigation protocol (Table 1). The highest number of bacteria was retrieved from root canals irrigated with sterile saline. The number of viable bacteria found in the 20/.04 and 25/.06 tapered canal preparations irrigated with sterile saline by SNI were 245 ± 13.75 and 279 ± 10.99, respectively. There was no significant difference between these groups. Following sterilization and re-inoculation of the same samples, NaOCl by SNI resulted in a significant reduction (87%; $P < 0.0001$) in viable bacteria (Table 3). Greater bacterial reduction occurred in canals irrigated with saline and PIPS (193.4 ± 19.1) compared to sterile saline and SNI (245.2 ± 13.75) in.04 tapered canal preparations, but the difference was not significant ($P = 0.0546$). The .06 tapered canals also showed similar results (Table 3). Irrigation with NaOCl exhibited no significant difference in viable bacterial counts obtained from minimal tapered or fully flared canals using SNI (Figure 1 & 2). The PIPS irrigation protocol resulted in 19.43 ± 6.13 viable bacteria in 20/.04 tapered canals, while the 25/.06 preparations showed 10.75 ± 4.35 bacteria. Significant differences in the number of viable bacteria were found between NaOCl delivered using either SNI or PIPS (Table 3). The 20/.04 minimal preparation with NaOCl and PIPS exhibited greater disinfection of the root canal system, but the difference in viable bacteria was not significant ($P = 0.1186$).

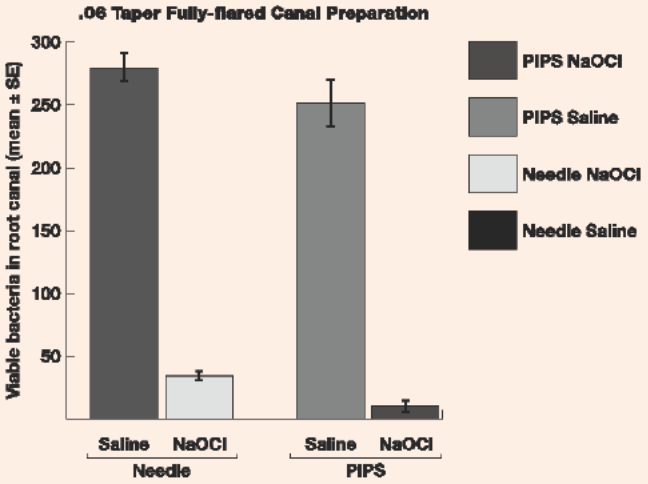

Figure 1: Demonstration of viable bacteria remaining in the root canals following the irrigation of a minimally shaped (#20/.04) preparation. Values are means and standard deviations. Bars with similar letters are not statistically significant (P > 0.05).

Table 3: Demonstration of values among root canal treatments exhibiting significant difference.

Root Canal Treatment	*P* Values
.04 SNI saline and .04 SNI NaOCl	< 0.0001
.04 PIPS saline and .04 SNI NaOCl	< 0.0001
.06 PIPS NaOCl and .06 SNI saline	0.0008
.04 PIPS NaOCl and .06 SNI saline	< 0.0001

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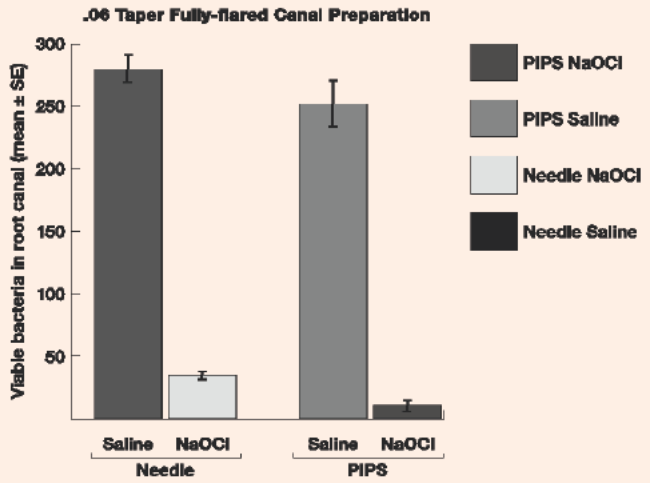

Figure 2: Demonstration of viable bacteria remaining in the root canals following the irrigation of a flared (#25/.06) preparation. Values are means and standard deviations. Bars with similar letters are not statistically significant ($P > 0.05$). Statistical significance between B, C ($P < 0.0008$).

Discussion

This study investigated the interaction of varying canal taper with the disinfection potential of PIPS laser-activated irrigation in comparison to SNI using sterile saline or sodium hypochlorite. While no statistical differences between the tapered preparations and their disinfection potential for either irrigation method existed, a tendency for significantly greater disinfection in the fully flared .06 tapered preparations occurred when PIPS was used. This may be due to minimal shaping to an .04 taper preparation may not permit an adequate volume of active irrigant to flow to the canal terminus and cause turbulent flow sufficient to detach sessile microbes. While intracanal tissue debris has been shown to be cleared from the isthmus at 2.6 times greater than for SNI [18], the isthmus remains an area from which existing organic debris, along with packed inorganic debris from instrumentation, can be difficult to remove. Eddy currents exist where the two canals merge into one, with a stationary area above which fluid interchange does not occur, leaving microbes present in complex nonseparated systems. This has previously been demonstrated in simulated lateral canals and isthmuses where ultrasonic activation of water or sodium hypochlorite can create stable bubbles, preventing biofilm removal in its entirety [25]. Alternatively, minimal tapered preparations prevent the collection of sufficient microbes in the aliquot from the mesial root canal system. The MTT assay measures the metabolic activity of microbes and is able to provide quantification over other methods, such as turbidity or plating. A standard curve of *E. faecalis* was performed using the assay with a known number of bacteria. Similar results showing improved disinfection capability of PIPS irrigation have been reported using different analytical methodologies [26-28]. The challenges of determining antibacterial efficacy are compounded by the difficulty in assessing what is actually being measured. Techniques which are commonly used, such as paper point sampling followed by CFU counting can determine general bacterial counting from the coronal third which can exclude information from the apical third, where disinfection is more indicative of irrigation efficiency and more difficult to access. Measurement of the apical third cleanliness has been attempted by direct-access methods, as well as split-model methods, both requiring structural modification of the dentin and apical complexities [17-31]. While the method used in this experiment contains similar shortcomings to the paper point/CFU method with regards to sampling location, it provides expeditious sample collection, reuse of samples in order for the same sample to serve as a control, and a well-known, reliable methodology for bacterial quantification. Complex nonseparated mesial roots of mandibular molars were prepared either to a 20/.04 or 25/.06 taper, to determine the *in vitro* disinfection using PIPS or SNI with sodium hypochlorite. The fully flared preparation provided the highest level of intra-canal disinfection with PIPS and sodium hypochlorite. Further studies are needed regarding taper preparation requirements for optimizing disinfection, as well as evaluation of the collection of apical bacteria from intact root canal systems.

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Efficacy of 4 Irrigation Protocols in Killing Bacteria Colonized in Dentinal Tubules Examined by a Novel Confocal Laser Scanning Microscope Analysis

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Abstract

Introduction: The aim of this study was to determine the efficiency of 4 irrigation systems in eliminating bacteria in root canals, particularly in dentinal tubules. **Methods:** Roots of human teeth were prepared to 25/04, autoclaved, and inoculated with *Enterococcus faecalis* for 3 weeks. Canals were then disinfected by (1) standard needle irrigation, (2) sonically agitating with EndoActivator, (3) XP Endo finisher, or (4) erbium:yttrium aluminum garnet laser (PIPS) (15 roots/group). The bacterial reduction in the canal was determined by MTT assays. For measuring live versus dead bacteria in the dentinal tubules (4 teeth/group), teeth were split open and stained with LIVE/DEAD BackLight. Coronal, middle, and apical thirds of the canal dentin were scanned by using a confocal laser scanning microscope (CLSM) to determine the ratio of dead/total bacteria in the dentinal tubules at various depths. **Results:** All 4 irrigation protocols significantly eliminated bacteria in the canal, ranging from 89.6% to 98.2% reduction ($P < .001$). XP Endo had the greatest bacterial reduction compared with other 3 techniques ($P < .05$). CLSM analysis showed that XP Endo had the highest level of dead bacteria in the coronal, middle, and apical segments at 50-μm depth. On the other hand, PIPS had the greatest bacterial killing efficiency at the 150-μm depth in all 3 root segments. **Conclusions:** XP Endo appears to be more efficient than other 3 techniques in disinfecting the main canal space and up to 50 μm deep into the dentinal tubules. PIPS appears to be most effective in killing the bacteria deep in the dentinal tubules. *(J Endod 2016;■:1–7)*

Key Words

CLSM, dentinal tubules, EndoActivator, MTT assay, PIPS, root canal disinfection, XP Endo

The main goal of chemomechanical treatment of the root canal system is to eliminate or reduce bacterial populations in the canal to a level that can allow periradicular tissue healing with a positive treatment outcome (1–3). To render the canal bacteria-free is challenging. Mechanical debridement alone is limited in reaching all the root canal spaces (4, 5). Previous studies have demonstrated that only 40%–60% of the cases can have a negative culture after cleaning and shaping of the canals (6–10). Recent endeavors on advancing regenerative endodontics further underscore the importance of effective root canal disinfection (11). Thus, strategies for root canal disinfection should be directed to use more effective irrigation activation techniques that may maximize root canal disinfection. Multiple activation methods have been proposed to improve the efficacy of irrigants, including sonic, ultrasonic, negative apical pressure irrigation, as well as laser activation (12).

EndoActivator (EA) (Dentsply, York, PA) is a battery-operated sonic handpiece that uses plastic tips to agitate irrigant solutions vigorously. The activator tips are available in 3 different sizes and produce 2000–10,000 cycles/min. It is recommended to use after cleaning and shaping of the root canal system to activate the irrigation solution (13). Photon-induced photoacoustic streaming (PIPS) has been recently introduced and gained attention because of its properties that appear to enhance the disinfection of the root canal system (14–16). PIPS operates by transferring the energy to the irrigation molecules, resulting in rapid and powerful shock waves, forcing the irrigant throughout the entire root canal system (17). XP Endo Finisher (FKG Dentaire, Switzerland) is also a new file that has been recently introduced to be used as a final disinfection step to disturb the bacterial biofilm. It is claimed by the manufacturer to provide an optimal cleaning of the root canal system while preserving dentin.

The effectiveness of multiple irrigation techniques on reducing the bacterial count in the main root canal space has been previously investigated (18, 19). More recent studies have visualized bacteria in dentinal tubules and determined its status (live or dead) by confocal laser scanning microscopy (CLSM) (14, 20). Zou et al (21) have shown that NaOCl can penetrate into the dentinal tubules

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at a range between 77 and 300 μm, depending on time, concentration, and temperature. There is a lack of studies providing quantitative assessment for the level of bacterial reduction in the dentinal tubules after disinfection. Therefore, the aim of this study was to quantify and compare the bacterial viability in root canals treated by 4 different irrigation systems: standard needle irrigation (SNI), EA, XP Endo, and PIPS. We measured the level of bacterial reduction in the main canal by using a chemical method and in the dentinal tubules by using CLSM analysis.

Materials and Methods

Specimen Preparation

Intact mandibular premolars and molars with no apical resorption were collected from the clinic in the Department of Oral and Maxillofacial Surgery and placed in phosphate-buffered solution (PBS). The tooth sample collection in this study conformed to exempt protocols approved by the Institutional Review Board of University of Tennessee Health Science Center (12-01937-XM). Molars were vertically split into mesial and distal roots by using a water-cooled high-speed bur. Only distal roots with single canals were used in this study. The configuration of the single canal was confirmed through high magnification and buccolingual and mesiodistal radiographs. Composite resin was used to build the remaining walls of the coronal portion of the tooth crown to provide a reservoir for the irrigant. The crowns of the teeth were adjusted to a standardized working length of 18 mm. Canals were instrumented by using rotary files up to 25/04 (Endo Sequence; Brasseler) while maintaining apical patency. Teeth were then autoclaved in PBS at 121°C for 20 minutes.

Canal Inoculation with *Enterococcus faecalis*

A standard suspension (1×10^8 cells/mL) of *E. faecalis* (ATCC 47077; Rockville, MD) was prepared from a 24-hour culture of bacteria grown in brain heart infusion (BHI; Difco). Each canal was filled to the orifice level with *E. faecalis* suspension by using sterile 1-mL insulin syringes with a 30-gauge needle. The root was then placed in a 15-mL tube containing 10 mL BHI broth and incubated at 37°C for 21 days in 100% humidity to allow colonization of the bacteria on the canal wall and into the dentinal tubules. Aliquots of culture medium (5.0 mL) were replaced with fresh medium every 3 days.

Disinfection Procedures

After 21 days, specimens were removed from the inoculation tubes, and the root apexes were sealed with composite resin in a clean environment laminar flow cabinet to prevent sample contamination. The canals were disinfected by using 4 different irrigation systems/groups described below (15 teeth/group). In each procedure, the canals were irrigated with 2 mL 17% EDTA for 1 minute by using a 30-gauge side-vented needle, followed by 3 mL 6% NaOCl at a flow rate of 2 mL/min with the following cycles: 30 seconds of 6% NaOCl irrigation (1 mL/30 sec) followed by 30 seconds of no irrigation. Procedures were performed by one board-certified endodontist except for the PIPS group. Below are the different treatment protocols.

