

Shock wave-enhanced emission photoacoustic streaming versus photon-induced photoacoustic streaming modes for clearing root canal bacteria using erbium-doped yttrium aluminum garnet lasers: An *in vitro* study

Xiao-Na Wang

Shanxi Provincial People's Hospital

Jing Shi (✉ crystalshi@163.com)

Shanxi Provincial People's Hospital

Research article

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Abstract

Background: The root canal cleaning efficacy of the photon-induced photoacoustic streaming (PIPS) mode in erbium-doped yttrium aluminum garnet (Er:YAG) lasers is controversial. Shock wave-enhanced emission photoacoustic streaming (SWEEPS), a novel mode in Er:YAG lasers, creates a strong shock wave in a narrow container. We accordingly hypothesized that it may effectively eliminate bacteria from narrow root canals. The present study aimed to compare the bacterial clearance efficacy between the SWEEPS and PIPS modes for Er:YAG lasers designed for root canal treatment.

Methods: The distal and palatal roots of 130 freshly extracted human molars were used. The smear layer was first removed by conventional root canal preparation. All samples were then sterilized in an autoclave. The samples were subsequently exposed to *Enterococcus faecalis* and incubated for 4 weeks to establish *E. faecalis* infection models. The models were divided into three groups according to the irrigation method (n = 40 per group): 3% sodium hypochlorite (NaOCl) activated using the SWEEPS Er:YAG laser mode, 3% NaOCl activated using the PIPS Er:YAG laser mode, and 3% NaOCl without activation.

Results: The bacterial clearance efficacy, based on the colony-forming unit count, was significantly higher in the SWEEPS group than in the PIPS and NaOCl groups. Moreover, scanning electron microscopy after irrigation revealed that the SWEEPS group had the least number of bacteria.

Conclusions: The SWEEPS mode is more effective than the PIPS mode for eradicating *E. faecalis* from root canals.

Background

Endodontic infection and periapical periodontitis are the most important pathologies involving the dental pulp, with the main cause being bacterial invasion of the root canal system. Therefore, their treatment is based on the elimination of harmful bacteria from the canals [1]. Root canal treatment (RCT) is the most common and effective treatment for these conditions. However, studies [2, 3] have demonstrated a failure rate of 4%–15%, even in cases involving uncomplicated treatment. In other words, periapical inflammation may persist or recur after treatment; both these events represent treatment failure. The complex anatomy of the root canal system is the most important reason for failure. Canal enlargement with modern instruments such as stainless steel and nickel–titanium files does not eliminate all microorganisms, particularly those in collateral and apically divergent canals. Therefore, improved cleaning efficacy is essential for a higher RCT success rate [4].

With the development of root canal irrigation technologies, different modalities have become available such as conventional needle irrigation (CI), manual–dynamic irrigation (MDI), passive ultrasonic irrigation (PUI), and laser-activated irrigation (LAI) [5-7]. Andrabi et al. [8] found that MDI allowed the irrigant solution to easily reach the apical third of the root through effective hydrodynamic activation. This method can also neutralize the vapor lock effect, thus improving the cleaning efficacy. Sluis et al. [9]

found that PUI was better than CI with regard to dentin fragment and planktonic bacteria elimination. The ultrasonic vibration-induced shear force eliminates dentin debris, residual bacteria, pulp tissue, and other infectious substances from the canals [10]. Moreover, the solution effectively enters the main canal and collateral canals, which improves the cleaning performance [11, 12]. However, complete bacterial elimination from the root canal system is not achieved with any of these techniques [13].

