"In-Vitro Evaluation of the Efficacy of PIPS Irrigation System on disinfection of Type 2 canal systems in Molars."

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ABSTRACT

Introduction: This in vitro study was designed to compare the antimicrobial effects of PIPS® laser activation of irrigant against established 3-week bacterial biofilms to traditional needle irrigation in root canal therapy. **Methods:** Twenty-four extracted human teeth of one common canal configuration (Vertucci Type II) were endodontically cleaned and shaped using two different preparations (flared #25/0.06 or non-flared #20/0.04). Teeth were inoculated with a 3-week plaque biofilm, disinfected with one of two irrigation protocols (NaOCl or saline), and one of two delivery methods (PIPS or needle irrigation), resulting in six teeth per group. PIPS groups were run in duplicate. During the period of biofilm formation, 0.5 ml of media was discarded and replaced with fresh culture media every 48 hours. Following the irrigation treatment, the number of viable bacteria in root canals was determined by MTT bacterial viability assay. Data was analyzed for statistical significance by ANOVA followed by Scheffe's *f*-test, with a p-value less than 0.05 considered significant.

Results: All saline groups showed significantly more bacteria remaining after irrigation than the corresponding sodium hypochlorite (NaOCl) groups. PIPS 0.06 NaOCl group showed significantly greater disinfection than the needle 0.06 NaOCl group. While PIPS 0.04 NaOCl had greater disinfection than its needle group counterpart, this was not significant ($p > 0.05$). All other groups showed no significant differences in viable bacteria enumerated from root canals.

Conclusions: The use of the PIPS laser technique with a novel irrigation protocol in an *in vitro setting* showed statistical significance in achieving higher disinfection in larger tapered preparations than needle irrigation. Higher disinfection (although not

significantly different) was found in in 0.04 tapered preparations that had undergone the PIPS technique compared to needle irrigation of 0.04 tapered preparations.

Keywords*:* irrigation, PIPS, contemporary irrigation techniques, needle, taper, root canal disinfection

Introduction:

The challenges of endodontic treatment have evolved with the profession's understanding of the complexity of root canal systems, root morphology, dentin structure, bacterial biofilms, irrigation fluid dynamics and canal shaping philosophies. Greater emphasis on dentin conservation, instrumentation safety, antimicrobial efficacy, and apical awareness have added refinement to the founding principles of root canal shaping, cleaning, and obturation that Schilder defined in his seminal work (1).

The goals of irrigation are both the bacterial disinfection and the removal of mechanical debris from smear layer and pulpal remnants. Studies have shown that one of the main challenges in successful canal treatment is the difficulty in delivery of antimicrobial irrigants to the apical third of the canal in a safe and effective manner (2, 3). By leaving bacterial contamination, apical lesion healing is expected to either take longer than expected or never resolve, leading to retreatment or extraction (4). While the adverse effect of remnant debris in the apical third is not as clearly known, it is considered a substance rich with bacterial biofilm within a space untouched by sealers, irrigants, or obturating materials, detrimental to a satisfactory outcome (5). When treating canals using rotary or hand instrumentation, the extent of apical debris that remains within the canal is significant, and may be removed to some degree with standard manual needle irrigation (6). Many alternative irrigation techniques intended to replace or serve

as adjuncts to needle irrigation have been investigated including varying forms of ultrasonic and sonic agitation, photo-activated dyes (PAD), photon-induced photoacoustic streaming (PIPS), and negative apical pressure devices.

Photon-induced photoacoustic streaming (PIPS) relies on a principle of creating a shockwave that travels through liquid media with short low-level laser energy, thereby removing the smear layer, and releasing energy further down the canal (7). Previous studies have shown that disinfection using the PIPS system is an efficient methodology as an adjunct to hypochlorite and EDTA irrigation (8, 9).

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 (10, 11). However, the application of differing taper sizes continues to be studied, with the goal of improving the disinfection of contaminated canal systems, as well as improving the efficiency of treatment. While conservation of apical size has been valued highly, an improvement of irrigant penetration to the apical third has been correlated with an increase in taper and apical dimension of rotary instrumentation (12, 13). With the introduction of alternative irrigation techniques, the canal taper and apical size decisions may take on a lesser importance if the hydrodynamic action capabilities offset the physical restrictions to fluid flow.

One associated effect with application of acoustic or photoacoustic waves on chemicals systems is sonochemistry, which may alter the chemical substantivity of endodontic irrigants. Previous studies have shown that ultrasound and laser activation significantly increase the reactivity of sodium hypochlorite (14, 15). One such laser-assisted technique is the PIPS® system that relies on cavitation from the pressure waves

caused by the 20mJ energy output with 50 microsecond frequencies through its proprietary tip, possibly resulting in a sonochemical adjunctive property to its mechanical action (16).

The objective of this study was to examine the disinfection of intentionally contaminated Type II mandibular molar canals within the middle/coronal third using minimal preparation techniques. The null hypothesis was that no difference existed between bacterial disinfection present at #20/0.04 taper and #25/0.06 flared taper preparations using PIPS irrigation.

