ORIGINAL ARTICLE

In vitro comparative efficacy of two pulse widths of SWEEPS for elimination of *Enterococcus faecalis* biofilm from the root canals

ABSTRACT

Aim: *This study aimed to compare the efficacy of two pulse widths of the shock wave enhanced emission photoacoustic streaming (SWEEPS) for elimination of Enterococcus faecalis (E. faecalis) biofilm from the root canals.*
Materials and Methods: *This in vitro experimental study was conducted on single-rooted single-canal extracted teeth. After cleaning and shaping and sterilization of root canals, they were inoculated with E. faecalis and were randomly assigned to 4 experimental groups (n=8) of (I) SWEEPS with ultra-short pulse (USP) mode and sodium hypochlorite (NaOCl) irrigation, (II) SWEEPS with super-short pulse (SSP) mode and NaOCl irrigation, (III) SWEEPS with USP mode and saline irrigation, and (IV) SWEEPS with SSP mode and saline irrigation, and 3 control groups (n=2) of bacterial inoculation with no disinfection (positive control), bacterial inoculation and disinfection without laser irradiation (negative control), and sterilization alone with no bacterial inoculation. Dentin chips were collected from the root canal walls, and E. faecalis colonies were counted after culture, and statistically analyzed by the Kruskal-Wallis and Dunnett tests (alpha=0.05).*
Results: *All experimental groups showed significantly lower colony count than the positive control group (P<0.05). Among the experimental groups, the highest reduction in colony count occurred in the USP-NaOCl, followed by the USP-saline, with no significant difference with the negative control group. The smallest reduction in colony count occurred in the SSP-saline group.*
Conclusion: *SWEEPS with the USP mode combined with NaOCl irrigation was effective for elimination of E. faecalis biofilm from the extracted root canals.*

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Introduction

Maximum elimination of bacteria from the root canal system is a major prerequisite for a successful root canal treatment. It has been well confirmed that microorganisms remaining in the root canal system are the main cause of endodontic treatment failure and endodontic infections (1, 2). Elimination of bacteria in organized biofilms adhering to the canal walls or those lodged in isthmi, accessory canals, and dentinal tubules is challenging (3). Different bacterial species such as *Enterococcus faecalis (E. faecalis)* are capable of resisting conventional syringe irrigation (CSI) and remain attached to dentin and penetrate deep into dentinal tubules. *E. faecalis* can penetrate into dentinal tubules by 800 µm due to its small size and form intra-radicular and extra-radicular biofilm. It is also resistant to pH alterations (4). In root canals with inflamed or necrotic pulp, bacterial biofilm is often found as a sessile and dense mass in the extracellular matrix, further enhancing bacterial resistance to disinfectants and mechanical debridement, compared with the planktonic form of bacteria. Evidence shows that mechanical root canal instrumentation disinfects only 50% to 75% of the canals at the end of the first treatment session. Although chemical disinfection of the root canals further improves the quality of disinfection, chemical agents still have difficulties in accessing the apical ramifications, isthmi, and accessory canals (5). It has been demonstrated that the penetration depth of sodium hypochlorite (NaOCl), which is commonly used for root canal irrigation, into dentinal tubules is 60 to 150 µm while *E. faecalis* can colonize the dentinal tubules by 600 to 1000 µm depth; other commonly used irrigants cannot penetrate deeper than 100 µm into dentinal tubules (6). The efficiency of root canal disinfection depends not only on the type of irrigant but also on its method of delivery into the canal and its activation (7). The commonly used root canal irrigation techniques include the CSI, passive ultrasonic irrigation, and laser-activated irrigation (LAI). In the CSI, the irrigant does not penetrate into the canals by more than 2 mm; therefore, the irrigant does not often reach the apical region and cannot penetrate into the dentinal tubules (8, 9). In the recent years, the efficacy of laser for activation of irrigants and its role in improving the quality of debridement of the canal walls have been interesting research topics (10). Currently, two methods are available for LAI of the root canals. In the first method, the fiber tip is inserted into the canal, is activated inside the canal and in the irrigating solution, and is subsequently removed slowly; alternatively, it may remain still in the canal or moved within a short distance inside the canal. This technique is known as LAI, which was first introduced with erbium, chromium:YSGG laser, and is commercially available under the brand name of Waterlase. In the second method, the fiber is used outside the canal, and is activated in the irrigant in the pulp chamber above the orifice. This technique was first introduced as photon-induced photoacoustic streaming (PIPS) and used Er:YAG laser. It is commercially available under the brand name of Light Walker Fotona (9, 11). Later on the PIPS technique was replaced with a newer technique known as the shock wave enhanced emission photoacoustic streaming (SWEEPS). In the PIPS technique, one single laser pulse is irradiated in the solution, and after its absorption by the solution, a vapor bubble forms at the end of the laser tip, which expands and bursts. In the SWEEPS technique, simultaneous and fast bursting of the formed bubbles creates a supersonic turbulent stream of irrigant. Also, the device tips used for PIPS and SWEEPS are different (12, 13). Ozkaya et al. (10) compared the efficacy of PIPS, Nd:YAG laser, and CSI for elimination of *E. faecalis* biofilm and reported significantly higher antibacterial and anti-biofilm effects of Er:YAG LAI on *E. faecalis*. Some others only assessed the efficacy of LAI or PIPS and made no comparison with other methods (14). To the best of the authors' knowledge, no previous study has compared two