Group 1: SNI. A 30-gauge side-vented needle was placed within 2 mm from the working length and moved in a vertical motion to avoid the needle being locked in the canal. To ensure length control, a stopper was placed on the needle at the required length.

Group 2: EA. The canal was first passively filled with irrigant. The irrigation needle was then placed at the pulp chamber level, and under constant irrigation, a yellow EA tip was placed in the canal 1 mm short of the working length, and irrigant was activated by following the method described above.

Group 3: XP Endo. In a manner similar to EA, the file was placed 1 mm short of the working length and operated by using a slow-speed motor at 900 RPM in a vertical motion. Similar to SNI, a stopper was adjusted at the required length for length control.

Group 4: PIPS. Fotona LightWalker Er:YAG (Fotona LLC, Dallas, TX) was set at the recommended settings (20 mJ, 15 Hz, 0/0 air/water). For this part of the study, a clinician with experience in using PIPS operated the instrument. The canal was first passively filled with irrigant as described above, and under constant irrigation, the PIPS tip was placed in the pulp chamber and was submerged in irrigant as described previously (14). The tip was left stationary and activated for the cycles described under constant irrigation, while ensuring the canal and pulp chamber remained passively filled with irrigant throughout irrigation. If the pulp chamber was seen without irrigant, the cycle was stopped and then continued after replenishing the pulp chamber with NaOCl.

E. faecalis Sampling

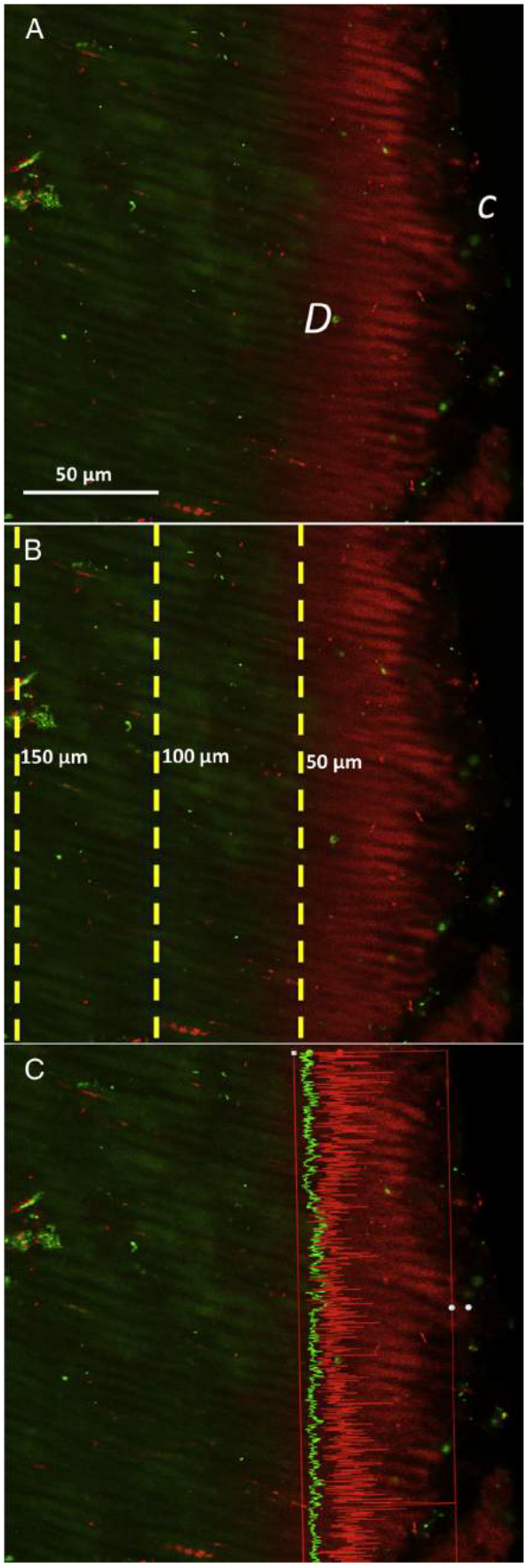
After the 21-day bacterial incubation, *E. faecalis* in the root canal of each tooth was sampled before (S1) and after (S2) disinfection. All materials and instruments used in the following sample collection were sterile. For collection of S1 samples, the BHI broth in the canal space was first aspirated and then filled with PBS. A Hedström instrument #25 was used to file the dentinal walls vigorously (20 strokes). Canal content was then aspirated by using 1-mL insulin syringes with a 25-gauge needle and transferred to a microcentrifuge tube. This procedure was repeated 3 times/canal, and the final volume of collected bacteria in PBS was 100 μL for each tooth/canal. After S1 sample collection, each tooth/canal was disinfected by one of the disinfection methods described above, followed by flushing the root canal with 1 mL 10% sodium thiosulfate to neutralize the NaOCl. The canal was then ready for S2 sample collection. Each canal was treated and bacterial samples were collected with the same manner as that for S1 sample collection.

MTT Assay

Collected bacterial samples (S1 and S2) from the canals were subjected to a standard MTT assay to detect the viable bacteria. Ten microliters of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) agent (Sigma-Aldrich, St Louis, MO) was placed in each of the microcentrifuge tubes containing the bacteria samples. Samples were vortexed and then incubated at 37°C for 4 hours. Then 110 μL isopropanol/HCl was added to each tube to solubilize the formazan dye. Microcentrifuge tubes were then spun for 5 minutes at 6000 RPM, and 190 μL supernatant was placed in a 96-well plate. The optical density was read at 570 nm by using a SpectraStar Nano spectrometer (BMGLabTech, Ortenberg, Germany). Two blanks (PBS only) were included for each group as a negative control. Two samples in the PIPS group were excluded because of errors during sampling.

CLSM

To study the ability of each disinfection method to eliminate/kill bacteria in the dentinal tubules, we used CLSM to directly visualize the live/dead bacteria in the tubules. Eighteen intact mandibular premolars were used for this part of the study. Teeth were instrumented, autoclaved, and then inoculated with *E. faecalis* for 21 days and disinfected with the above-mentioned procedures



and techniques (4 teeth/group). Two teeth served as controls. After the 21-day bacterial inoculation, 1 tooth was autoclaved and used as a negative control. The other tooth did not receive canal disinfection and was used as a positive control. Teeth were then decoronated, and the roots were split vertically into 2 halves as described by Al Shahrani et al (14). Briefly, a groove was cut along the long axis of the tooth without penetrating the root canal system. By using a chisel, the root was split open into 2 pieces. All samples were visually inspected to select 1 sample from each group with comparable canal dimensions for CLSM analysis. Samples were then stained by using LIVE/DEAD BackLight Bacterial Viability Kit (Molecular Probes, Inc, Eugene, OR) for 15 minutes, according to the manufacturer's instructions. A Zeiss LSM 510 confocal microscope (Carl Zeiss, Oberkochen, Germany) set at the excitation/emission wavelengths of 480/500 nm for SYTO 9 and 490/635 nm for propidium iodide was used to inspect the tooth samples (×20 with an additional ×2 zoom). CLSM images were acquired by the software Zen V. 2 (Carl Zeiss) at a resolution of 1024 × 1024 pixels.

CLSM Analysis

We used a method similar to that described by Ma et al (20). Three random locations on the root were scanned at each part, coronal, middle, and apical (total of 9 images/tooth), at 10-μm-deep scans (1-μm step size, 10 slices/scan; each scan took ~20 minutes). The 10 slices of images were then stacked into 1 image (ie, each image represented 10 slices/scan area). For each stacked image, 3 measurements were taken, representing 3 different depth levels: 50 μm, 100 μm, and 150 μm deep into the dentinal tubules as displayed in Figure 1. All the scanned points along the dashed line of the particular depth level were measured by Zen 2 software (Zeiss). At each depth level, the software measured the intensities of red (dead bacteria) and green fluorescence (live bacteria), eg, at 50-μm level as shown in Figure 1*C*, the red fluorescence intensity is higher than green. The measured red/green fluorescence intensities were used to calculate the percentage of dead bacteria over both dead and live bacteria.

Statistical Analysis

For MTT assay, the approximated bacterial reduction was calculated for each group by using the following formula:

$$\textit{Bacterial reduction percentage} = \frac{S1 - S2}{S1} \times 100\%$$

Shapiro-Wilk test was performed to determine whether the samples were normally distributed by groups. Because the data were not normally distributed, Kruskal-Wallis test was used to test the overall difference among groups. Dwass-Steel-Critchlow-Fligner multiple comparison procedure was used to perform the pairwise comparison.

For CLSM analyses, the percentage of red to red-green combined was calculated for each group:

Figure 1. Method of confocal imaging analysis of live/dead bacteria in dentinal tubules. (*A*) Representative image after image stacking showing the canal side *C* and dentin side *D*, revealing dead bacteria (*red*) and live bacteria (*green*). (*B*) At different depth planes within the dentinal tubules, measurements were taken at 50, 100, and 150 μm deep into the dentinal tubules. (*C*) Representative image profiling on ZEN software showing measurement of *red* and *green* fluorescence intensities along the line at 50 μm depth level.

$$percent = \frac{intensity\ of\ red}{intensity\ of\ red + intensity\ of\ green} \times 100$$

Three factors of analysis of variance were fit by using treatment groups (SNI, EA, XP Endo, or PIPS), locations (coronal, mid-root, or apical), and depth points (50 μm, 100 μm, or 150 μm) as factors. Pairwise comparisons were performed by using tests of contrasts between the tested groups at different locations and depth points. Tukey-Kramer method was used to control experiment-wise type I error rate at 0.05.

SAS 9.4 (SAS Institute, Inc, Cary, NC) software was used for all analyses. The difference was considered to be significant when the *P* values were less than .05.

Results

Bacterial Elimination Efficiency in Canal Space

The MTT assay measured the viable bacteria from the sample collection before and after root canal disinfection. Results are displayed in Figure 2. The median percentage reduction ranged between 89.6% and 98.2% relative to S1. Pairwise comparison showed statistically significant difference in the bacterial reduction efficiency between XP Endo and PIPS ($P < .05$) as well as between XP Endo and EA ($P < .05$). There was no significant difference in the bacterial reduction between SNI, PIPS, and EA ($P > .05$).

Efficiency of Killing Bacteria in Dentinal Tubules

We applied CLSM to detect live versus dead bacteria in the dentinal tubules after staining. As shown in Figure 3, representative images depict live/dead bacteria in the dentinal tubules of different segments (coronal, middle, and apical) in roots treated by the 4 disinfection procedures. The negative control showed no live bacteria in the dentinal tubules, whereas dead bacteria were present.

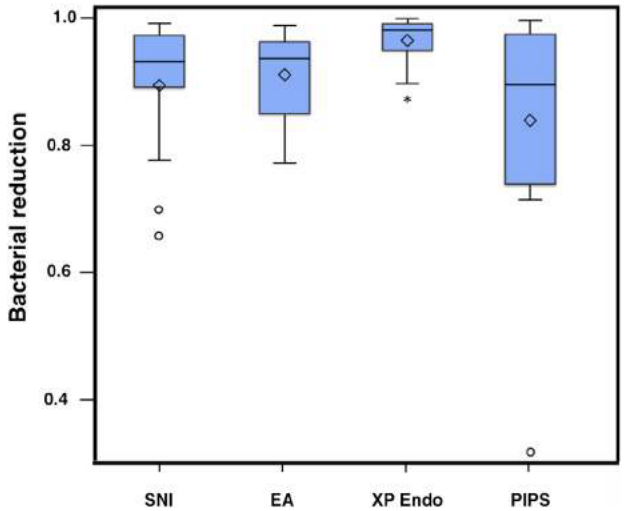


Figure 2. Efficiency of bacterial elimination in the main canal by different disinfection methods. *Box plots* showing level of bacterial reduction percentage by groups ($n = 15$ per group, except PIPS group $n = 13$). Live bacteria collected from sampling before (S1) and after (S2) disinfection were detected and measured by MTT assays. Bacterial reduction levels were calculated and plotted as depicted. *Significance ($P < .05$) compared with all other groups. *Middle line in the box*, median; ◇, mean; ○, individual observations that may be potential outliers.

In contrast, the positive control had many live bacteria in the dentinal tubules.

The percentage of dead bacteria (red) over total bacteria was calculated along the vertical lines placed on each image as described in Figure 1. The measurement along the lines ranged between 2770 and 3059 points/image. The results from analyzing those images are presented in Figure 4. There was no statistically significant difference between EA and XP Endo at the 50-μm and 100-μm levels in the apical segment ($P > .05$). There was no statistically significant difference between XP Endo and SNI at the 100-μm level in the mid-root segment or at the 150-μm level in the apical segment ($P > .05$). All the other groups showed a statistically significant difference between each other at every dentinal tubule depth level and canal segment (coronal, middle, and apical) ($P < .05$). Overall, XP Endo had the highest level of dead bacteria in the coronal, middle, and apical segments at 50-μm depth. On the other hand, PIPS had the greatest bacterial killing efficiency at the 150-μm depth in all 3 root segments.