Materials and Methods

Sample collection:

Tooth collection conformed to the protocols approved by the Institutional Review Board of the University of Tennessee Health Science Center and no identifiable data was associated with samples obtained (IRB 10-00832-XP). Intact extracted mandibular molars with non-damaged apices, similar root lengths, and Vertucci type II anatomical configurations (evaluated with digital radiographs) were selected. A type II anatomy is defined as having two separate canals that exist within the chamber, which remain separate in the cervical third of the root and join to a common apical foramen in the apical third (17).

Biofilm formation in root canals:

A mixed bacterial plaque sample was collected from laboratory personnel, according to an established protocol, and grown in Todd Hewitt broth (THB; Difco Labs, Detroit, MI) at 37°C for 24 hours in the presence of 5% CO_2 (18). A standard bacterial suspension ($1x10^8$cells/ml) was prepared by measuring the optical density (OD at 600 nm) and confirmed by the plate dilution method.

Tooth preparation:

All teeth had coronal wells naturally present, or were added with composite in the coronal segment in order to formulate a well for irrigant as a requirement of the PIPS system, according to a previously established methodology (16). Well heights (chamber heights) were of similar vertical dimension, in a realistic anatomical dimension, to achieve similar canal lengths and volumes of irrigant. Wells were left unsealed, with only the distal canal orifice sealed with Fuji II LC glass ionomer (GC America, Alsip, IL).

Cleaning and shaping following one of two protocols:

Pre-flared #25/0.06 groups – Canals were prepared to their apical terminus and a minimum pathway developed with size 10 k-files. Working length was established as patency less 0.5 mm. Canals were prepared using orifice openers and a crown down method utilizing a full series (#40/0.06 to #25/0.06) of Profile Vortex nickel-titanium rotary files (Dentsply Tulsa Dental, Tulsa, OK). The tooth was only considered for this group if the #25/0.06 rotary file had resistance when preparing the apical third.

"Minimally-shaped" #20/0.04 groups - Canals were prepared to their apical terminus and a minimum pathway developed with size 10 k-files. Hand K-files up to a size 20 were worked to the indicated working length of patency less 0.5 mm. The canal space was prepared with Profile Vortex nickel-titanium rotary files with a 0.04 taper #30-#20. The #20/0.04 rotary was required to have resistance when preparing the apical third in order to qualify for the group.

The teeth were divided into two main groups (#20/0.04 taper or #25/0.06 taper preparations), and four subgroups each comprised of an irrigation method and an irrigation protocol:

Subgroup 1 (6 teeth) (Control Group –PIPS®) Irrigation using PIPS laser system with sterile saline and 17% EDTA.

Subgroup 2 (6 teeth) (Experimental Group-PIPS) Irrigation using PIPS laser system with 6.0% sodium hypochlorite and 17% EDTA.

Subgroup 3 (6 teeth) (Control group-Needle) Irrigation using 30-gauge ProRinse™ needle with saline and 17% EDTA.

Subgroup 4 (6 teeth) (Experimental group-Needle) Irrigation using 30-gauge ProRinse™ needle using 6.0% sodium hypochlorite and 17% EDTA.

Specimen (extracted teeth) sterilization:

All prepared teeth were kept in sterile saline and sterilized by steam autoclave. Sterilized teeth were then immediately placed into sterile glass vials containing 15 ml of sterile bacterial culture media (Todd Hewitt Broth) and incubated at 37ºC for 24 hours to confirm the sterility of the teeth.

Infection protocol:

All prepared teeth were infected by injecting 40 µl of the standard bacterial suspension ($1x10^8$ cells/ml) into each tooth, with special emphasis on placement in both mesial canals. A 3-week biofilm protocol was followed, similar to an established protocol, with the modification of fresh THB (0.5 ml) media added every 2 days during the incubation cycle (18).

Irrigation

The irrigation protocol is summarized in Table 1. Sterile saline (Baxter International Inc., Deerfield, IL), 6.0% sodium hypochlorite (prepared from 8.25% stock solution) and 17% EDTA (Roth International Ltd., Chicago, IL) were used as irrigating solutions.

After the 4-week incubation cycle, each tooth was removed from the vial and an apical barrier of yellow prosthodontic “sticky wax” (Kerr Dental Laboratory Products, Orange, CA) was placed on the apical 5 mm of the external root surface. A Fotona Lightwalker laser (Fotona D.D., Ljubljana, Slovenia) was used for the PIPS procedure, with irrigation performed by two operators; one operating the irrigation device, while the

other delivered irrigant to the chamber. The PIPS laser tip was moved within the chamber, avoiding touching the tip to the wall and the floor, and keeping the tip in a constant circular motion within the chamber. Standard manual needle irrigation with a 30 gauge close-ended, side-vented needle was performed by placing the needle tip as apical as possible, short of working lengths, and expressing irrigant with an up and down movement within the canal system. Both irrigation methods were used in conjunction with their respective irrigation protocols (Table 1).

