Effectiveness of photon-initiated photoacoustic streaming in root canal models with different width or taper

Cheng Wen¹, Yuanyuan Kong¹, Jian Zhao¹, Yang Li¹, Ya Shen², Xuechao Yang¹, Qianzhou Jiang¹*

¹Department of Endodontics, Affiliated Stomatology Hospital of Guangzhou Medical University, Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medical, Guangzhou, Guangdong, 510182.
²Division of Endodontics, Faculty of Dentistry, The University of British Columbia (UBC), 2199 Wesbrook Mall, Vancouver V6T 1Z3, Canada

Key words: PIPS, width, taper, root canal ·

Correspondence author:
*Qianzhou Jiang
Department of Endodontics, Affiliated Stomatology Hospital of Guangzhou Medical University, Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medical, Guangzhou, Guangdong, 510182.
Tel: 020-61350524 E-mail: jqianzhou@126.com
ABSTRACT

**Background:** To evaluate the sterilization effect of photon-initiated photoacoustic streaming (PIPS) in different widths or different tapers root canal systems.

**Methods:** Artificial root canal samples (n=480) were randomly divided into 3 groups (n=160/group). The canals were prepared into size #10/.02, #25/.02 or #25/.06. Four different irrigation solutions were activated with conventional needle irrigation (CNI) or laser-activated irrigation (LAI). Bacterial suspensions and biofilms were assessed with adenosine 5'-triphosphate (ATP) assay kit and fluorescent microscopy.

**Results:** When the root canal taper is 0.02, size #10 with LAI had a significant reduction of the bacteria than #25 with CNI ($P < .05$). When the apical width is 25, taper 0.02 with LAI had a significant reduction of the bacteria than taper 0.06 with CNI ($P < .05$). 5.25% NaOCl attained superior antibacterial and bacteriostatic effects as compared to the other irrigants, followed by 2% NaOCl. 2% and 5.25% NaOCl combined with PIPS can effectively enhance the long-term antibacterial effect of root canal after incubation for 6 hours.

**Conclusions:** Compared with CNI, PIPS have stronger ability to remove bacteria in root canals with a small preparation width and a small taper. PIPS with 2% and 5.25% NaOCl can have superior antibacterial and bacteriostatic effects.

**Key words:** PIPS, width, taper, root canal ·

**Background**

Eliminating infection within a canal is crucial to the success of a root canal treatment, which is currently carried out through a variety of chemomechanical techniques(1). The purpose of mechanical preparation, is to form a good shape in the root canal system, which is conducive to root canal irrigation and root canal filling (2). In recent years, mechanical preparation of root canals has made great progress, however, they were still unable to completely eradicate the bacteria within the root canal (3, 4). Irrigation is complementary to instrumentation in facilitating the removal of bacteria, debris, and the smear layer (5, 6). Previous studies have shown that instrumentation and irrigation
with NaOCl eliminated bacteria in 50–75% of infected root canals at the end of the first treatment session, whereas the remaining root canals contain recoverable bacteria (7). The photon-initiated photoacoustic streaming (PIPS) with a Er:YAG laser with a low pulse energy (20 mJ) and a short pulse duration (50 μs) has been introduced in root canal treatment to facilitate remove bacterial in root canal system(8). Matsumoto et al. (9) observed that cavitation bubbles could clean the apical region even when the PIPS laser tip was not inserted to the work length of root canal system. PIPS have shown promise in eliminating the smear layer and dentinal debris (10, 11). It has been well documented that the Er:YAG laser irradiation using PIPS significantly improved the disinfection efficacy of NaOCl irrigation(12, 13). Our previous research also shows that PIPS with 2% or 5.25% NaOCl can effectively improve the removal of bacteria in the root canal (14),

Studies have shown that a larger root canal preparation width and taper can improve the effectiveness of root canal irrigation, but optimization of the mechanical efficacy of irrigation provided by enlargement of canals may result in weakening of the root structure (15, 16). With the development of minimally invasive treatments, recent research has focused on methods to reduce mechanical preparation and explore more effective chemical preparation methods (17-19). Korkut et al.(20) illustrated that Er:YAG laser with PIPS activation resulted in cleaner root canal walls compared with Nd:YAG and diode laser groups. But it is unclear whether PIPS can effectively irrigate in root canals with a small taper and width. Therefore, the present study aim to evaluate the sterilization effect of PIPS laser-activated irrigation with NaOCl from root canals in different widths and tapers.