In 2009, Blanken et al. [14] introduced the laser-activated irrigation (LAI) technology. The radiation emitted by the laser is strongly absorbed by the aqueous solution, and LAI is achieved by cavitation. Vapor bubbles are formed at the tip of the fiber, expand, and then collapse [15,16]. This continuous collapse of bubbles produces local shock waves and causes significant fluid movement. Then, the subsequent laser pulse creates secondary cavitation bubbles, which lead to acoustic streaming of the solution throughout the entire root canal system [17,18]. Photon-induced photoacoustic streaming (PIPS) by a single pulse Er:YAG laser is one of the applications of LAI [19]. It uses low pulse energy (10 or 20 mJ) with a short pulse length (50 μ s), resulting in high peak powers and efficient cavitation [20]. Several studies [14-26] have demonstrated its effectiveness. The latest development in LAI for root canal irrigation is shock wave enhanced emission photoacoustic streaming (SWEEPS) by a double pulse Er:YAG laser. This double pulse of laser light is the most significant difference between SWEEPS and PIPS. The SWEEPS mode uses double pulses to create a series of bubbles, which are timed to appear such that secondary bubbles lead to a collapse of existing bubbles. It results in more vigorous shock waves and enhanced photoacoustic streaming compared to that in PIPS mode [27,28]. The shock wave and enhanced photoacoustic streaming can pump out the infectious material in the more hidden parts of the canal, achieving a better irrigation efficacy compared to that with PIPS [28-31]. However, till now data from independent research on SWEEPS activation has not been published.

Thus, in the present study we assumed that SWEEPS activation is more effective than PIPS and compared the reduction rate of bacteria in root canal systems before and after irrigation.

Methods

Sample collection and preparation

The ethics committee of Shanxi Provincial People's Hospital approved the study protocol [Provincial Medical Opinions (2019) No. 3]. Palatal roots of the maxillary first molars and distal roots of the mandibular first molars were used for this *in vitro* study for the following reasons. First, the required sample capacity of this experiment was large, so palatal and distal roots were collected to ensure a sufficient sample size. Second, the diameter of the palatal and distal roots is relatively large, which is convenient for the preparation of the *Enterococcus faecalis* biofilm model. The selected teeth were untreated and had completely formed apices and root canals. All samples were scanned using KaVo 3D eXam cone-beam CT (KaVo, Germany) to observe the morphology of the root canal system from different directions. Type I (1-1) palatal and distal root canal systems were selected, excluding curved and other root canal system types. Any calculi and periapical soft tissues were first removed by using an ultrasonic

scaler. All samples were then placed in a saline-filled test tube and stored at 4 °C until use. For the study, the teeth were decoronated using a diamond bur. The standard length of the remaining root was 12 mm. The working length (WL) was set as the standard length minus 1 mm (i.e., 11 mm). K-files (#10 and #15; Dentsply Maillefer, Ballaigues, Switzerland) were used to create a glide path for the WL, and ProTaperNext rotary files (Dentsply Sirona, York, PA, USA) were used to shape the canals up to size X3 (0.3 mm, 7%) according to the manufacturer's instructions. The shaping of all root canals in this study was performed by the same operator. First the 10 # and 15 # K-files were used in order to establish the root canal access, and then X1, X2, and X3 Ni-Ti files were used to shape the root canal in turn; all shaping methods and parameters were selected in accordance with the manufacturer's instructions. Using a Gates Glidden drill #5, a groove 4 mm in length, 1 mm deep, and 0.4 mm wide was made in the wall of each root canal. This groove served as a coronal reservoir for irrigated placement. After the use of each instrument, the canals were irrigated with 2.5 mL 3% sodium hypochlorite (NaOCl) solution (Sigma-Aldrich Corporation, St. Louis, MO, USA), which was delivered via a syringe with a 27-G side-vented needle. After completing the preparation, the canals were sequentially irrigated with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA; Ultradent Products, Inc., South Jordan, UT, USA) and 5 mL of 3% NaOCl for 5 minutes each; this ensured removal of the smear layer. The canals were then flushed for 15 minutes with 20 mL of 0.9% physiological saline for the removal of any residual EDTA or NaOCl solution. The samples were dried at room temperature. To prevent bacterial leakage, we sealed the apical third of all roots with a composite resin and coated the entire root surface with nail polish. Finally, five teeth were randomly selected and subjected to scanning electron microscopy (SEM) (EVO MA10; ZEISS, Oberkochen, Germany) for confirmation of smear layer removal.

The following scoring criteria were used for smear layer removal: 1, no smear layer, 100% dentinal tubules open; 2, small amount of scattered smear layer, 80% dentinal tubules open; 3, thin smear layer, 60% dentinal tubules open; 4, a portion of the root canal wall covered with a thick smear layer; and 5, root canal wall completely covered by a smear layer. Two experts in the field of dental pulp disease evaluated the SEM images using a double-blind method. A score of 3 or lower met the smear removal standard. SEM images were used for two reasons—first, the smear layer was composed of assorted debris and infectious substances; however, in this study the single bacterial infection model (i.e. the *E. faecalis* infection model) had to be established. Thus, it was important to remove as much of the smear layer as possible to minimize the possibility of the presence of bacteria other than *E. faecalis* in the root canal system. Secondly, the presence of the smear layer in would have hindered the colonization of *E. faecalis* in the dentin tubules and led to failure of establishing the *E. faecalis* infection model.