Discussion

This study compared 4 different irrigation protocols in their abilities to eliminate bacteria in the root canal space as well as their lateral penetration capabilities into the dentinal tubules to kill bacteria. The study presents a novel method to analyze CLSM images that allowed us to calculate the percentage of dead bacteria in the dentinal tubules at coronal, middle, and apical segments of the canal and at different depths into the tubules. The data reflect the levels of efficiency of the different irrigation protocols tested in killing the bacteria that colonized deep into the dentinal tubules.

E. faecalis was chosen for our present study because it has been repeatedly isolated from the root canal system in failing endodontic cases (22–24). In addition, *E. faecalis* can penetrate into the dentinal tubules (25, 26) and form biofilms, which are more resistant to canal disinfection (27, 28). Previous studies that used CLSM either did not quantify the dead bacteria (14–16) or only quantified the dead bacteria across the entire image (20). In addition, samples were always selected from the coronal and middle thirds of the canal but not the apical third (20). Although the apical third has more peritubular dentin and a decreased number of dentinal tubules (29), peritubular dentin was observed only in the apical 0–1.3 mm of the canal (30). At levels coronal to that (1.5–3 mm), tubular dentin can still be observed. Our present study addressed this issue by using CLSM imaging analysis capacity to compare the percentage of dead bacteria up to 150 μm deep into the dentinal tubules at the coronal, middle, and apical thirds of the canal.

For bacterial reduction efficiency in the main canal space, XP Endo showed a significantly higher bacterial reduction (98.2%) compared with PIPS (89.6%) and EA (93.3%). In terms of the efficiency of killing the bacteria in dentinal tubules, XP Endo treated teeth also showed the highest percentage of dead bacteria at a 50-μm depth in the coronal, middle, and apical thirds of the canal, ranging between 78% and 82%. It may have been that mechanical effects of the file during operation together with its irrigation agitation facilitated the removal of bacteria on the canal wall and killing of the bacteria at a depth of 50 μm. However, this ability dropped in the middle and apical thirds at the 100-μm and 150-μm levels in comparison with PIPS ($P < .001$). The results agree with Pedullà et al (18) and Brito et al (19) that there is no significant difference between PIPS or EA and SNI in bacterial reduction in the main root canal space. The results disagree with other studies in which the

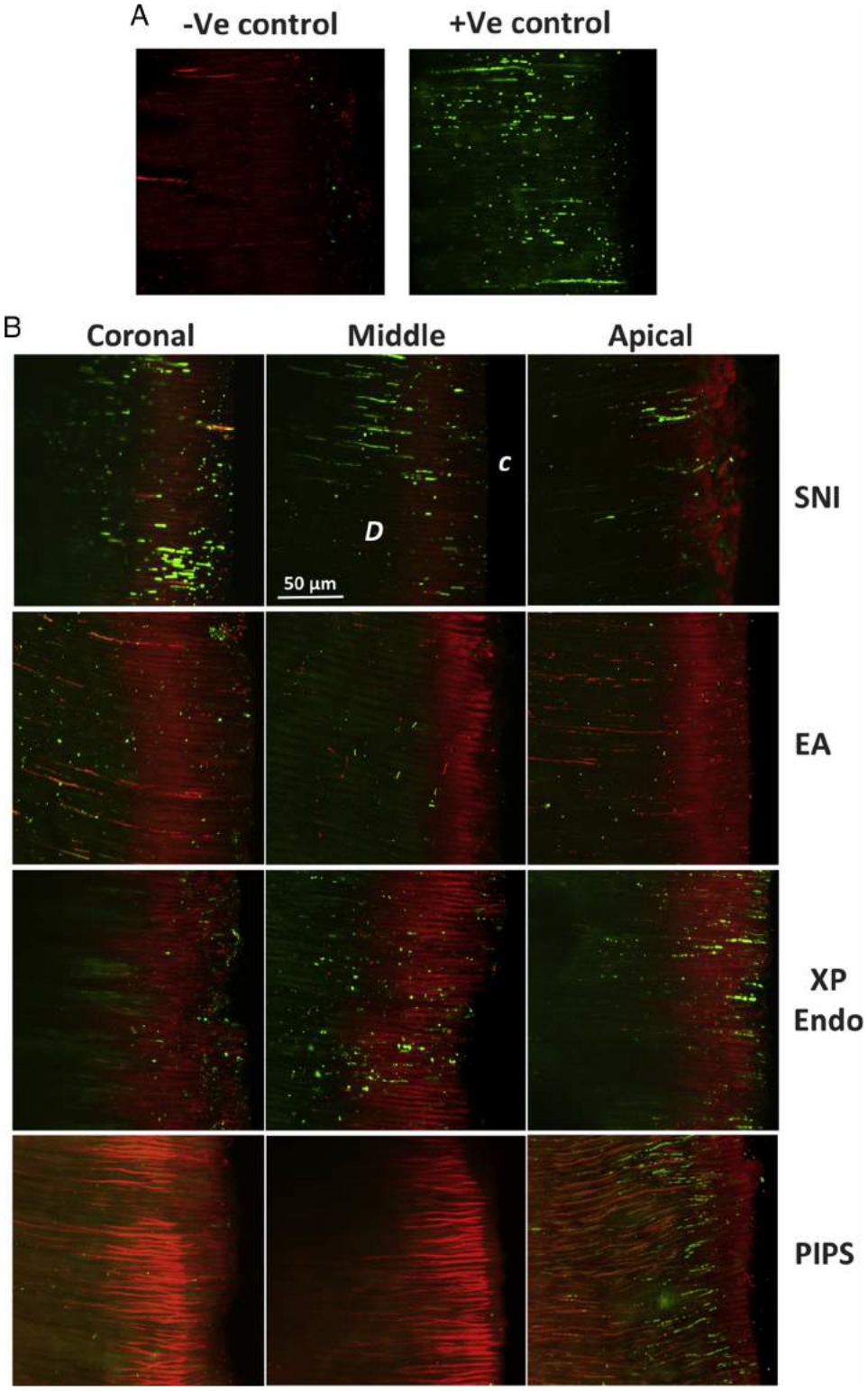


Figure 3. CLSM analysis of live/dead bacteria in dentinal tubules. (*A*) Positive and negative control sample images. *Red* indicates dead bacteria (Ve control), and *green* indicates live bacteria (+Ve control) in dentinal tubules. Negative control was inoculated as for experimental samples and then autoclaved; positive control was inoculated but not disinfected. (*B*) Representative stacked images from coronal, middle, and apical thirds of the root showing dentinal tubules in samples treated by different irrigation methods: SNI, EA, XP Endo, and PIPS. C, canal side; D, dentin.

bacteria collected from the root canal were plated to form colonies so that the number of viable bacteria recovered was counted. Such a method yielded a result that showed a higher efficiency in disinfecting the root canal by PIPS than SNI (14, 31). Difference in results may stem from difference in canal size and selection of the tooth model between the studies. PIPS provided the maximum bacterial reduction

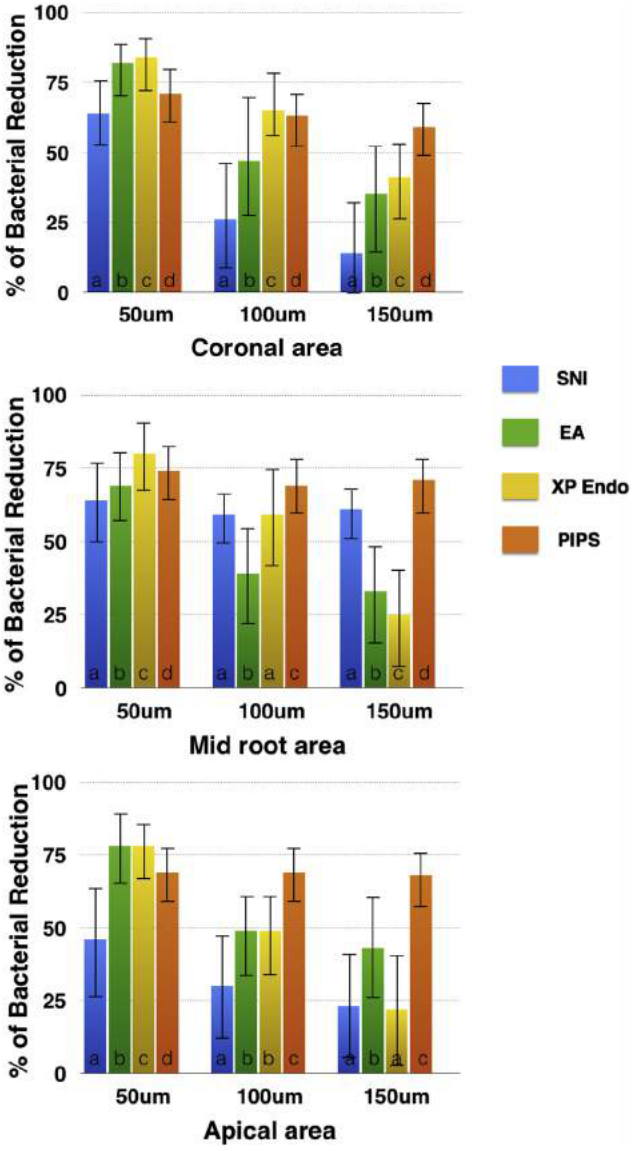


Figure 4. Quantitative analysis of dead bacteria in dentinal tubules detected by CLSM. Graphs showing percentage of dead bacteria at coronal, middle, and apical thirds of the canal at different depths into the dentinal tubules by using different canal irrigation techniques. Different letters (a, b, c, and d) indicate significance within each depth group.

within the dentinal tubules, ranging between 60% and 70% at the 150-μm level along the entire canal length (P <. 0001). Such superior performance may be attributed to the cavitation bubbles produced by PIPS generating a shock wave effect, which allowed a deeper penetration of NaOCl into the dentinal tubules, thus creating better disinfection (17).

In this study, dead bacteria were observed in samples of all irrigation groups at different depths in dentinal tubules and segments of the root where we examined. The results agree with Zou et al (21) that NaOCl can manage to penetrate deeply into the dentinal tubules. However, the percentage of dead bacteria decreased at deeper levels of tubules. Each irrigation protocol showed different effectiveness in killing bacteria at different segments within the root canal system. PIPS provided a steady performance along the entire canal length and depth. XP Endo had the maximum bacterial reduction at the coronal segment, followed by mid-root and then the apical segment. On the other hand, EA had an improved performance at the apical third in comparison with mid-root, which may be attributed to the maximum oscillation of the amplitude (antinode) formed at the activator tip located in the apical third of the canal (13). On the contrary, SNI had its best disinfection at the mid-root segment. This may stem from the increased irrigant velocity as it leaves the irrigation needle and flows coronally (32), allowing deeper penetration and disinfection at the mid-root segment. As the velocity drops, its penetration efficiency is reduced at the coronal area.

In conclusion, our present study provides insight into the killing abilities of the bacteria colonized in the main canal space or that have penetrated into the dentinal tubules throughout the canal length by various clinical disinfection protocols. XP Endo appears to be stronger than PIPS and EA in disinfecting the main root canal space and up to 50 μm deep into the dentinal tubules. PIPS appears to be more effective than XP Endo and EA in deeper disinfection of the dentinal tubules. Combination of most efficient canal disinfection protocols will likely yield better endodontic outcomes, particularly in the cases for regenerative approaches. Further studies are required to determine their efficiency in different canal anatomies and larger canal models.

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

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Laser-activated irrigation within root canals: cleaning efficacy and flow visualization

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Abstract

de Groot SD, Verhaagen B, Versluis M, Wu M.-K, Wesselink PR, van der Sluis LWM. Laser activated irrigation within root canals: cleaning efficacy and flow visualization. *International Endodontic Journal*, **42**, 1077 1083, 2009.

Aim To test *ex vivo* the efficiency of laser activated irrigation in removing dentine debris from the apical part of the root canal and to visualize *in vitro* the fluid dynamics during the activation of the irrigant by laser, using high speed imaging at a relevant timescale.

Methodology Root canals with a standardized groove in one canal wall filled with dentine debris were irrigated with syringe irrigation, ultrasonically or laser activated irrigation (LAI) using 2% sodium hypochlorite as irrigant. The quantity of dentine debris after irrigation was determined. Visualization of the fluid dynamics during activation was achieved using a high speed camera and a glass model.

Results Laser activated irrigation was significantly more effective in removing dentine debris from the apical part of the root canal than passive ultrasonic irrigation or hand irrigation when the irrigant was activated for 20 s.

Conclusions The *in vitro* recordings suggest that streaming, caused by the collapse of the laser induced bubble, is the main cleaning mechanism of LAI.

Keywords: irrigation, laser, root canal, ultrasound, visualization.