**Methods**

**Artificial root canal block**

The artificial root canal blocks (n=480) were made of resin with a curved root canal and a coronal reservoir resembling a pulp chamber (Endo Training Block; Dentsply Maillefer, Ballaigues, Switzerland). The root canals had a 16.5 mm working length with
a degree of curvature of 45.2°, a taper of 0.02 and a radius of canal curvature of 6.1 mm (21).

**Canal preparation**

The blocks were divided into three groups, each group was prepared using files with different widths or tapers (n=160/group).

**#10/.02 group.** The root canal was prepared by K-file (Dentsply Tulsa Dental). It was placed to the working length and a reciprocal action was used until it fit loosely in the canal.

**#25/.02 group.** The root canal was prepared by K-file. A #10/.02 K-file was placed to the working length. A #15/.02 K-file, #20/.02 K-file and #25/.02 K-file were used sequentially until they fit loosely in the canal.

**#25/.06 group.** The root canal was prepared using MTWO files (VDW, Munich, Germany) adapted to an electric motor (VDW) in rotary movement. The following files were used: size #10/.04, #15/.05, #20/.06 and #25/.06. The instrumentation was performed in a gentle in-and-out motion, taking the files to the working length.

The three groups were subdivided into four subgroups (n=40) based on the irrigation solution used: subgroup A. distilled water; subgroup B. 1% NaOCl; subgroup C. 2% NaOCl; subgroup D. 5.25% NaOCl. Half of samples in each subgroup were irrigated with conventional needle irrigation (CNI) and half were irrigated with laser-activated irrigation (LAI) (n=20).

**Bacterial inoculation of the root canals**

*Enterococcus faecalis* (ATCC 29212) was grown in brain heart infusion (BHI) broth (hopebio, Qingdao, China). Single colonies were inoculated with 5 mL BHI in an aerobic chamber for 24 h at 37 °C. A $5 \times 10^8$ CFU/mL, which was equivalent to 0.5 McFarland, was prepared. Then 20 μL bacterial suspension was added into the root canal systems. The #25/.02 and #25/.06 root canal blocks were immersed in the 5 mL fresh BHI for 48 h at 37 °C aerobically. #10/.02 root canal blocks were immersed in the 5 mL fresh BHI for 7 days under the same conditions. The bacterial suspension was
refreshed for each 24 h. Before irrigation, the root canal blocks were washed with 1 mL distilled water three times. Sterile paper points were inserted into the root canal system and left in the canal for 1 min to collect planktonic bacteria. Samples from each canal were collected into separate Eppendorf tubes (S0 sample).

**Root canal irrigation**

For CNI, a 30-gauge needle tip was inserted into each root canal up to bend of root canal system. The root canals were irrigated for 60 s with 3 mL of irrigate solution.

For LAI, the tip was left stationary in the pulp chamber and activated while ensuring the canal and pulp chamber remained passively filled with irrigation solution throughout irrigation. The irrigant was activated by a 2,940 nm Er:YAG laser (AT Fidelis; Fotona, Ljubljana, Slovenia) equipped with a handpiece (R14-PIPS, Fotona) holding a 400-μm-diameter quartz tip (XPulse 400/14, Fotona). The tip was applied with 0.3 W, 15 Hz, and 20 mJ per pulse, as recommended by the manufacturer, without water/air spray. The fiber tip was placed in the pulp chamber. The irrigation in the root canals were activated for 30 s with 3 mL of irrigate solution (22).

After irrigation, all samples were irrigated with 1 ml distilled water to remove residual irrigation solution from the root canal. Subsequently, 10 samples were performed to similar S0 sampling procedures to obtain S1 samples. Other 10 samples were re-incubated for 6 hours. Subsequently, similar S0 sampling procedure was performed at the root canals to obtain the S2 sample.

**Adenosine 5'-triphosphate (ATP) assay kit analysis**

ATP in the root canal system was measured as previously reported (23) using an ATP assay kit (Beyotime, China, S0026) according to the manufacturer's instructions. Each sample of bacteria was collected by sequential placement of sterile paper points in the root canal prepared before irrigation (S0), after irrigation (S1), and after incubation (S2). The paper points were put into the root canal, where it remained for 1 min to obtain a sample of bacteria and then transferred to a 1.5 mL Eppendorf tube of 200 μL of lysis buffer with 0.025 g of glass beads (D3350-01, Omega Biotek Inc, America), and
centrifuged at 12,000 g/min for 5 min at 4 °C for supernatant collection. Then, the ATP detection solution was prepared. The 20 μL of ATP detection solution was mixed with 80 μL diluted solution into detection tube and placed at room temperature for 5 min. Finally, 20 μL of sample was added into ATP detection solution and quantified using ATP fluorescence detector (Lux-T020, China).