pulse widths of SWEEPS for elimination of *E. faecalis* biofilm. Thus, this study aimed to compare the efficacy of two pulse widths of SWEEPS for elimination of *E. faecalis* biofilm from the extracted root canals under in vitro conditions.

Materials and Methods

This in vitro, experimental study was conducted on single-rooted single-canal maxillary incisors and canine teeth, and mandibular incisors, canines, and premolar teeth that had been extracted due to poor periodontal prognosis or as part of orthodontic treatment. The study protocol was approved by the ethics committee of the university (IR.IAU.DENTAL.REC.1399.308).

Eligibility criteria
The inclusion criteria were extracted single-rooted single-canal teeth with sound, caries-free straight roots with no history of endodontic treatment, and no apical resorption. Teeth with calcifications, coronal caries, fracture, or curved roots were excluded.

Sample size
The sample size was calculated to be 8 in each of the 4 experimental groups, and 2 in each of the 3 control groups according to a study by Korkut et al, (5) assuming α=0.05, =0.2, mean standard deviation of the log colony count to be 0.73, and effect size of 0.65 using one-way ANOVA power analysis of PASS 11. The teeth were selected by convenience sampling.

Specimen preparation
The roots were cleaned from the periodontal ligament residues and calculus by a curette. The teeth were then decoronated such that 13 mm of the root length remained. Next, a #15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was used to ensure apical patency. The root canals were subsequently prepared to F3 by ProTaper rotary system (Dentsply Maillefer, Ballaigues, Switzerland). After using each file, the root canals were rinsed with 2 mL of 2.5% NaOCl by using a syringe and a 27-gauge needle. The smear layer was removed by using 5.25% NaOCl for 1 minute, followed by a rinse with saline and subsequent irrigation with 17% EDTA for 1 minute. A final rinse with saline was also performed for 1 minute. Glass ionomer was applied on the root surface to seal the apical end and accessory canals. The teeth were then placed in microtubes, and autoclave-sterilized at 121°C for 20 minutes (7).