The samples were subsequently placed in glass test tubes filled with 0.9% physiological saline sterilized at 121 °C in a 1.5-Mpa autoclave (LS-150LD; Binjiang Medical Equipment Ltd., Jiangyin, China) for 30 minutes. An inoculating loop was used to collect a loopful of the liquid near the root canal in the test tube. This liquid was inoculated on a sterile plate with blood agar medium and placed in a Tri-Gas incubator (HF-100; Heal Force Bio-meditech Holdings, Ltd., Shanghai, China) for 24 hours. The effects of sterilization were determined by observing the colony growth on the plate.

Establishment of the *E. faecalis* infection models

A standard *E. faecalis* strain (ATCC 29212), which was procured from stocks in the Microbiology Laboratory of Shanxi Provincial People's Hospital, was activated and formulated into a bacterial suspension, the concentration of which was adjusted to 1.0 MCF on an electronic turbidimeter (BioMerieux, Mercy l'Etoile, France). Five tooth samples were placed in a glass tube containing 1 mL of *Enterococcus* broth (HB0133-2; Haibo Biotechnology Co., Ltd, Qingdao, China) and 1 mL of the *E. faecalis* suspension (in total, 25 tubes), which were incubated for 4 weeks at 37 °C in a Tri-Gas incubator (HF-100; Heal Force). Every 48 hours, the liquid in the tube was changed. At the time of culture solution replacement, 1 mL of liquid near the root canal was collected and incubated for 24 hours on a plate containing blood agar medium. The presence of other bacteria was ruled out by analyzing the formed colonies using a fully automated rapid mass spectrometry detection system (Microflex LT/SH; Bruker Daltonik, Bremen, Germany). After 4 weeks, *in vitro E. faecalis* infection models were successfully established. To confirm *E. faecalis* colonization, five samples were randomly selected and observed by using SEM.

Bacterial sampling and counting before irrigation

In a biosafety cabinet (HFSafe 1200; Heal Force Bio-meditech Holdings, Ltd.), the culture solution in the canals was carefully blotted by using sterile paper tips. The canals were then rinsed with 1 mL of 0.9% sterile saline to flush out unattached bacteria. Thereafter, three sterile paper tips saturated with 0.9% saline were successively inserted up to the WL and repeatedly rubbed against the inner canal walls. After 1 minute, the paper tips were placed in 1 mL of 0.9% sterile physiological saline and shaken on a vortex mixer (XW-80A; Jingke Industrial Co., Ltd., Shanghai, China) for 5 minutes for deployment of the bacterial suspension. This suspension was serially diluted with physiological saline (up to 10^{-6}), with a volume ratio of 1:10. To count the bacteria, 0.1-mL aliquots containing appropriate dilutions of each sample were spread onto blood agar plates and incubated for 24 hours at 37 °C in the Tri-Gas incubator (HF-100; Heal Force Bio-meditech Holdings, Ltd.). The colony-forming unit (CFU) number in the entire plate was then counted and recorded.

Irrigation protocols

We randomly divided the 120 roots into two experimental groups and one control group (n = 40 each) according to the irrigation protocol: 3% NaOCl activation with an Er:YAG laser (LightWalkers ATS; Fotona, Ljubljana, Slovenia) using the SWEEPS mode for 60 seconds (SWEEPS group); 3% NaOCl activation with an Er:YAG laser (LightWalkers ATS) using the PIPS mode for 60 seconds (PIPS group), and 3% NaOCl irrigation without activation for 60 seconds (control group).