**Fluorescence microscope**

The processed samples were stained with the LIVE/DEAD BacLight Bacterial Viability Kit (L7012, Life, USA) for 15 min, following the manufacturer’s instructions. Before dying, the models were stained with 20 μL of the staining solution in a dark chamber. All blocks were rinsed with distilled water for 1 min and observed with a fluorescence microscope (BX43, OLYMPUS, Japan). The fluorescence microscope images were analyzed by Image Pro Plus (version 6.0; Media Cybernetics, Inc., Rockville, MD), which calculated average fluorescence density to quantify the number of live bacteria. Apical, middle, and coronal thirds were established by marking the roots into 3 levels at 0-3, 3-6, and 6-9 mm from the apical foramen.

**Statistical analysis**

Statistical analysis was performed using SPSS Statistics Version 17.0 (IBM SPSS Inc, Chicago, IL, USA). The normality of data distributions was assessed. For data distributed normally, results were presented with means and standard deviations (SD), and one-way analysis of variance (ANOVA) was used. For data not following a normal distribution, among-group comparisons were assessed by using the Kruskal-Wallis test; within-group comparisons were assessed by the Dunn’s multiple comparison test. For all tests, statistical significance was set at α = 0.05.

**Results**

1. **Before irrigation**

   Before root canal irrigation, the ATP value and average fluorescence density. They
were no statistically significant in each group \( P > .05 \).

2. **After irrigation**

2.1 **different width root canal models**

ATP levels were shown in Fig. 1 VII. The canals instrumented to size #10/.02 in the LAI group had a significant reduction of ATP value than those instrumented to #25/.02 in CNI \((P < .05)\). The group irrigated by LAI exhibited greater ATP value reduction compared to CNI \((P < .05)\) in same width.

A larger reduction of average fluorescence density after irrigation was observed in coronal (Fig. 1 I), middle (Fig. 1 II) and apical regions (Fig. 1 III) of the LAI group than in the CNI group \((P < .05)\) in same width. The value of average fluorescence density in the coronal (Fig. 1 IV), middle (Fig. 1 V) and apical (Fig. 1 VI) regions. Size #25/.02 in the LAI group had the most reduction of bacteria than other groups \((P < .05)\). The canals instrumented to size #10/.02 in the LAI group had a significant reduction of bacteria than those instrumented to #25/.02 in CNI \((P < .05)\), except 2% NaOCl subgroup in apical region and 5.25% NaOCl subgroup in middle and apical regions \((P > .05)\).

2.2 **different taper root canal models**

ATP levels were shown in Fig. 2 VII. The canals instrumented to #25/.02 in the LAI group had a significant reduction of ATP value than those instrumented to #25/.06 in CNI \((P < .05)\). Irrigation by LAI resulted in greater ATP value reduction compared to irrigation by CNI \((P < .05)\) in same taper.

A larger reduction in average fluorescence density after irrigation by LAI was observed in coronal (Fig. 2 I), middle (Fig. 2 II) and apical regions (Fig. 2 III) \((P < .05)\) in same taper. The value of average fluorescence density in the coronal (Fig. 2 IV), middle (Fig. 2 V) and apical (Fig. 2 VI) regions. The canals instrumented to #25/.02 in the LAI group had a significant reduction of bacteria than those instrumented to #25/.06 in CNI \((P < .05)\). Size #25/.06 in the LAI group had the most reduction of bacteria than other groups \((P < .05)\).

3. **After incubation**
3.1 different width root canal models

ATP levels were shown in Fig. 3 VII. After incubation, the ATP value of all subgroups significantly increased. Size #10/.02 had lower increase of ATP value than #25/.02 ($P < .05$), except 1% NaOCl subgroup with LAI ($P > .05$). The group irrigated with 5.25% NaOCl with LAI had the least increase than other subgroups ($P < .05$).

A greater increase of average fluorescence density after incubation for 6 h was found in coronal (Fig. 3 I), middle (Fig. 3 II) and apical regions (Fig. 3 III). The value of average fluorescence density in the coronal (Fig. 3 IV), middle (Fig. 3 V) and apical (Fig. 3 VI) regions. Size #10/.02 with LAI had lower increase of average fluorescence density than #25/.02 with CNI in three regions ($P < .05$), except for the 1% NaOCl subgroup in middle region ($P > .05$). No significant differences were observed between subgroups in the apical region ($P > .05$).