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Introduction

An important procedure during root canal treatment is the irrigation of the root canal. Syringe irrigation is the standard procedure but unfortunately, syringe irrigation is not effective in the apical part of the root canal (Ram 1977, Salzgeber & Brilliant 1977, Abou Rass & Patonai 1982, Druttman & Stock 1989) and in isthmuses or oval extensions (Lee *et al.* 2004, Burleson *et al.* 2007). Therefore, acoustic and hydrodynamic activation of the irrigant have been developed (Weller *et al.* 1980, Lumley *et al.* 1991, Lussi *et al.* 1993), which have been shown to contribute to the cleaning efficiency (Lumley *et al.* 1991, Lussi *et al.* 1993, Roy *et al.* 1994). The physical mechanisms underlying these cleaning procedures, however, are not well understood (van der Sluis *et al.* 2007a).

Laser activated irrigation (LAI) has been introduced as a powerful method for root canal irrigation (Blanken & Verdaasdonk 2007, George & Walsh 2008, George *et al.* 2008). The laser radiation produces transient cavitation in the liquid through optical breakdown by strong absorption of the laser energy (Blanken & Verdaasdonk 2007). LAI can result in smear layer removal from the root canal wall, but also cause extrusion of irrigant through the apex (George & Walsh 2008, George *et al.* 2008). However, the removal of dentine debris from the root canal by LAI has not yet been studied. Furthermore, Blanken & Verdaasdonk (2007) suggested repeating

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their visualization experiment with a single high speed camera recording, visualizing a single pulse, to improve the understanding of the cavitation process.

The purpose of this study was to evaluate *ex vivo* the removal of artificially placed dentine debris in standardized root canals by syringe irrigation, passive ultrasonic irrigation (PUI) and LAI. LAI was also visualized *in vitro* using high speed imaging at a timescale relevant to the cleaning process (μs). The resulting flow is theoretically described using a fluid dynamical model.

Materials and methods

Dentine debris removal

Maxillary canines with straight root canals were decoronated; the length of the remaining root was 15 mm for all teeth. The roots were then embedded in self curing acrylic resin (Ostron 100, GC Tokyo, Japan) and then split longitudinally through the canal in mesio distal direction. To remove the imprint of the root canal, both halves were ground with sandpaper and fixed with four screws (see Fig. 1a). Then, the root canals were prepared by K files hand instruments (Dentsply Maillefer, Ballaigues, Switzerland) and mechanically driven Race NiTi instruments (FKG Dentaire, La Chaux de Fonds, Switzerland), to a length of 15 mm, size 35 and 0.06 taper resulting in a standardized root canal. To verify the standardization of the models, the canal diameter of six randomly chosen models was measured at 2, 6 and 10 mm from the apical end of the canal, using a KS100 Imaging system 3.0 (Carl Zeiss Vision GmbH, Halbermoos, Germany). At 2 mm, the average canal diameter was found to be 0.47 ± 0.02 mm (diameter of the Race NiTi instrument: 0.47 mm); at 6 mm the average canal diameter was 0.71 ± 0.02 (0.71) and at 10 mm the diameter was 0.94 ± 0.02 (0.95). These measured values demonstrate that the root canals were indeed uniform and standardized.

The coronal 3 mm of the canal was enlarged by a no. 23 round bur (Dentsply Maillefer) with a diameter of 2.3 mm, simulating a pulp chamber. A standard

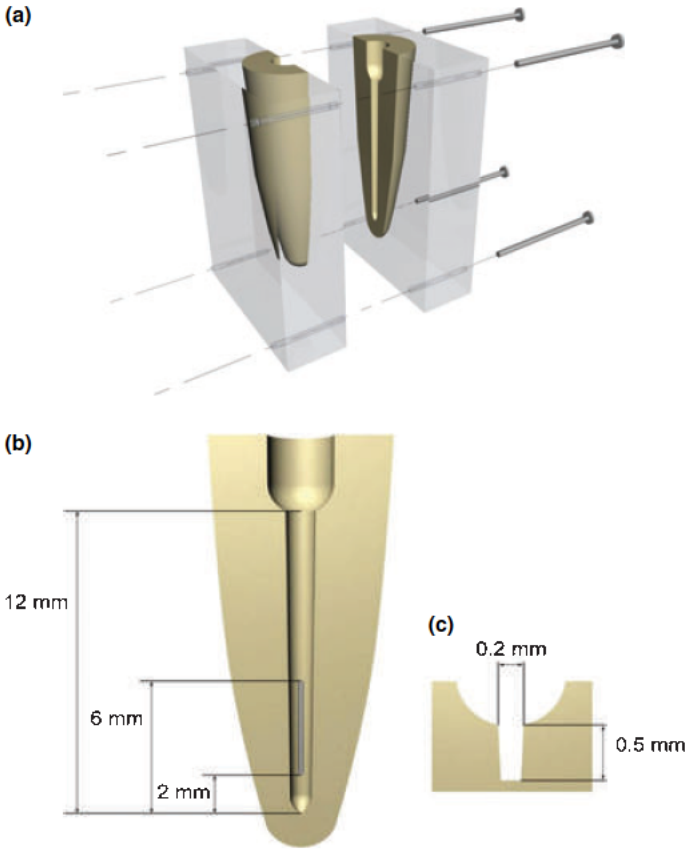

Figure 1 Schematic representations of the standardized root canal model (a), its groove (b), and its cross section (c).

groove of 4 mm in length, 0.5 mm deep and 0.2 mm wide, situated at 2 6 mm from working length, was cut in the wall of one half of each root canal with an ultrasonically driven tip (Fig. 1b,c) (P5 Booster, Sat elec, Acteongroup, Mérignac cedex, France). The dimension of the groove was comparable with that of an oval extension of a root canal. Each groove was filled with dentine debris mixed with 2% NaOCl to simulate a situation in which dentine debris accumu lates in uninstrumented canal extensions (Lee *et al.* 2004). This model was introduced to standardize the root canal anatomy and the amount of dentine debris present in the root canal before the irrigation proce dure, in order to increase the reliability of dentine debris removal evaluation. The methodology is sensi tive and the data are reproducible (van der Sluis *et al.* 2007b).

Three irrigation protocols were tested. In all groups, the needle, wire and fibre were inserted 1 mm short of the working length and were moved slowly up and down 4 mm in the apical half of the root canal; the activation time was 20 s, the total irrigation time was 50 s and the total irrigant volume was 4 mL. In group 1 (n 20) syringe irrigation with 4 mL of 2% NaOCl solution was performed with a 10 mL syringe and a 30 gauge needle (Navitip, Ultradent, South Jordan, UT, USA). In group 2 (n 20), the 2% NaOCl solution was activated by ultrasound using PUI. A stainless steel noncutting wire (size 20) (Irrisafe, Satelec, Acteon group) was used, driven by an ultrasonic device (Suprasson Pmax Newtron, Satelec, Acteongroup) at power setting 'blue 4' (frequency 30 KHz, displace ment amplitude *ca.* 30 μm according to the manu facturer). Subsequently the canal was flushed with 2 mL of 2% NaOCl solution using a 10 mL syringe and a 30 gauge needle. In group 3 (n 20), the 2% NaOCl solution was activated by laser radiation (KEY2 laser, KaVo Dental GmbH, Biberach, Germany) from an optical fibre laser tip with outer diameter 280 μm and length 30 mm (type Gr. 30 × 28, Kavo Dental GmbH). Calibration by the manufacturer showed that the optical fibre has a reduction factor of 0.36, which results in a fluence of 146 mJ mm^{-2} for a laser pulse energy setting of 100 mJ. The Er:YAG laser emits at a wavelength of 2.94 μm which coincides with the major absorption band of water (Robertson & Williams 1971). A pilot study demonstrated that the optimal settings for dentine debris removal from the root canal are a low power setting of 80 mJ per pulse and a pulse repetition frequency of 15 Hz. Finally, the canal was flushed with 2 mL of 2% NaOCl solution using a 10 mL syringe and a 30 gauge needle.

After irrigation the root canals were dried with paper points. Subsequently, the two halves were separated and the amount of debris in the groove was evaluated. Before and after the irrigation, a digital image was taken of the groove, using a Photomakroskop M400 microscope with a digital camera (Wild, Heerbrugg, Switzerland) at 40× magnification. The quantity of dentine debris in the groove before and after irrigation was scored double blind and independently by three dentists using the following scores: score 0: the groove is empty, score 1: less than half of the groove is filled with dentine debris; score 2: more than half of the groove is filled with dentine debris; score 3: the groove is completely filled with dentine debris. The differences in dentine debris scores between the different groups were analysed by means of the Kruskal Wallis and Mann Whitney tests (level of significance α 0.05).

High-speed imaging experiments

An optical setup was constructed in order to visualize the effect of the Er:YAG laser radiation in an artificial root canal containing water or NaOCl. Optical record ings were made at a pulse repetition rate of the Er:YAG laser of 1 Hz and a pulse energy between 80 and 250 mJ per pulse. The laser fibre tip was inserted up to 1 mm from the apical end of a glass root canal model. The canal was 12 mm in length with an apical diameter of 0.35 mm and taper 0.06. Imaging was performed using a high speed camera (FastCam APX RS, Photron, Tokyo, Japan), recording at a frame rate of 14 000 frames per second, attached to a microscope with 12× magnification (SZX12, Olympus, Tokyo, Japan). The root canal model was illuminated in bright field by a continuous wave light source (ILP 1, Olympus).

Results

Dentine debris removal

The debris scores before and after irrigation are presented in Table 1. The difference between the groups was statistically significant (Kruskal Wallis test, $P < 0.0001$). The debris score in group 3 was signif icantly lower than group 2 (P 0.002) and group 1 ($P < 0.0001$), and the score in group 2 was signifi cantly lower than group 1 ($P < 0.0001$).

Table 1 Dentine debris score in the groove after the irrigation procedures per group (no. cases and percentage of total; 20 cases in total for each irrigation procedure)

Score:	0 *n* (%)	1 *n* (%)	2 *n* (%)	3 *n* (%)
Syringe irrigation	0	0	4 (20%)	16 (80%)
Ultrasonic irrigation	6 (30%)	8 (40%)	6 (30%)	0
Laser activated irrigation	16 (80%)	4 (20%)	0	0

Scoring system: 0: the groove is empty; 1: less than half of the groove is filled with debris; 2: more than half of the groove is filled with debris; 3: the complete groove is filled with debris.

High-speed imaging experiments

The high speed recordings of the laser activity inside the artificial (glass) canal showed that irrigant was vapourized by the laser pulse energy and that a large vapour bubble was created at the fibre tip, similar to that observed previously (Lauterborn 1972). The bubble grew with a velocity of the order of 1 m s^{-1} during the pulse duration (see Fig. 2 and video clips S1 and S2); a higher energy laser pulse corresponded to a longer growth time of the bubble. When the laser pulse ended, the bubble collapsed with a velocity of the order of 1 m s^{-1}. Upon collapse, a shockwave was generated (Holzfuss *et al.* 1998), whose negative pressure tail caused secondary cavitation in the root canal with a relatively large bubble near the collapse site (which was usually at the apex). The cavitation bubble then collapsed again and this cycle repeated for a number of times, until it was damped out within a few milliseconds (6 ms at 250 mJ per pulse). Smaller bubbles with a typical diameter of 10 μm remain buoyant for a longer time (even up to the next pulse), also at the apical end of the root canal.

The laser induced bubble grew predominantly in the coronal direction, as there was a confinement at the apex. The depth reached by this bubble depended on the position of the fibre and the laser energy, but never fully extended to the apex. It was observed that when a small bubble was present at the apex, it grew during the collapse phase of the laser induced bubble and

Figure 2 (Video clips S1 and S2) Visualization of the laser generated vapor bubble. The laser energy was 60 mJ per pulse in (a) and 250 mJ per pulse in (b). Image sequence is from left to right. The interframe time is 140 μs. Panel *p* in (a) shows a sketch of the setup, with 1) the root canal model, 2) the laser fiber tip (outer diameter 280 μm), 3) the laser induced cavitation bubble, and 4) a stable cavitation bubble at the apex.

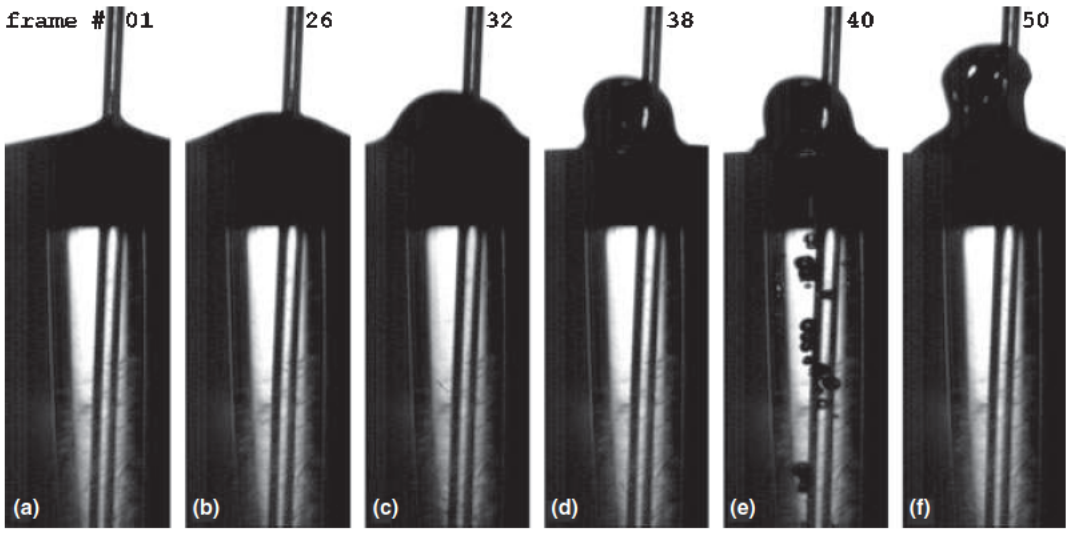

Figure 3 (Video clip S3) Pinch off at the free surface at the coronal part of the glass root canal model. Secondary cavitation bubbles are formed (in *e*) upon passage of a shockwave generated by the vapor bubble inertial collapse at the laser fiber tip. The frame rate is 14,000 frames/second.

collapsed and renucleated in anti phase with the laser induced bubble (Fig. 2a (indicated with the no. 4 in panel *p*), whereas in Fig. 2b this bubble was not present).

