Photomedicine and Laser Surgery
Volume 31, Number 8, 2013

Pp. 371–377
DOI: 10.1089/pho.2013.3515

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

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Abstract

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

Introduction

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

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

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

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

Materials and Methods

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

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

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

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

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

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

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

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

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

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

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

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

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

Results

Antibacterial effect

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

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

Smear layer removal

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

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

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

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

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

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

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

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

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

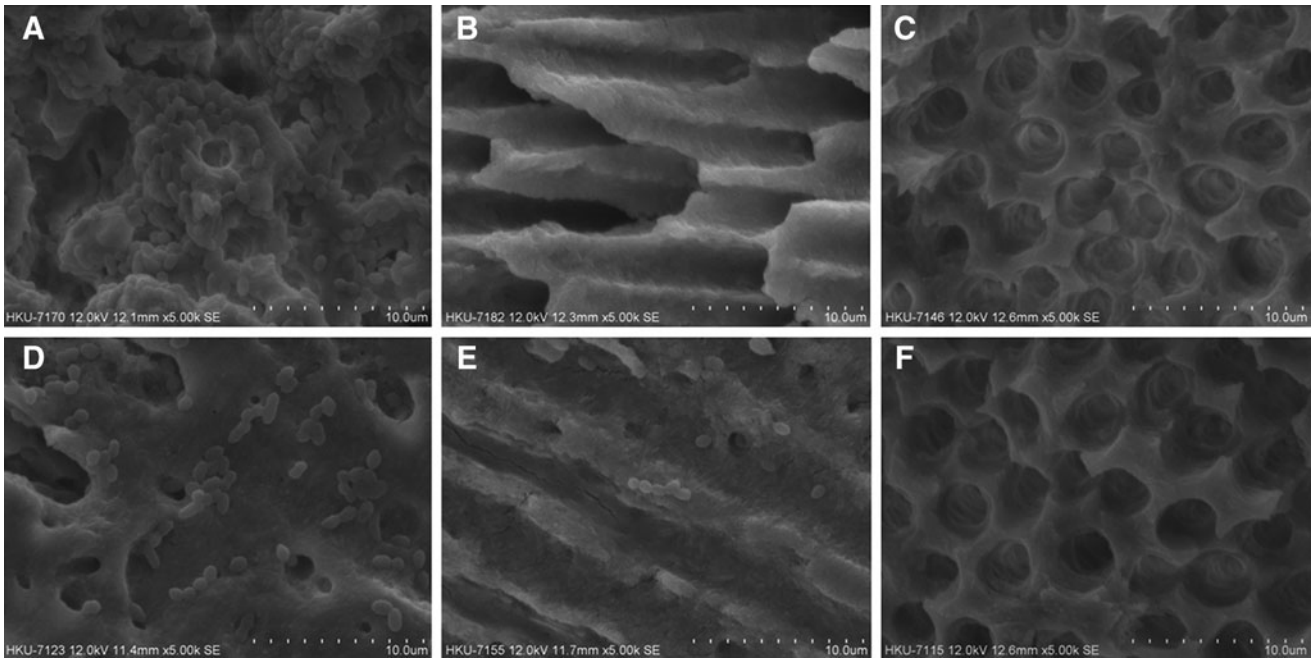

FIG. 1. Scanning electron microscopic (SEM) observation on *Enterococcus faecalis* infected root canals after treatment. **(A)** From the sterile saline (NS) group, a significant number of bacteria surround and reside in the dentinal tubules. **(B)** From the sodium hypochlorite (NaOCl) group and **(C)**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