3.2 different taper root canal models

ATP levels were shown in Fig. 4 VII. Size #25/.02 had lower increase of ATP value than #25/.06 ($P < .05$), except distilled water and 5.25% NaOCl subgroup with LAI ($P > .05$). The 5.25% NaOCl subgroup had the least increase in ATP value than other subgroups ($P < .05$).

A greater increase of average fluorescence density after incubation for 6 h was found in coronal (Fig. 4 I), middle (Fig. 4 II) and apical regions (Fig. 4 III). The value of average fluorescence density in the coronal (Fig. 4 IV), middle (Fig. 4 V) and apical (Fig. 4 VI) regions. The canals instrumented to size #25/.02 in the LAI group had a smaller increase than those instrumented to #25/.06 in CNI in the three regions ($P < .05$). In apical region, #25/.06 in LAI had a greater increase than #25/.02 in LAI ($P < .05$).

Discussion

Large taper instruments have been widely used by dentists in recent years, which can prepare the root canal faster and better, and make the root canal filling more convenient. However, some studies have shown that the large taper root canal
preparation instruments to increase the diameter of the upper 1/3 section of the root canal cavity and make the dentin wall thinner (24). Longitudinal fissure (25), which eventually leads to tooth extraction. The taper of the root canal preparation directly affects the flexural strength of the tooth. If the taper of the root canal preparation is greater than 0.08, the pressure resistance of the root canal wall will be reduced. Scanning electron microscope observation showed that the isolated tooth root with a taper of 0.08 had more surface cracks than the isolated tooth root with a taper of 0.02, 0.04 and 0.06 (26). The penetration depth of Enterococcus faecalis in the dentin tubules was about 300 μm. If only the root canal preparation was used to remove the biofilm on the wall of the root canal, a large amount of tooth tissue would be lost, which would increase the risk of root fracture. Therefore, it was very important to improve the efficiency of chemical irrigation in the process of root canal therapy. So, in the process of mechanochemical irrigation, can auxiliary irrigation increase irrigation efficiency, reduce excessive root canal preparation and reduce the risk of root fracture?

PIPS was an auxiliary root canal irrigation method developed in recent decades. The root canal disinfection of PIPS came from the cavitation effect (19), acoustic current effect (27) and thermal effect (28). When the PIPS laser tip is placed in the medullary cavity, the liquid in the root canal produces large and round water vapor bubbles under the action of the laser, and the total volume of the liquid can be expanded to 1600 times the original, which makes it easier for the irrigation fluid to reach the apical region (29). After the cavitation expands, it becomes unstable and bursts. Shear force is generated on the root canal wall, which reshapes the root canal surface and removes microorganisms in the root canal (30). The previous studies showed that PIPS were more effective in removing bacteria from the root canal system than conventional needle irrigation (11, 31). Our previous experimental results also showed that PIPS had greater antibacterial and bacteriostatic effects for E. faecalis compared with CNI in straight root canal system (14). However, the bactericidal effect of PIPS on small taper and small width root canals has not been studied.

When the taper of root canal is the same, size #10/.02 in the LAI group had a significant reduction of ATP value than size #25/.02 in CNI ($P < .05$). The results
showed that PIPS was better than CNI for root canal systems with a smaller taper. Even if the root canal was only dredged with #10/.02 and irrigated with NaOCl + PIPS, the sterilization effect was better than size #25/.02 with NaOCl + CNI. After incubation for 6 hours, size #10/.02 had lower increase of ATP value than #25/.02 with LAI or CNI ($P < .05$). Size #10/.02 in LAI group had a significant reduction in bacteria than #25/.02 in CNI group in the coronal region and middle regions ($P < .05$), which was consistent with ATP experiment. Cheng X et al. (32) found that the disinfection efficacy of the #15/.04 irrigated by NaOCl + Er: YAG group was similar to that of the #40/.04 irrigated by NaOCl group and the SEM results showed that there were still Enterococcus faecalis in 200-300 μm, which indicated that in the root canal model (#15/.04), Er: YAG laser and NaOCl treatment could effectively kill Enterococcus faecalis in root canal, but it could not completely remove Enterococcus faecalis in deep dentin. When the width of root canal is the same, size #25/.02 in the LAI group had a significant reduction of ATP than size #25/.06 in CNI ($P < .05$) and size #25/.02 had lower increase than #25/.06 with LAI or CNI ($P < .05$) after incubation for 6 hours. When we used PIPS as an auxiliary irrigation, even if the root canal is only prepared to #25/.02, the sterilization effect was significantly better than that of root canals prepared to #25/.06 without PIPS, which was also consistent with ATP experiment. Therefore, PIPS also could remove bacteria from root canals with smaller width.