It was observed that the laser induced bubble grew larger when NaOCl was used as an irrigant solution. Consequently, it had a longer collapse time as com pared with having water as an irrigant. It was also found that a higher amount of smaller bubbles were present after laser activation when using NaOCl as the irrigant solution.

Because of the impulsive growth of the laser induced bubble the fluid was pushed outward at the free surface at the coronal part (see Fig. 3 and Video Clip S3). For a laser energy exceeding 120 mJ per pulse it was observed that some fluid was ejected from the root canal, leaving less irrigant in the root canal.

Discussion

The results of the *ex vivo* experiments demonstrate that within the time frame of 20 s, LAI is more effective in removing dentine debris from an artificial groove in the apical part of the root canal than ultrasonically activated (PUI) or syringe activated irrigation.

The high speed recordings have shown that vapou rization of the irrigant causes a large bubble to grow, which then collapses and renucleates a few times. During this process, secondary cavitation bubbles are formed. The fluid flow associated with such an inertial collapse, combined with acoustic streaming resulting from the oscillations of smaller bubbles, could explain the cleaning efficacy of LAI; however, a more detailed study is required to elucidate the principal cleaning mechanism. The secondary cavitation bubbles can also assist in the cleaning of the root canal wall, as they are excited by the bubble collapse of the consecutive laser pulse. As the flow does not penetrate all the way into the apex, a trapped bubble in the apex (most likely a remainder of previous laser pulses) could assist in the cleaning of the apical part of the root canal.

The irrigant flow in the root canal due to the collapsing laser induced bubble can be modelled by a flow in concentric annuli for heights above the insertion depth of the fibre. For the typical flow velocity of 1 m s^{-1} (value obtained from the high speed record ings by measuring the bubble wall displacement between consecutive frames), the Reynolds number $Re \quad Ud\rho/\mu$ (with U the flow velocity, d the distance between the cylinders, ρ the density of the liquid and μ the dynamic viscosity) for a flow in annuli is of the order of 300. According to Rothfus *et al.* (1950), the transition to turbulence occurs over the range 2100 3700, therefore the flow in this problem is treated as laminar flow.

Rothfus *et al.* (1950) also give the laminar flow velocity distribution for flow in concentric annuli:

$$u(r) = 2u_{av}\frac{\left[r_1^2 \quad r^2 + \frac{r_2^2 \quad r_1^2}{\ln\frac{r_2}{r_1}}\ln\frac{r}{r_1}\right]}{r_2^2 + r_1^2 \quad 2r_m^2} \tag{1}$$

where r_m is the radius of maximum velocity, given by:

$$r_m = \left[\frac{r_2^2 \quad r_1^2}{2\ln\frac{r_2}{r_1}}\right]^{\frac{1}{2}} \tag{2}$$

Using $\tau = \mu\frac{\partial u}{\partial r}$ the shear stress for laminar flow in annuli is given by:

$$\tau(r) = 2\mu u_{av}\frac{\left(\frac{r_2^2 - r_1^2}{r} \quad 2r\ln\frac{r_2}{r_1}\right)}{(r_2^2 + r_1^2)\ln\frac{r_2}{r_1} \quad r_2^2 + r_1^2} \tag{3}$$

Using standard values for density ρ 1000 kg m^{-3} and dynamic viscosity μ 1×10^{-3} m^2 s^{-1}, and a measured average velocity u_{av} 5 m s^{-1} and cylinder radii r_1 140 μm (inner) and r_2 300 μm (outer), the shear stress on the inner wall is 496 N m^{-2} and on the outer wall 436 N m^{-2}. These values are one order of magnitude lower than the shear stress generated by a laser induced cavitation bubble of radius 0.75 mm next to a single wall, which is reported to generate a shear stress of up to 3.5×10^3 N m^{-2} (Dijkink & Ohl 2008). No quantitative data on the adhesion strength of dental intracanal biofilms to dentine or its failure shear stress is available in the literature.

Figure 4 shows the velocity profile calculated with the theory described above in a tapered canal with a cylinder inserted, assuming an average velocity of 5 m s^{-1} at the fibre tip (taken from experiment). The profile on the left of the inner cylinder is the velocity profile; the profile on the right is the shear stress distribution. The plot clearly shows that on the inner cylinder (the laser fibre) the shear stress is higher than on the outer cylinder (the root canal wall).

The root canal diameter increases with height, therefore the average velocity decreases with height. This results in the shear stress being highest next to the tip of the laser fibre. LAI is therefore expected to be most effective in the region close to the fibre tip, with decreasing efficiency away from the tip.

Using a 27G needle and a volume flow rate of 0.30 mL s^{-1} (Boutsioukis *et al.* 2007) it follows that the typical fluid velocity in syringe irrigation is of the order of 1 m s^{-1} at the needle orifice, which is the same order of magnitude as the flow velocities developed with LAI. Likewise for PUI with u $\omega\varepsilon_0{}^2/a$ (Ahmad *et al.* 1988; ω oscillation frequency, ε_0 oscillation amplitude and a file radius) a typical fluid velocity of the order of 1 m s^{-1} was found. One possible explanation for the improvement in cleaning efficacy with LAI is the impulsive nature of the laser generated bubble dynamics. Because of the pulsations the fluid becomes accelerated at every pulse and the acceleration gives rise to inertial forces, whereas a steady streaming as in syringe irrigation and PUI only exerts viscous stress. This would also explain why the irrigation duration is an important factor and why a high pulse repetition rate of the laser is more efficient than a lower one, as found in the pilot study.

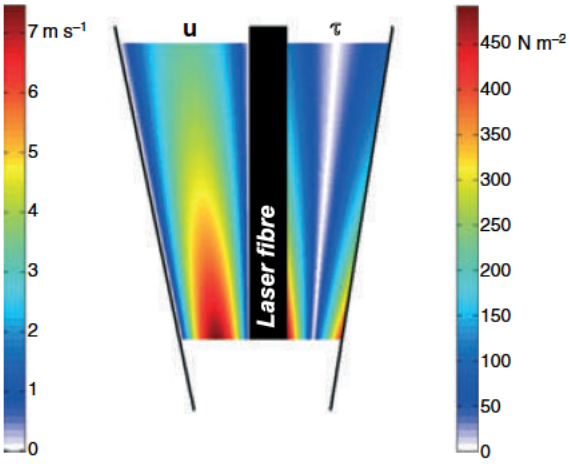

Figure 4 Average velocity profile (left) and sheer stress distribution (right) between two concentric cylinders of which the outer cylinder represents the tapered root canal wall. The average velocity at the laser fiber tip is set at 5 m s^{-1}. The region below the laser fiber tip is intentionally left blank, as details of the streaming pattern in the apical part are missing and are part of a future study.

Previous studies have shown side effects caused by the use of these types of lasers in the root canal. Carbonization of the root canal and cracks were observed when laser tips were used in the root canal (Matsuoka *et al.* 2005). Kimura *et al.* (2002) have shown a temperature increase of the root canal wall of 3 6 °C. The current study did not monitor these side effects, because the aim of this study was clarification of the fluid mechanical working mechanisms.

Conclusion

Laser activated irrigation was more effective in removing the artificially placed dentine debris from the root canal than syringe irrigation or PUI when the irrigant was activated for 20 s.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Video Clip S1. Visualization at the apex of the root canal, laser intensity 60 mJ/pulse (*apex energy60mj. wmv*).

Video Clip S2. Visualization at the apex of the root canal, laser intensity 250 mJ/pulse (*apex energy250mj. wmv*).

Video Clip S3. Visualization at the corona of the root canal, laser intensity 250 mJ/pulse (*corona energy250mj.wmv*)

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

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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*.

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 standard ized 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 irri gated 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 ste reomicroscope 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 stream ing provided complete removal of $Ca(OH)_2$ from artifi cial 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 patho gens (Athanassiadis *et al.* 2007). Research has shown that remnants of $Ca(OH)_2$ on dentine walls can affect

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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

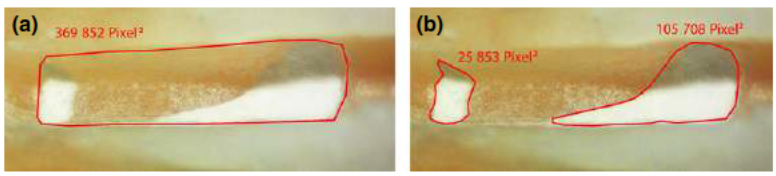

Figure 1 Demonstration of the pixel count of $Ca(OH)_2$ remaining on the artificially created grooves; (a) overall groove surface and (b) the remaining $Ca(OH)_2$.

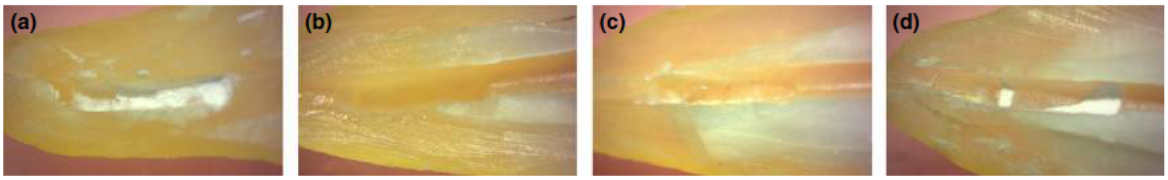

Figure 2 Representative images for the groups; (a) needle irrigation, (b) Photon initiated photoacoustic streaming (PIPS) *note the complete removal*, (c) ultrasonic irrigation and (d) sonic irrigation.

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).

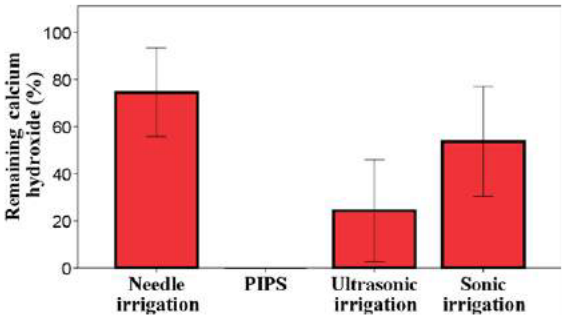

Figure 3 Graphical demonstration of the percentage of the $Ca(OH)_2$ according to the groups. Photon initiated photoacoustic streaming (PIPS) was statistically superior to the other groups ($P < 0.05$). Ultrasonic irrigations was also superior to the sonic and needle irrigation ($P < 0.05$).

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

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Abstract

Arslan H, Capar ID, Saygili G, Gok T, Akcay M. Effect of photon initiated photoacoustic streaming on removal of apically placed dentinal debris. *International Endodontic Journal*.

Aim To compare the efficacy of photon induced photoacoustic streaming (PIPS) technique with con ventional, 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 per formed up to size 40 on 48 extracted single rooted teeth using ProTaper rotary instruments. The speci mens were then split longitudinally, and a standard ized 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× magnifi cation, 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 con ventional 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 stre aming was more effective than conventional, sonic and ultrasonic irrigation in the removal of apically placed dentinal debris.