Bacterial inoculation
Pure *E. faecalis* was obtained from the Pasteur Institute of Iran and cultured on blood agar for 24 hours. Microbial suspension was prepared by mixing the pure culture with 2 mL of saline. Next, the root canals were filled with 10 µL of the microbial suspension by an insulin syringe and incubated at 37 °C and 100% humidity for 28 days. Microbial inoculation of the canals was repeated every 2 days by using fresh microbial suspension during this time period. The roots (n=38) were then randomly assigned to 4 experimental groups (n=8), and 3 control groups (n=2).
In group 1, the SWEEPS technique was performed using Er:YAG laser (Light Walker, Fotona, Ljubljana, Slovenia) with 2940 nm wavelength, super-short pulse (SSP) mode with 50 µs pulse width, 0.3 W power, 15 Hz frequency, and 20 mJ pulse energy by using a laser tip with 600 µm diameter. The laser tip was positioned in the coronal part of the canal orifice. Saline was injected into the canal by a 27-gauge irrigation needle and laser was irradiated for 40 seconds. In group 2, Er:YAG laser was used with the same parameters reported for group 1. However, NaOCl was injected into the canal (instead of saline) by a 27-gauge irrigation needle.In group 3, Er:YAG laser was used with the same parameters reported for group 1, but in ultra-short pulse (USP) mode with 25 µs pulse width.
In group 4, Er:YAG laser was used with the same parameters reported for group 3 but NaOCl was injected into the canal as irrigant by a 27-gauge irrigation needle.
In group 5 (positive control), bacterial inoculation was performed but with no

subsequent disinfection protocol. In group 6 (negative control), bacterial inoculation was performed and the disinfection protocol was carried out by root canal irrigation with NaOCl injected into the canal with a 27-gauge needle. However, no laser irradiation was performed. In group 7, sterilization was performed with no bacterial inoculation.

Microbiological analysis

Each root was transversely divided into three sections of coronal third, middle third, and apical third. A low-speed endodontic hand-piece, a #4 Gates-Glidden drill, and #30 and #35 Hedstrom hand files were used to collect 0.01 g dentin chips from the root canal walls containing biofilm to assess the antimicrobial efficacy of the tested modalities. For colony counting, dentin chips were placed in sterile test tubes containing 2 mL of saline and mixed for 20 seconds. Next, they were diluted 10 times, and 10 µL of each suspension was cultured on brain heart infusion agar and incubated at 37 °C for 24 hours. The number of *E. faecalis* colonies was then counted and reported for each group.

Statistical analysis

Due to non-normal distribution of data as shown by the Kolmogorov-Smirnov test, comparisons were made by the Kruskal-Wallis and Dunnett tests. Level of statistical significance was set at 0.05.

Results

Table 1 presents the measures of central dispersion for the colony count in the study groups. The colony count was the highest in the positive control group, and the lowest in the USP-NaOCl and negative control (NaOCl) groups. The Kruskal-Wallis test showed a significant difference in colony count among the groups (P=0.005). Thus, pairwise comparisons were performed by the Dunnett test (Table 2). The results showed that all experimental groups had significantly lower colony count than the positive control group (P<0.05). The negative control group showed significantly lower colony count than all other groups (P<0.05) except for USP-NaOCl and USP-saline groups (P>0.05). The USP-NaOCl group showed significantly lower colony count than all other groups (P<0.05) except for negative control and USP-saline groups (P>0.05). The SSP-NaOCl group indicated significantly lower colony count than all other groups (P<0.05) except for USP-saline group (P>0.05). The USP-saline group had significantly lower colony count than all other groups (P<0.05) except for the negative control, SSP-NaOCl, and USP-NaOCl groups (P>0.05). Also, SSP-saline group had significantly lower colony count than the positive control group and significantly higher colony count than all other groups (P<0.05).

Discussion

This study compared the efficacy of two pulse widths of SWEEPS for elimination of *E. faecalis* biofilm from the extracted root canals under in vitro conditions. The results showed that the USP mode, especially in combination with NaOCl irrigation, had greater antimicrobial effects on *E. faecalis* than the SSP mode. Also, lack of a significant difference in colony count between the SSP-NaOCl and USP-saline groups indicated that in case of unavailability of NaOCl, the USP mode along with saline irrigation can have the same optimal efficacy for elimination of *E. faecalis* as SSP-NaOCl. However, its efficacy would be definitely lower than that of USP-NaOCl.