The parameters for the SWEEPS and PIPS modes are presented in Table 1. In the SWEEPS group, the canals were first subjected to 3% NaOCl (2 mL each) activation using the SWEEPS mode for 20 seconds, followed by a rest interval of 20 seconds, 0.9% saline (2 ml each) activation using the SWEEPS mode for 20 seconds, and then another rest interval of 20 seconds. This procedure was repeated three times. The

same regimen was followed in the PIPS group, where NaOCl was activated using the PIPS mode. In the control group, the canals were subjected to three 20-second cycles of 3% NaOCl (2 mL each) irrigation via a syringe with a 27-G side-vented needle without any activation, 0.9% sterile physiological saline irrigation (2 mL each) for 20 seconds, and a rest period of 20 seconds.

The No. 99128 fiber tip was used in the SWEEPS mode. This fiber tip has a diameter of 0.60 mm, a length of 12 mm, and a flat end. The No. 89036 fiber tip was used in the PIPS mode. This fiber tip has a diameter of 0.60 mm and a length of 9 mm. Unlike that of the fiber tip used in SWEEPS mode, the end of the fiber tip in PIPS mode is tapered. During activation, the fiber tip was placed into the coronal reservoir which was 10 mm from the WL. The SWEEPS and PIPS fiber tips are shown in Figure 1a and b.

Bacterial sampling and counting after irrigation

The method used for bacterial sampling and counting after irrigation was the same as that used before irrigation. On the basis of the obtained values, we calculated the bacterial reduction rate by using the following formula: bacterial reduction rate (%) = $(E - F)/E \times 100$. In this paper, *E* and *F* represent the number of bacterial colonies before and after irrigation, respectively. Based on the bacterial reduction rate, the bacterial clearance efficacy of each method was evaluated. For more intuitive demonstration of the bacterial clearance efficacy, the number of remaining bacteria in the irrigated samples from the three groups was observed by SEM.

Statistical analysis

All data were statistically analyzed using SPSS, version 22.0 (IBM, Chicago, IL, USA). We used the Kruskal–Wallis test to detect statistically significant differences between groups. Intergroup comparisons were conducted using nonparametric one-way analysis of variance. The bacterial reduction rate are presented as medians and interquartile ranges. A *p*-value of ≤ 0.05 was considered statistically significant.

Results

Scanning electron microscopy images before irrigation

Figure 2 shows an SEM image of a middle canal after smear layer removal. Figure 3 shows SEM images of a successfully established *E. faecalis* infection model.

Scanning electron microscopy images after irrigation

The SEM images for the SWEEPS group showed nearly complete *E. faecalis* elimination from the canals (Fig. 4a), whereas the SEM images for the PIPS group showed some residual bacteria (Fig. 4b). In the control group, a large number of residual bacteria were visible (Fig. 4c). Overall, the SEM images indicated a qualitative decrease in the bacterial counts in the SWEEPS and PIPS groups relative to the counts in the control group (Fig. 4).

The bacterial reduction rate calculated from the CFU counts before and after irrigation are shown in Table 2. All irrigation regimens significantly reduced bacterial growth ($p < 0.001$). The bacterial reduction rate of 88.6%, 58.5%, and 34.4% in the SWEEPS, PIPS, and control groups, respectively, were significantly different ($p < 0.05$).

Discussion

In the present study we confirmed our hypothesis that SWEEPS activation is more effective than PIPS. Our comparison of the bacterial clearance efficacy between the SWEEPS and PIPS modes for root canal irrigation using Er:YAG lasers revealed that the SWEEPS mode resulted in near-complete elimination of *E. faecalis* from root canals via enhanced pressure waves travelling at shock speeds. Previous studies [32, 33] have demonstrated high detection rates for *E. faecalis* in root canals with secondary infection. Owing to its special properties, *E. faecalis* forms a single bacterial biofilm on root canal surfaces. Therefore, an *E. faecalis* infection model is often used as a standard model for evaluation of the cleaning ability of irrigation protocols

In the SWEEPS mode, a pair of individual laser pulses is emitted to accelerate the effects of fluid cavitation dynamics. [34] An initial laser pulse is emitted at time T_0 , and the initial vapor bubble and smaller secondary vapor bubbles are simultaneously formed at the fiber tip. These bubbles gradually expand until they reach the maximum volume at time T_{max} . The bubble volume then gradually decreases. During their collapse, subsequent laser pulses are delivered to the solution at time T_{opt} . At that point, several secondary bubbles are triggered. The pressure waves created by these bubbles induce the violent collapse of the initial and secondary bubbles, and thereby accelerate fluid motion. Powerful shock waves are eventually generated throughout the root canal, which significantly improves clearance efficacy [20, 27]. In the present study, this efficacy was proven by the bacterial reduction rates and SEM images, which showed that the SWEEPS mode caused near-complete elimination of *E. faecalis* from the observed canals.