Keywords: EndoActivator, endodontics, photoa coustic 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 (Tora binejad *et al.* 2003, Mancini *et al.* 2009), improved irrigation agitation methods such as sonic and ultra sonic 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

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(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

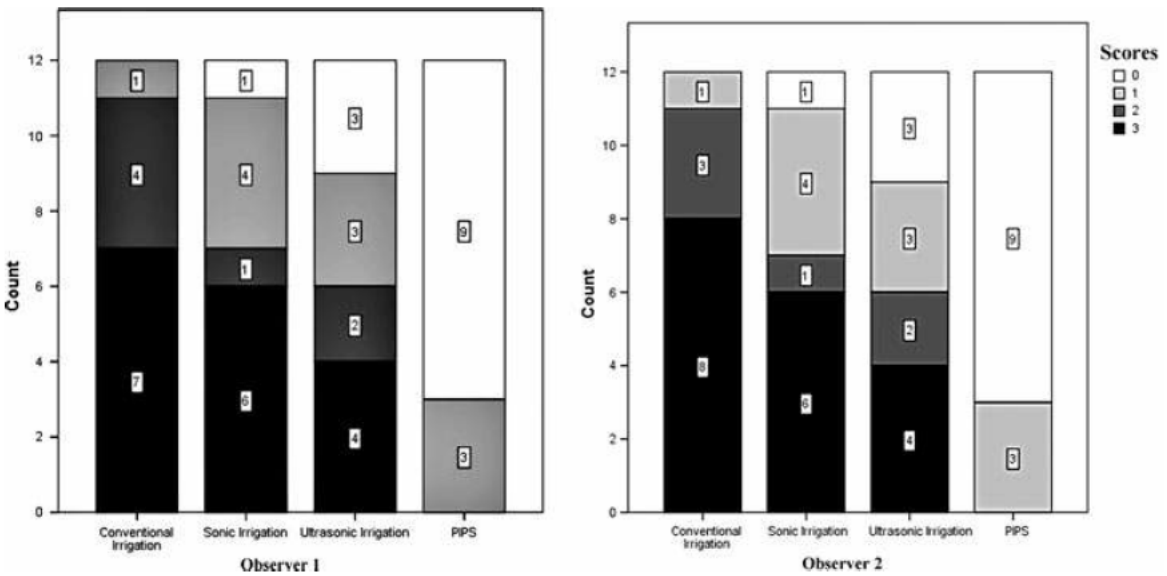

Figure 1 Distribution of scores for removal of apically placed dentinal debris after agitation with different protocols according to Observers 1 and 2.

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

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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 $2.21 \pm 0.46\,mm^3$ 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,

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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 ~ 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 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}

$^{I\,III}$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,

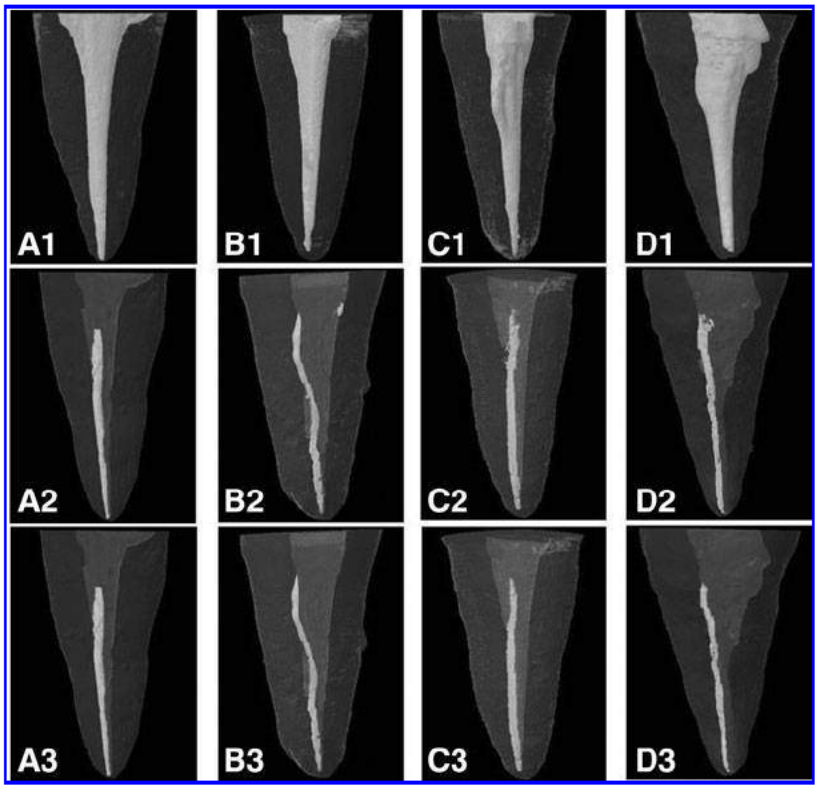

FIG. 1. Three-dimensional reconstruction of micro-CT scans showing filling material after obturation, mechanical retreatment, and additional activation of NaOCl with different devices. Control (A1, after obturation; A2, after mechanical retreatment; A3 after additional irrigating procedure); EndoActivator (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).

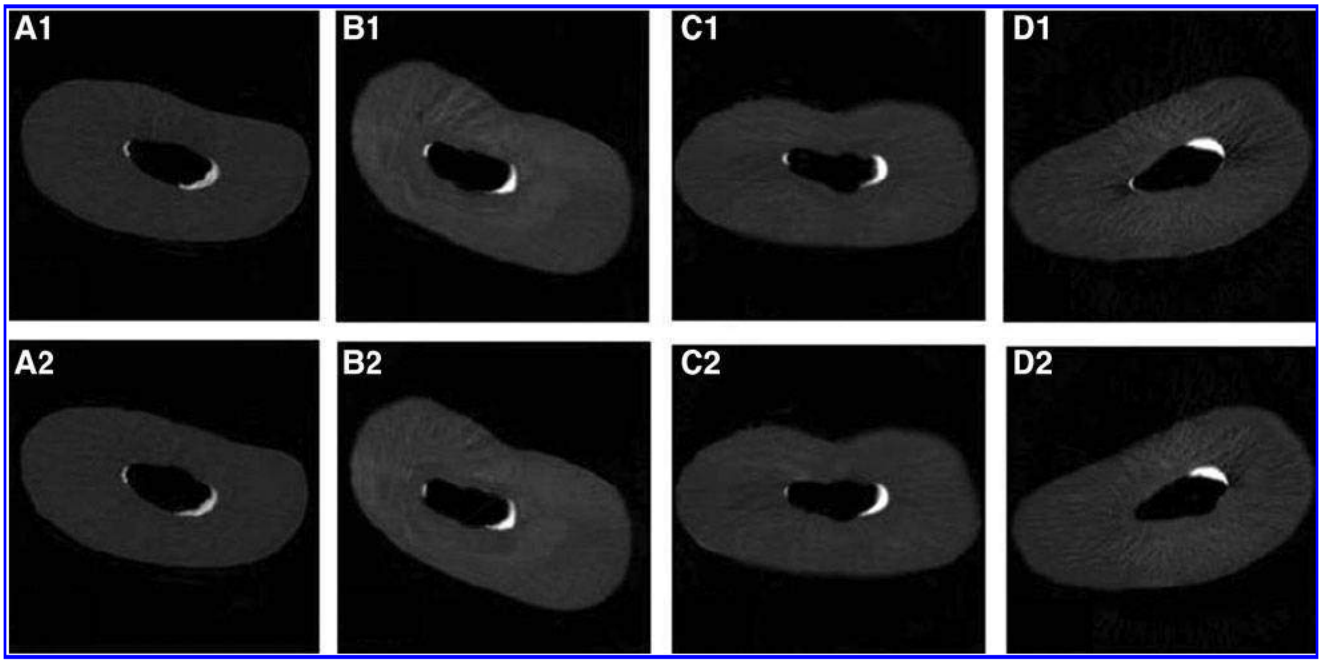

FIG. 2. Cross-sectional micro-CT image of residual filling material before and after additional activation of NaOCl with different devices. Control (A1, before irrigation; A2, after irrigation); EndoActivator (B1, before irrigation; B2, after irrigation); Ultrasonic (C1, before irrigation; C2, after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

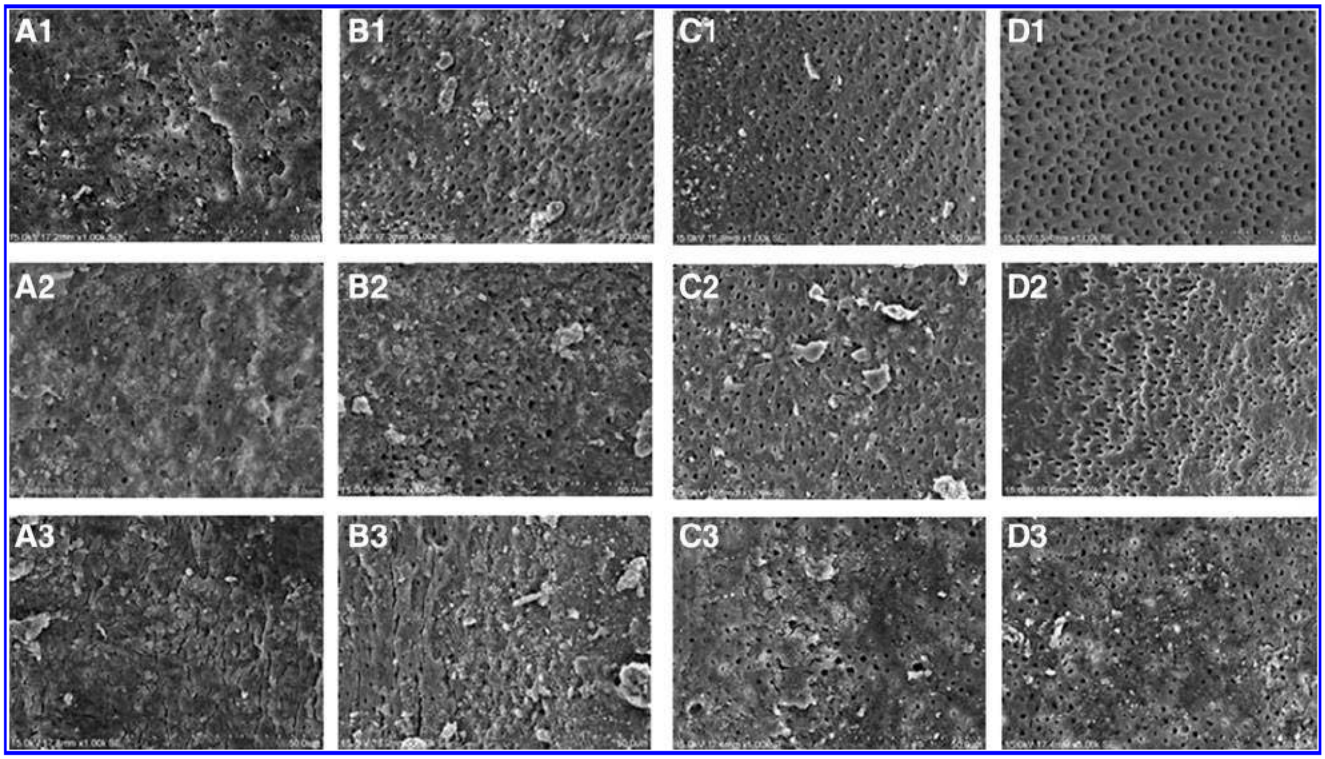

FIG. 3. Scanning electron microscopy (SEM) images of residual filling material in the coronal, middle, and apical thirds of the root canal after additional activation of NaOCl. Control (A1, coronal third; A2, middle third; A3, apical third); sonic (B1, coronal third; B2, middle third; B3, apical third); 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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The efficacy of photon-initiated photoacoustic streaming in the removal of calcium silicate-based filling remnants from the root canal after rotary retreatment

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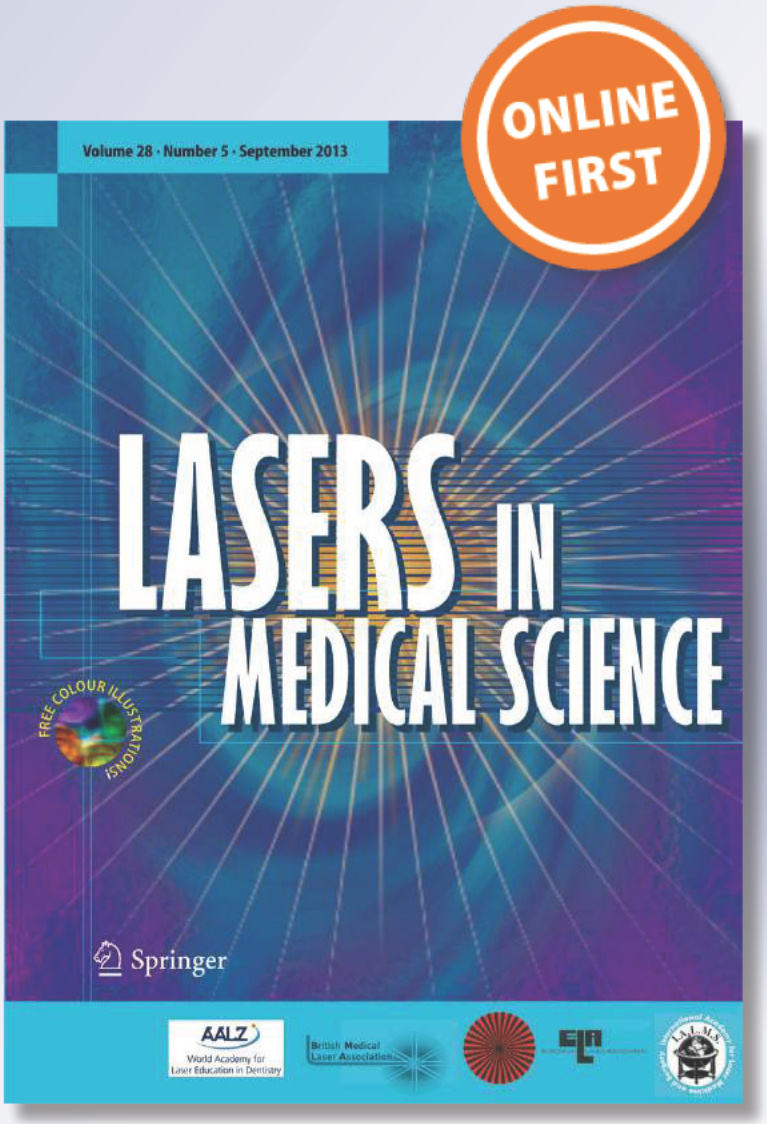