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

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

Discussion

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

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

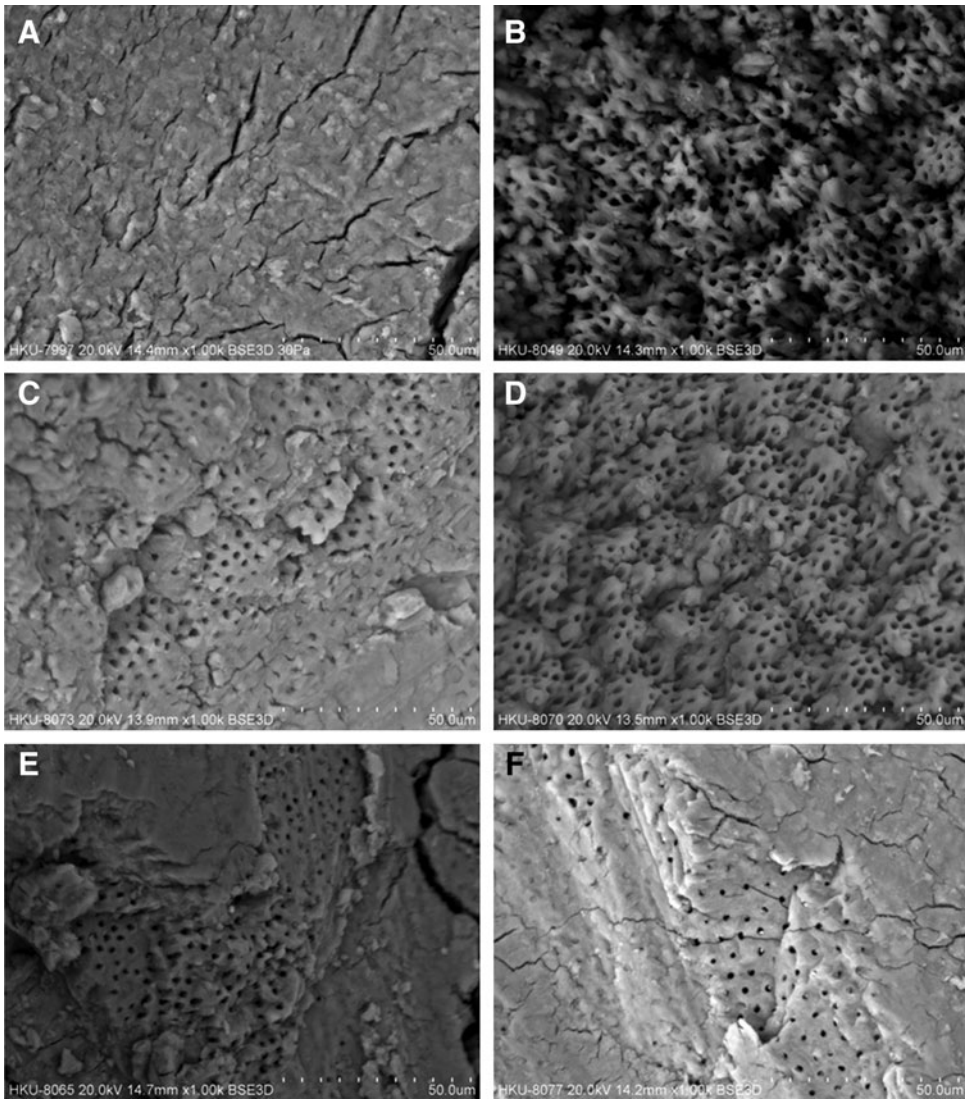

FIG. 2. Scanning electron microscopic (SEM) observation of smear layer in each group. **(A)** From the sterile saline (NS) group, a homogeneous smear layer covers the entire root canal, with no open dentinal tubules. **(B)** From the sodium hypochlorite (NaOCl) group, smear layer in the coronal third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

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

Table 2. Quantitative Evaluation of Smear Layer Removal

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

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

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

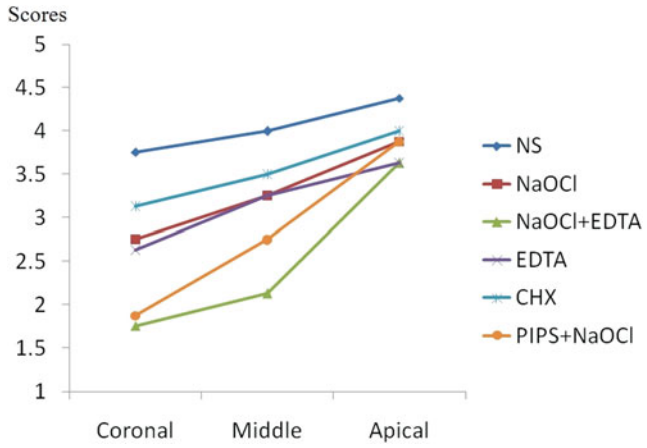

FIG. 3. A quantitative evaluation of smear layer at different positions within the root canals. All debridement properties were less effective in removing smear layer in the apical third.

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

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

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

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

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

Conclusions

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

Acknowledgments

This study was supported by the National Natural Science Foundation of China (NSFC) Grant 81000428.

Author Disclosure Statement

No competing financial interests exist.

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