

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 photoacoustic (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 photoacoustic (PIPS) irrigation, Biofilm; type 2 canal system, mixed bacterial oral plaque, MTT assay

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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 24hrs in the presence of 5% CO₂.²⁴ A standard bacterial suspension (1×10⁸ cells/ml) was prepared by measuring the optical density at 600nm 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 37°C for 24hours. 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 40μl of the standard bacterial suspension (1×10⁸ 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 0.5ml 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 18ml of irrigant (saline or 6% NaOCl), 6 ml of sterile saline, 6 ml of 17% EDTA and 18ml of additional saline delivered over a period of 6 minutes (Table 2), a protocol documented previously.¹⁸ The 9mm long, 600μm 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 2940nm wavelength Er: YAG laser (LightWalker DT, Fotona, Ljubljana, Slovenia), at 15Hz and 20mJ 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 withdraw 10 μ l 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 μ l 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 560nm 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 Scheffé'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 ($= 0.0546$). 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 ($P = 0.1186$).

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

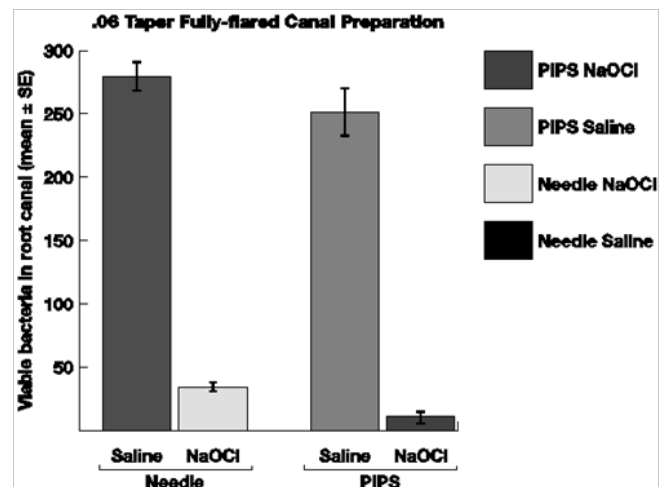


Figure 1 Demonstration of viable bacteria remaining in the root canals following the irrigation of a minimally shaped (#20/.04) preparation. Values are means and standard deviations. Bars with similar letters are not statistically significant ($P > 0.05$).

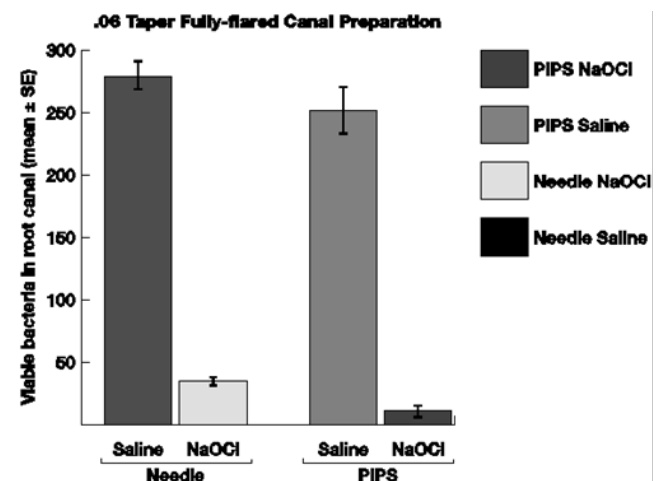


Figure 2 Demonstration of viable bacteria remaining in the root canals following the irrigation of a flared (#25/.06) preparation. Values are means and standard deviations. Bars with similar letters are not statistically significant ($P > 0.05$). Statistical significance between B, C ($P < 0.0008$).

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 SNI¹⁸, 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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Conflicts of interest

The authors declare there is no conflict of interest.

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