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ORIGINAL ARTICLE

The efficacy of photon-initiated photoacoustic streaming in the removal of calcium silicate-based filling remnants from the root canal after rotary retreatment

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Abstract The aim of the study was to evaluate the efficacy of photon-initiated photoacoustic streaming (PIPS) in the removal of filling remnants from root canals after rotary phase of retreatment and to examine the difference in the amount of residual material considering the type of sealer. Thirty-six extracted single-rooted human teeth were instrumented and randomly divided into three groups according to the filling material used: group 1: EndoSequence BC Sealer (Brassler, USA), group 2: MTA Fillapex (Angelus Solucoes Odontologicas, Londrina, Brasil), and group 3: AH Plus sealer (Dentsply DeTrey, Konstanz, Germany). Cold lateral condensation technique was used. After 2 weeks, the root canals were retreated with a rotary phase retreatment system (ProTaper Universal Retreatment, Maillefer, Ballaigues, Switzerland), followed by Er:YAG laser-activated irrigation (photon-initiated photoacoustic streaming, PIPS). The specimens were scanned in a micro-computed tomographic (micro-CT) device after root canal filling, after the rotary retreatment, and after the PIPS. There was significant reduction in the amount of filling material after the rotary phase of retreatment in all groups ($p < 0.05$), the highest in the MTA Fillapex group ($p < 0.001$) and no difference between the EndoSequence BC and the AH Plus ($p = 0.608$). There was significant reduction of the filling remnants after the PIPS in all groups ($p < 0.05$). The MTA Fillapex was the most easily removed during rotary phase of the retreatment, and there were no differences in the amount of the remaining filling material between EndoSequence BC and the AH Plus groups after rotary phase of the retreatment. The PIPS improved the removal of filling remnants in all groups.

Keywords Calcium silicate · Retreatment · ProTaper · Laser-activated irrigation · Micro-CT

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Introduction

The most common reasons for root canal treatment failure are incomplete cleaning and disinfection of root canals, inadequate filling of root canals (unfilled, overfilled, or incomplete filling), and unsatisfactory or untimely coronal tooth restoration [1–4]. Root canal retreatment involves a reopening of an endodontically treated tooth and removal of previous filling material (sealer and gutta-percha) in order to shape and disinfect the root canal system to allow the healing of periradicular lesion [5]. It can be accomplished using solvent and hand, rotary, reciprocating, or ultrasonic instruments [6–9]. Rotary nickel-titanium (NiTi) instruments have been shown to be more efficient and less time consuming compared to hand instruments [10–12]. However, regardless of the retreatment technique used, substantial amount of filling material remained on the root canal walls [13–15]. Although it has not been proven yet that complete removal of filling material is crucial for a positive outcome of the endodontic retreatment [3], filling remnants can prevent efficient root canal irrigation and disinfection by leaving microorganisms or necrotic material protected by the material. Furthermore, filling remnants

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can negatively affect the bond strength of other sealers to root canal walls [16].

In the past decade, new silicate-based root canal filling materials—MTA Plus (Prevest Denpro, Heidelberg, Njemačka), MTA Fillapex (Angelus, Londrina, Brazil), iRoot SP (Innovative Bioceramix, Vancouver, Kanada), and EndoSequence BC (Brassler, Savannah, SAD) have been intensively researched. They are bioactive, non-toxic, biocompatible, have dimensional stability, and cause less pain and inflammation in the case of overfilling [17–21]. They offer superior bond strength to dentin compared to zinc oxide–eugenol-based cements [22]. Some of them stimulate biomineralization which strengthens the bond between dentin and sealer even more [23, 24]. Those features explain the recent reports about difficult complete removal of calcium-based sealers using rotary techniques [25, 26].

Laser-activated irrigation (LAI) is a photo-thermal, photo-acoustic, or photo-mechanic activation of irrigant in root canal system using erbium-lasers (Er:YAG, Er, Cr:YSGG laser). If Er:YAG laser is used with small energy (20 mJ) and very short pulses (50 μs), intracanal cavitations and shock waves generated as a result of photo-acoustic and photo-mechanical effect; the protocol is called photon-initiated photoacoustic streaming (PIPS) [27]. Until now, the efficacy of the PIPS has been extensively researched in the removal of the smear layer, debris, and biofilm [28–30]. However, there have been only two studies so far on the use of the PIPS in root canal retreatment, both of them evaluating the retreatment of epoxy resin-based sealer [31, 32].

The aim of this study was to evaluate the effect of the PIPS in the removal of the remnants of three filling materials (epoxy resin and two calcium silicate-based materials) after a rotary phase of the retreatment. The difference in the amount of the remaining filling materials after rotary phase of the retreatment was analyzed and compared.

Materials and methods

Selection and preparation of samples

The study was approved by the local Ethical Committee No 05-PA-26 11/2015. The study sample consisted of 36 straight single-rooted human mandibular and maxillar second incisors and second premolars. The teeth with previous endodontic treatment, root caries, external resorption, or more than one root canal were not included in the study. The teeth were stored in a 0.5% chloramine-T solution at 4 °C until use.

The teeth were accessed with a water-cooled diamond fissure bur no. 016 (Komet, Rock Hill, SC, USA). The tooth crown was horizontally cut with the same bur. In order to establish the working length (WL) of 16 mm, the K-file size 10 was inserted into the apical foramen and deducted 1 mm from the canal length. Canal patency was confirmed by inserting a size 10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) through the apical foramen before and after canal preparation.

The root canals were prepared by a single operator with the ProTaper Next (PTN) rotary instruments (Dentsply, Maillefer, Ballaigues, Switzerland) driven by a torque-controlled motor (Wave One, Maillefer, Ballaigues, Switzerland) at a speed of 300 rpm. The instruments PTN X1, X2, and X3 (master apical file, MAF) (30/0.07) were used up to the WL. Between each instrument, the root canals were irrigated with 1 ml 2.5% NaOCl using a 30 G needle and a disposable syringe (BD, Microlance, Becton Dickinson, Madrid, Spain). A coronal reservoir for the irrigant was prepared by a water-cooled Gates Glidden bur size no. 5 (VDW, Münich, Germany) placed 5 mm into the canal [30]. The smear layer from the root canal walls was removed by the final irrigation protocol: 1 mL 15% ethylenediaminetetraacetic acid (Calsinase, Lege artis, Dettenhausen, Germany) during 1 min, 1 mL 2.5% NaOCl (30 s), and 1 mL saline solution (30 s) [30]. The root canals were dried with sterile X3 paper points (ProTaper Next, Maillefer).

Root canal filling

The prepared samples were randomly divided in three experimental groups according to the filling material used ($n = 12$). The canals were obturated using cold lateral condensation technique.

Group 1

The root canals were filled with a calcium silicate-based sealer and gutta-percha points covered by bioceramic (EndoSequence BC, Brassler, Savannah, USA). The sealer was filled in the root canal with the corresponding plastic extension tip. The master gutta-percha point size no. 30 (EndoSequence, Brassler, Savannah, SAD) was inserted into the WL. For lateral condensation, a hand spreader size no. 25 (Anataeos, München, Germany) and additional gutta-percha point size no. 20 (EndoSequence BC) were used. The gutta-percha points were covered with the sealer by dipping them into the sealer before insertion in the canal.

Group 2

The samples were filled with the MTA Fillapex material (Angelus Solucoes Odontologicas, Londrina, Brazil) and gutta-percha points (DiaDent, Seoul, Korea).

After mixing the two pastes, according to the manufacturer's instructions, the material was inserted in the canal with

master gutta-percha point size no. 30 (DiaDent), which was placed to the WL. A hand spreader size no. 25 (Anataeos, München, Germany) and additional gutta-percha point size no. 20 (DiaDent, Seoul, Korea) were used for lateral condensation.

Group 3

The samples were filled with AH Plus material (Dentsply DeTrey, Konstanz, Germany) and gutta-percha points. The filling technique was same as in the group 2.

The access cavities of all the samples were closed with temporary material (Caviton, GC, Tokyo, Japan). The samples were stored at 37 °C and 100% relative humidity for 2 weeks to allow complete setting of the sealer.

Root canal retreatment

The retreatment procedure was performed with the ProTaper Universal rotary instruments (Maillefer, Baillaigues, Switzerland). The working parameters were speed 300 rpm and torque 2 N/cm^2. The D1 instrument was used for the removal of the material from the cervical part of the canal, and instruments D2 and D3 were used for the removal of the material from the middle and apical third of the canal. The root canals were further prepared with instruments X3 (30/0.07) and X4 (40/0.06) to the WL [12]. No solvent was used during the retreatment [12]. Each instrument was used for the instrumentation of the three root canals after which it was discarded. The rotary phase of the retreatment was considered complete when each instrument reached the WL for five times.

After the retreatment, the root canals were irrigated with 1 ml 2.5% NaOCl using 30 G needle and syringe, then canals were filled with 15% EDTA, left in canal for 3 min, and finally rinsed with 1 mL 2.5% NaOCl. The canals were dried with sterile paper points (ProTaper Universal, Maillefer) [12].

Photon-initiated photoacoustic streaming

After mechanical retreatment phase, the root canals were irrigated with 2.5% NaOCl and activated by the Er:YAG laser (2940 nm, LightWalker, Fotona, Ljubljana, Slovenia) according to the protocol of photon-initiated photoacoustic streaming (PIPS) [30]. The root canals were continuously irrigated with 5 mL 2.5% NaOCl using 30 G needle (Becton Dickinson, Madrid, Spain), which was placed in the access cavity. The solution was activated for 60 s with the endodontic laser tip (diameter 600 μm, Fotona, Ljubljana, Slovenia), which was placed at the access opening in the pulp chamber, and remained stationary during activation. The laser parameters were pulse energy, 20 mJ; frequency, 15 Hz; pulse duration, 50 μs; and energy density, 2.06 J/cm^2 [30].

Micro-CT scanning and evaluation

Specimens were scanned in a micro-CT device after the filling procedure, after the rotary retreatment (analyzed the difference of the filling remnants after rotary retreatment), and after the PIPS (analyzed the difference after the PIPS).

The volume of gutta-percha was measured with an industrial micro CT (Nikon XT H 225, USA) using a target with 0.7 μm focal spot size and 400 mm × 300 mm 14 bit flat panel detector with 127 μm pixel size. The samples were measured at 80 kV and 60 μA using 1600 projections at 1 s exposure time. The geometrical magnification was ≈ 100 yielding structural resolution of 1.2 μm. All samples were measured at the same position and at the same radiation settings. Similar postprocessing was performed on all measurement sets: beam hardening reduction was performed using Hanning filter, noise reduction was applied using median filter, and surface detection was made using an adaptive search algorithm (Volume Graphics VGMax 2.2). During analysis, the filling material was treated as an inclusion in base tooth material; this was possible because of very distinct grayscale values of tooth and filling material (10,000 and 40,000, respectively). Grayscale value of tooth was used as base material, and then a simple threshold algorithm was used to detect any occurrence of gutta-percha in interior tooth volume. Results are expressed as reduction of filling material volume on canal walls after the PTU retreatment and after the PIPS.

Statistical analysis

The results between the groups were analyzed by the Kruskal-Wallis test with additional post-hoc Mann-Whitney *U* test. All *p* values lower than 0.05 were considered statistically significant. The program IBM SPSS Statistics verzija 23.0 (www.spss.com) was used.

Results

Micro-CT scans of the filling remnants on root canal walls after the PTU retreatment and after the PIPS for all three groups are shown in Figs. 1, 2, and 3.