Evaluation of antimicrobial efficacy of the irrigation systems:

The antimicrobial efficacy of each of the irrigation techniques was assessed by withdrawing 30 µl of fluid from the canal systems following the final saline rinse of each tooth with sterile 30 gauge ProRinse needles and sterile tuberculin syringes. The fluid from each tooth was placed in a sterile 96-well microtiter plate. The viable bacteria were enumerated by MTT assay (Roche Diagnostics Corp., Indianapolis, IN). 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 were incubated with solubilizing solution (provided in the kit) overnight at 37°C and the absorbance was measured at 560 nm using BMG Spectrostar spectrophotometer (BMG Lab tech, Cary, NC).

After each experiment, the teeth were sterilized, and checked for sterility by incubating the teeth in fresh THB for 24 hours at 37°C. Each experiment was repeated twice.

Results:

The number of viable bacteria obtained from root canals was found to be different depending upon the irrigation protocol. The highest number of bacteria was obtained from the root canals harvested with saline. The number of viable bacteria found in root canals irrigated with saline had 245 ± 13.75 and 279 ± 10.99 in 0.04 and 0.06 tapered preparations, respectively. When similar infected teeth were irrigated with NaOCl about 87% fewer viable bacteria were detected (Table 2). Fewer numbers of bacteria were found in canals when irrigated with PIPS/saline compared to needle/saline irrigation, but the difference was not significant ($P < 0.0544$). Treatment of the canals with NaOCl resulted in significant reduction in viable bacteria ($P < 0.0001$). The present results did not exhibit any difference ($P < 0.0546$) between 0.04 and 0.06 taper preparations and also between 06-PIPS and needle/ NaOCl irrigation ($P < 0.296$). Significant reduction in viable bacteria was found between 0.06 NaOCl PIPS and needle irrigation ($P < 0.0008$; Table 2). The results of the study demonstrated higher disinfection rate in larger tapered 0.06-PIPS than the needle irrigation but not between 0.04 PIPS and needle irrigation methods.

Discussion:

This study determined the causal relationships of varying taper dimensions on disinfection potential of a photon-induced photoacoustic-streaming device compared to common needle irrigation techniques. The data showed no differences between taper sizes and their disinfection potential for both comparable methods, but showed a significant tendency for higher disinfection between 0.06 taper groups with the PIPS system compared to needle irrigation. This finding suggests two salient points regarding the methodology used: (1) the limited opening of the non-flared 0.04 taper preparation

may not allow for either the delivery of an adequate volume of active irrigant, or (2) that the sampling technique in itself allows for limited collection ability in smaller tapered systems. Similar results showing improved disinfection capability of laser-activated irrigation have been reported using different analytical methodologies (9, 14).

The challenges facing measurement of 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, but often can exclude information about the apical third, where disinfection is both 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, but require the structural modification of the dentin and apical complexities that are being investigated (19-22). While the method used in this paper contains similar shortcomings to the paper point/CFU method with regards to sampling location, it provides the benefit of allowing a more expedient sample collection in combination with a well-known, more reliable methodology for bacterial quantification.

One aspect which affects the clinical use of such an irrigation protocol described are the limitations of delivery of 48 ml through a small bore needle with a closed end. The force required to drive such an amount of liquid may well be considered exhausting, especially regarding the delivery of such a volume to multiple canals or multiple teeth. The usage of automated delivery mechanisms such as pump-driven devices would be essential to the further study of high-volume needle irrigation regimens. Other areas of concern are the limited data on extrusion of irrigant within apexo-mimetic conditions,

and the apical influence of the generated photoacoustic shock waves from the PIPS laser method.

In conclusion, in this *in vitro* study, the use of the PIPS laser with the 48 ml volume protocol showed a statistically significant higher disinfection in 0.06 tapered preparations, and higher disinfection (although not significantly different) in 0.04 tapered preparations. Further studies are needed regarding taper preparation requirements for successful disinfection, as well as evaluation of the collection of apical bacteria from intact root canal systems.

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

Saline protocol	*Hypochlorite protocol*
6ml Saline over 30 sec	6ml Hypochlorite over 30 sec
6ml Saline over 30 sec	Wait 30 seconds
6ml Saline over 30 sec	6ml Hypochlorite over 30 sec
6ml Saline over 30 sec	Wait 30 seconds
6ml 17% EDTA over 30 sec	6ml Hypochlorite over 30 sec
6ml Saline over 30 sec	Wait 30 seconds
6ml Saline over 30 sec	6ml Saline over 30 seconds
6ml Saline over 30 sec	6ml 17% EDTA over 30 seconds
	6ml Saline over 30 seconds
	6ml Saline over 30 seconds
	6ml Saline over 30 seconds

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

Mean number of viable bacteria ± Standard error in root canals

	Needle Saline 04	**Needle NaOCl 04**	**PIPS Saline 04**	**PIPS NaOCl 04**	**Needle Saline 06**	**Needle NaOCl 06**	**PIPS Saline 06**	**PIPS NaOCl 06**
Mean Bacteria ± Standard Error	245.2 ± 13.75a	32.71 ± 6.01a	193.4 ± 19.1	19.43 ± 6.13	279 ± 10.99	34.33 ± 4.07	251.14 ± 18.6	10.75 ± 4.35

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