These results indicated that the PIPS allowed a less apical preparation to reach an effective disinfection effect, which may prevent the excess loss of dental tissues, conserve the structural integrity of endodontically treated teeth, and therefore be a promising procedure for minimally invasive endodontics (MIE) (9, 19, 33, 34).

After incubation for 6 hours, a lower amount of bacterial growth in LAI, which may be due to the death of a large number of bacteria after irrigation with PIPS. 2% and 5.25% NaOCl with PIPS had a lower amount of bacterial growth after incubation than other subgroups. Those results of this study were in agreement with the findings of a previous study examining the efficacy of the PIPS method in the destruction of bacterial biofilm (14). We observed more bacteria were produced in the #25/.06 group. The larger the volume of culture medium, the more bacteria grew. This showed that the biofilm...
formed by *E. faecalis* remained in the root canal system even under the mechanical and chemical effects of root canal preparation and irrigation (35). PIPS combined with NaOCl cannot completely remove bacteria in root canal, which was consistent with some research results (13, 36).

The present study has a limitation of design, using an artificial root canal model without the dentinal tubule component of teeth, and may not accurately reflect the magnitude of adhesion of biofilms to the surface; 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**

1. PIPS can improve the ability to remove bacteria in root canals with a small width and a small taper.
2. PIPS with 2% and 5.25% NaOCl can have immediate bactericidal effect and long-term antibacterial effect.

**Abbreviations**

PIPS: photon-initiated photoacoustic streaming; CNI: conventional needle irrigation; or LAI: laser-activated irrigation; ATP: adenosine 5'-triphosphate; BHI: brain heart infusion; MIE: minimally invasive endodontics.

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**Author contributions**

Q.Z.J. contributed to conception and design of the study; C.W. and J.Z. performed most of the experiments; Y.Y.K. and Y.L. carried out data analysis. Y.S. and X.C.Y. participated in drafting of the manuscript and critical revision of the draft. All authors have read and approved the final version of the manuscript.
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Availability of data and materials
The raw data are available from the authors to any author who wishes to collaborate with us.

Ethics approval and consent to participate
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Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Endodontics, Affiliated Stomatology Hospital of Guangzhou Medical University, Guangzhou Key Laboratory of Basic and Applied Research of Oral Regenerative Medical, Guangzhou, Guangdong, 510182. 2Division of Endodontics, Faculty of Dentistry, The University of British Columbia (UBC), 2199 Wesbrook Mall, Vancouver V6T 1Z3, Canada
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Figure Legends

**Fig. 1** Representative microscopic images of each subgroup prepared by #10/.02 and #25/.02 after irrigation with CNI and LAI in the coronal region (I), middle region (II) and apical region (III). The value of average fluorescence density in the coronal (IV), middle (V) and apical (VI) regions. ATP levels were shown in VII. *p<0.05, **p <0.01, ***p <0.001.

**Fig. 2** Representative microscopic images of each subgroup prepared by #25/.02 and #25/.06 after irrigation with CNI and LAI in the coronal region (I), middle region (II) and apical region (III). The value of average fluorescence density in the coronal (IV), middle (V) and apical (VI) regions. ATP levels were shown in VII. *p<0.05, **p <0.01, ***p <0.001.

**Fig. 3** Representative microscopic images of each subgroup prepared by #10/.02 and #25/.02 after incubation for 6 hours with CNI and LAI in the coronal region (I), middle region (II) and apical region (III). The value of average fluorescence density in the coronal (IV), middle (V) and apical (VI) regions. ATP levels were shown in VII. *p<0.05, **p <0.01, ***p <0.001.

**Fig. 4** Representative microscopic images of each subgroup prepared by #25/.02 and #25/.06 after incubation for 6 hours with CNI and LAI in the coronal region (I), middle region (II) and apical region (III). The value of average fluorescence density in the coronal (IV), middle (V) and apical (VI) regions. ATP levels were shown in VII. *p<0.05, **p <0.01, ***p <0.001.