Cheng et al. (16) evaluated the antimicrobial efficacy of Er:YAG LAI by using NaOCl against *E. faecalis* and showed that it was capable of complete elimination of *E. faecalis* biofilm from the root canal walls and may be used as a successful protocol in endodontic treatments. Thapak et al. (17) compared the efficacy of Er:YAG laser, sonic irrigation, and CSI for smear layer removal, and reported that Er:YAG laser left the lowest amount of residual smear layer in the apical third of the root canals. Korkut et al. (5) compared the smear layer removal efficacy and antibacterial activity of Er:YAG (PIPS), Nd:YAG, and diode lasers and the CSI and showed that diode and

Table 1

Measures of central dispersion for the colony count in the study groups

Groups	Mean	Standard error	Minimum	Maximum
Control	3.03	0.06	2.95	3.15
SSP/NaOCl	0.40	0.01	0.38	0.44
SSP/Saline	1.25	0.11	1.05	1.45
USP/NaOCl	0	0.00	0.00	0.00
USP/Saline	0.27	0.04	0.22	0.37
NaOCl	0	0.00	0.00	0.00

Table 2

Pairwise comparisons of the groups regarding colony count by the Dunnett test

Groups	Control	SSP/ NaOCl	SSP/ Saline	USP/ NaOCl	USP/ Saline	NaOCl
Control	-	0.001	0.005	0.002	0.000	0.002
SSP/NaOCl		-	0.049	0.009	0.445	0.009
SSP/Saline			-	0.037	0.037	0.037
USP/NaOCl				-	0.119	1
USP/Saline					-	0.119
NaOCl						-

Er:YAG (PIPS) lasers were significantly more effective than the CSI with NaOCl and Nd:YAG laser for smear layer removal and reduction of *E. faecalis* count. Olivi and DiVito (12) evaluated the clinical techniques and protocols of PIPS and observed that photons are emitted with very low energy and microsecond pulse rates. Irradiation of irrigant with a laser pulse warms it up, forming a vapor bubble at the end of the laser tip, which further expands and bursts, forming a second bubble. Resultantly, a turbulent photo-acoustic stream moves the irrigant in the three-dimensional root canal space. They added that the speed of waves in the PIPS technique in areas close to the laser tip is 20 times the rate in ultrasonic irrigation; in farther areas from the laser tip, this rate is 10 times higher than the rate in ultrasonic irrigation. Kihara et al. (18) analyzed the irrigant stream in LAI and assessed the effect of position of laser tip in this regard. They found that placement of laser tip in the pulp chamber resulted in formation of a fast stream in the entire root canal system immediately after development of the vapor bubble. In contrast, placement of the tip in the apical part of the root canal led to formation of a fast stream due to development of secondary cavitation bubbles, which was limited to the apical region. As mentioned earlier, numerous studies have reported significantly improved antimicrobial activity against *E. faecalis* in Er:YAG LAI, and the SWEEPS technique has shown superior efficacy in elimination of *E. faecalis* compared with the conventional techniques (2, 5, 10, 12, 13,16, 19-21). Comparison of the efficacy of two different pulse widths of SWEEPS was the main strength of the present study, which has not been evaluated in any previous study. Thus, there was no similar study to compare our results with.

In vitro design, which limits the generalizability of the findings, evaluation of only single-rooted and single-canal teeth, and assessment of only one type of microorganism (*E. faecalis*) were among the

limitations of this study. Future studies are required on other tooth types with anatomical complexities, and other microorganisms Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

Conclusions

Within the limitations of this in vitro study, the results showed stronger antibacterial activity of the USP than the SSP mode against *E. faecalis* biofilm in extracted root canals. It appears that application of SWEEPS with the USP mode combined with NaOCl irrigation would be effective for elimination of *E. faecalis* biofilm from the root canal system. Moreover, in cases where NaOCl cannot be used (as in open apex teeth) and there is a risk of extrusion into the periapical tissue and hypochlorite accident, USP-saline can serve as an effective alternative for elimination of *E. faecalis.*

Clinical Relevance

This study aims to find the best parameters of SWEEPS technique for disinfection of root canals by eliminating E. faecalis.

Conflicts of Interest

The authors declare no conflict of interest.

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