Table 1 shows minimal, median, and maximal values of the remaining filling material after rotary PTU retreatment and PIPS, for all groups. There were no statistically significant differences in the baseline filling volume between the three materials ($p > 0.05$), indicating the approximately similar volume of the root canals in all groups. There were statistically significant differences

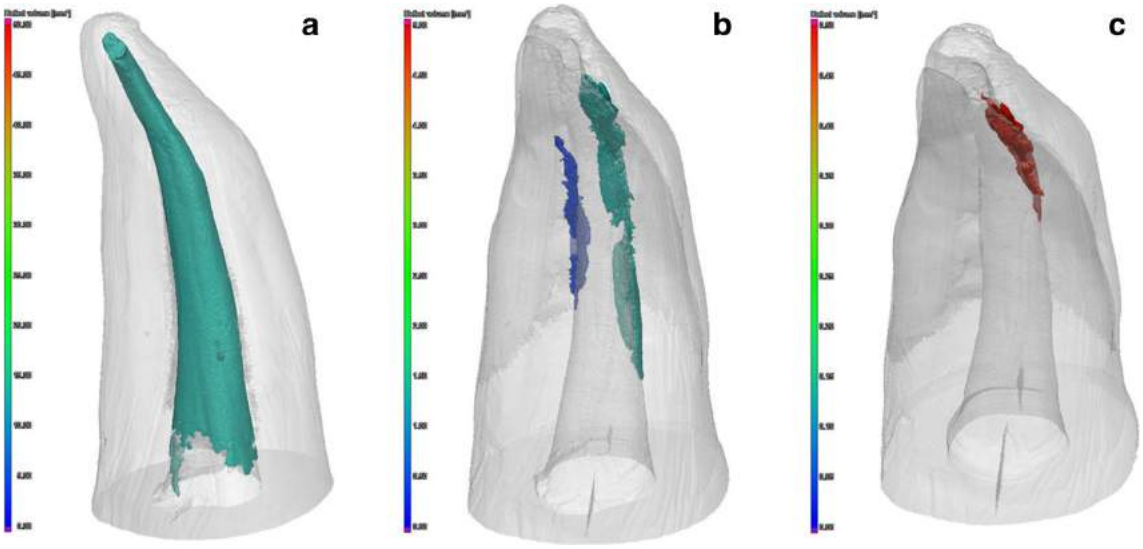

Fig. 1 Three dimensional model of a tooth with filling remnants (colored according to the volume of material under investigation) in EndoSequence BC group. **a** After obturation. **b** After the ProTaper retreatment. **c** After the PIPS

($p < 0.001$) in the volume of the remaining material between the groups, after the PTU retreatment and the PIPS.

Table 2 shows the reduction of the filling volume after the PTU retreatment (compared to the initial volume) and after the PIPS (compared to the volume after the PTU retreatment) with the level of significance (p). There was a statistically significant reduction of the volume of all three filling materials after the PTU retreatment and after the PIPS ($p < 0.05$). After the PTU retreatment, the MTA Fillapex group showed the least amount of the remaining material ($p < 0.001$) compared to other materials. There were no statistically significant differences in the amount of the remaining filling materials between EndoSequence BC and the AH Plus group after rotary phase of the retreatment ($p > 0.05$). The PIPS was the most

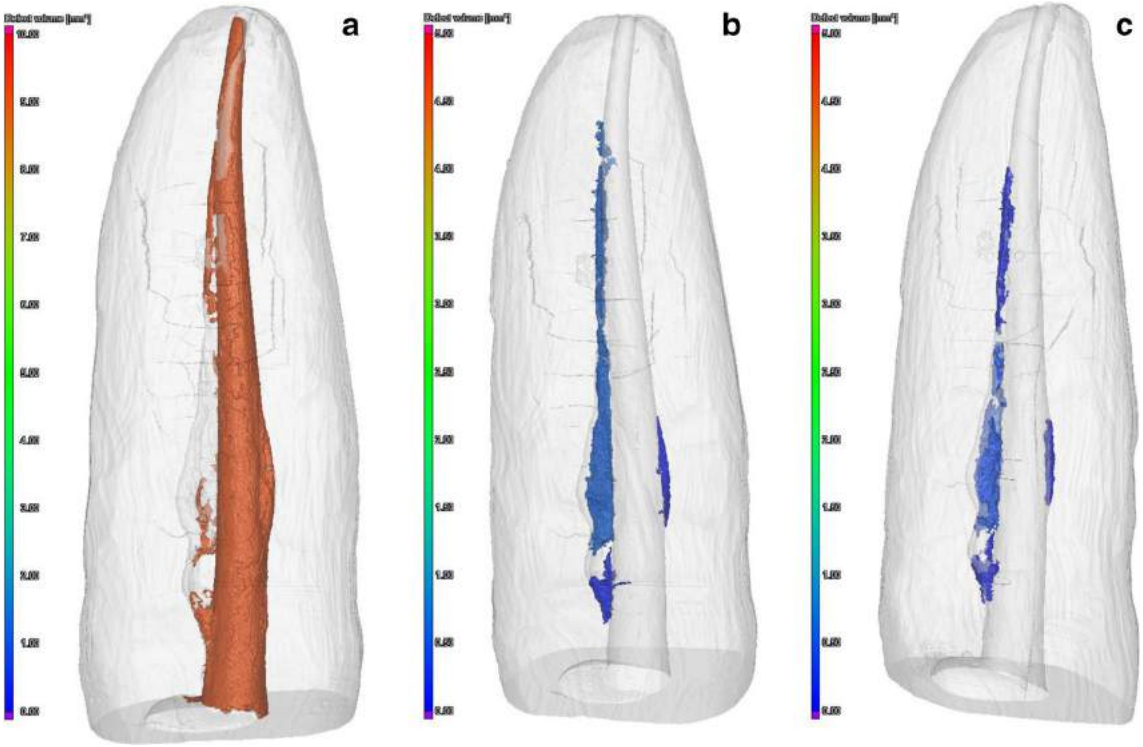

Fig. 2 Three dimensional model of a tooth with filling remnants (colored according to the volume of material under investigation) in MTA Fillapex group. **a** After obturation. **b** After the ProTaper retreatment. **c** After the PIPS

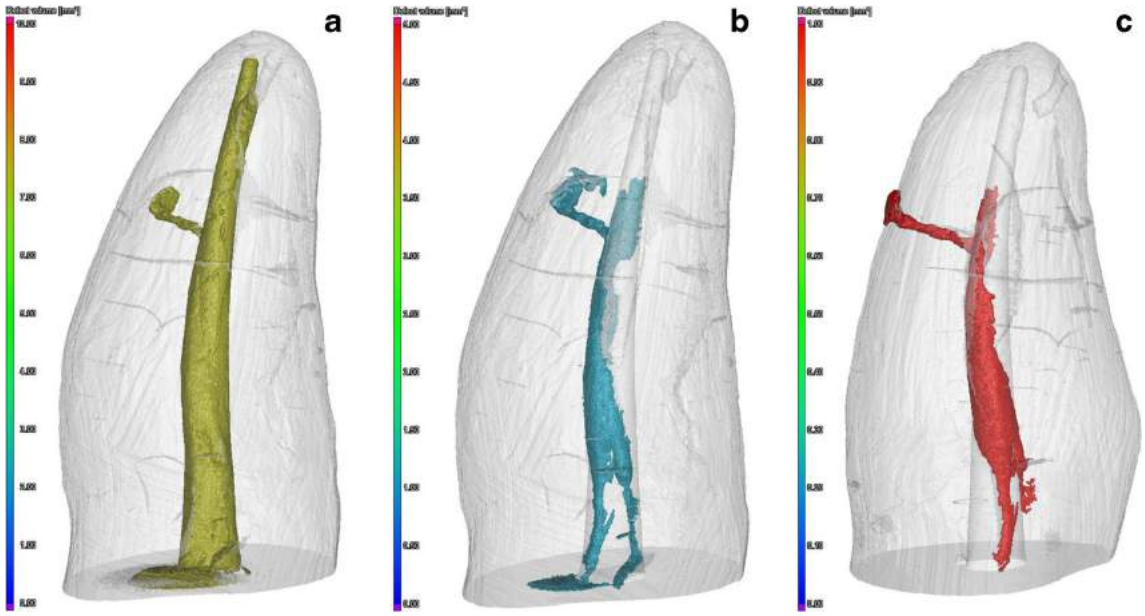

Fig. 3 Three dimensional model of a tooth with filling remnants (colored according to the volume of material under investigation) in AH Plus group. **a** After obturation. **b** After the ProTaper retreatment. **c** After the PIPS

successful in the removal of the MTA Fillapex material ($p < 0.05$), followed by the EndoSequence BC, and the least removed, AH Plus material ($p < 0.05$).

Discussion

Previous studies have shown that filling material often remained on the root canal walls regardless of the retreatment technique used [3, 9, 11, 33]. This was confirmed in our study, in which bioceramic material and epoxy resin material could not be removed completely by the use of rotary retreatment technique without using a solvent. However, the results have to be perceived carefully considering the fact that the partial tooth crown removal, which was performed in order to standardize the samples, could potentially facilitate root canal retreatment and underestimate the volume of filling remnants.

After the rotary retreatment, the least amount of the remaining material was recorded in group filled with MTA Fillapex (complete removal of the material in one sample). Similar results were reported by Prasanna et al. [14], who found greater removal of MTA Fillapex with ProTaper rotary retreatment technique compared to MTA Plus. The authors explained their results by the lower adhesion capacity [34] and questionable biomineralization [14] of the MTA Fillapex in root canal due to the low concentration of the MTA (13.2%). Probably, it is too small concentration of MTA for the initiation of biomineralization, which could be the reason of its lower bond to the root canal walls [14].

The results of this study did not show the difference in the retreatability of the EndoSequence BC and AH Plus filling material using rotary technique. Several previous studies also showed limitations of rotary and reciprocating retreatment of the calcium silicate-based filling material from the root canal

Table 1 Volume of filling remnants (mm^3) after the obturation, the ProTaper retreatment, and the PIPS

Group		Minimum	Maximum	Percentiles		
				25th	50th (median) mm^3	75th
Baseline volume	EndoSequence	5.430	13.440	5.750	6.640	12.095
	MTA Fillapex	4.130	13.740	7.433	8.600	12.830
	AH Plus	5.380	16.600	6.455	7.570	11.550
ProTaper	EndoSequence	0.370	2.810	1.270	1.480	2.470
retreatment	MTA Fillapex	0.000	0.810	0.018	0.210	0.393
	AH Plus	0.920	4.690	1.270	1.460	2.710
PIPS	EndoSequence	0.000	1.090	0.220	0.480	1.045
	MTA Fillapex	0.000	0.320	0.008	0.075	0.165
	AH Plus	0.730	4.400	1.170	1.390	2.540

Table 2 Reduction of filling material volume (in %) on canal walls after the ProTaper retreatment (in comparison to the volume after obturation) and after the PIPS (in comparison to the volume after ProTaper retreatment)

Group		Minimum	Maximum	Percentiles			*p*
				25th	50th (Median)	75th	
EndoSequence	Reduction of the filling volume after the ProTaper (%)	63.03%	93.43%	67.89%	78.55%	88.55%	0.008
	Reduction of the filling volume after the PIPS (%)	81.43%	100.00%	81.51%	96.43%	97.80%	
MTA Fillapex	Reduction of the filling volume after the ProTaper (%)	90.58%	100.00%	96.05%	97.73%	99.70%	0.012
	Reduction of the filling volume after the PIPS (%)	96.28%	100.00%	98.11%	99.25%	99.94%	
AH Plus	Reduction of the filling volume after the ProTaper (%)	69.89%	92.60%	70.65%	72.86%	84.36%	0.008
	Reduction of the filling volume after the PIPS (%)	71.45%	94.13%	71.98%	74.16%	85.59%	

compared to resin and zinc-oxide-based materials [12, 13, 26]. Kim et al. [26] evaluated the retreatment ability of the EndoSequence BC and AH Plus material with rotary technique by scanning electron microscopy. They concluded that both materials showed similar retreatment characteristics due to the similar penetrability in dentinal tubules. Ersav et al. [35] also did not find a difference in the retreatment ability between AH Plus and EndoSequence BC material, evaluated by a radiograph. The similar results of the abovementioned studies were probably due to the similar adhesion and sealing ability of the AH Plus and the bioceramic material [36, 37].

The use of Nd:YAG and Nd:YAP lasers in root canal retreatment has been evaluated and described in the late 90s [38, 39]. Although the wavelengths of these lasers are well absorbed in pigmented material (gutta-percha), the problem was the photo-thermal effect of the irradiation on the gutta-percha which resulted in carbonization and partial dissolution of the material and dentin causing its difficult removal from the canal [39]. In this study, we evaluated the efficacy of the Er:YAG laser in combination with NaOCl (PIPS). The PIPS protocol is based on the creation of photo-acoustic shock waves in the irrigant in root canal which causes rapid movement of the fluid and the secondary cavitation effect without thermal effect [27]. The results of this study showed significant additional removal of the three tested filling materials after the PIPS protocol. Although the highest total reduction rate was recorded in the MTA Fillapex group (median 99.25%), it is apparent (Table 2) that the EndoSequence BC showed bigger reduction, after the PIPS, compared to the reduction rate after the PTU (from 78.55 to 96.43%). Therefore, from the clinical point of view, the PIPS could be a valuable additional technique for the retreatment of the bioceramic material.

According to our knowledge, there have been only two studies published so far on the use of the PIPS in the root canal retreatment [31, 32], both of them evaluated the removal of epoxy resin-based sealer. Keles et al. [31] compared the PIPS, the Er:YAG laser-activated irrigation, and the Nd:YAG laser in the removal of AH Plus material and gutta-percha after rotary retreatment. Their results showed significant additional removal of the material in all groups; however, the most efficient protocol was with the deep position (3 mm from the WL) of the fiber tip in canal. In another study by Jiang et al. [32], the PIPS was more efficient compared to passive ultrasonic irrigation and sonic irrigation in the removal of the AH Plus material after ProTaper retreatment.

Conclusion

Using the rotary retreatment technique, the MTA Fillapex was most easily removed from the root canal, and there was no difference between removing the EndoSequence BC and the epoxy resin material.

The PIPS improved the removal of the remaining filling material after the retreatment with rotary instruments.

Compliance with ethical standards

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

Ethical approval The study is an ex vivo study and does not include human participants.

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