Notably, shock waves cannot be increased in narrow spaces such as root canals simply by increasing the energy of a single Er:YAG laser pulse [20]. An increase in the energy of a single pulse will enlarge cavitation bubbles and slow bubble oscillation dynamics at higher laser energies. The optimal shaping of the laser pulse emission temporally by using the SWEEPS technique enables amplification of the photoacoustic streaming effects [35,36].

We believe that the SWEEPS mode will improve the root canal cleaning efficacy and aid in direct smear layer removal. One limitation of this study was that we did not study the ability of the SWEEPS mode to remove the smear layer because of time constraints and other factors. Future studies are necessary to clarify this aspect.

Conclusion

When using Er:YAG laser irrigation, the SWEEPS mode may be superior to the PIPS mode in terms of bacterial clearance from infected root canals. The outcomes of root canal treatment can be negatively affected if residual bacteria grow and cause problems such as inflammation. In this scenario, the patient would require repeat treatment, which can be prevented by more thorough cleansing of the infected canal(s). Our findings regarding the efficacy of the SWEEPS mode for Er:YAG laser irrigation could potentially improve the outcomes of root canal treatment and prevent the need for re-treatment. In addition, they could form a basis for the development of new strategies for root canal irrigation in clinical practice. We hope to monitor the patients treated with the SWEEPS mode in order to evaluate its long-term effectiveness.

Declarations

Ethical approval and consent to participate: The ethics committee of Shanxi Provincial People's Hospital approved the study protocol [Provincial Medical Opinions (2019) No. 3]. The need for informed consent was not applicable for this type of study.

Consent for publication: Not applicable.

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

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Authors' contributions: XNW: experimental operation, data analysis, statistical analysis, article writing and modification; JS: project design and guidance, article writing and modification. All authors read and approved the final manuscript.

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Abbreviations

CFU, colony-forming unit

CI, conventional needle irrigation

EDTA, ethylenediaminetetraacetic acid

Er:YAG, erbium-doped yttrium aluminum garnet

LAI, laser-activated irrigation

MDI, manual–dynamic irrigation

NaOCl, sodium hypochlorite

PIPS, photon-induced photoacoustic streaming

PUI, passive ultrasonic irrigation

RCT, root canal treatment

SEM, scanning electron microscopy

SWEEPS, shock wave-enhanced emission photoacoustic streaming

WL, working length.

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Tables

Table 1. Parameters for Er:YAG laser irrigation of root canals using the shock wave-enhanced emission photoacoustic streaming mode and the photon-induced photoacoustic streaming mode

Mode	Pulse (mJ)	Frequency (Hz)	Power (W)	λ (μm)	Water and air
SWEEPS	20	15	0.3	2.94	Turn off
PIPS	20	15	0.3	2.94	Turn off

Er:YAG: erbium-doped yttrium aluminum garnet, *PIPS*: photon-induced photoacoustic streaming, *SWEEPS*: shock wave-enhanced emission photoacoustic streaming

Table 2. Statistical analysis of colony-forming units for the evaluation of bacterial clearance in different root canal irrigation groups

	No. (median)	Q (P ₂₅ -P ₇₅)	Range		c ²	p
			Min	Max		
SWEEPS	40	100.00% **	97.94%-100%	42.86%-100%		
PIPS	40	85.56% *	64.59%-99.84%	0.00%-100%	50.14	<0.001
Control	40	64.34%	0.00%-79.43%	0.00%-100%		

Er:YAG: erbium-doped yttrium aluminum garnet, *PIPS*: photon-induced photoacoustic streaming, *SWEEPS*: shock wave-enhanced emission photoacoustic streaming

For SWEEPS, 3% sodium hypochlorite (NaOCl) was activated with the Er:YAG laser in the shock wave-enhanced emission photoacoustic streaming mode. For PIPS, 3% NaOCl was activated with the Er:YAG laser in the photon-induced photoacoustic streaming mode. For Control, 3% NaOCl was not activated.

* Indicates a significant difference from the value for group control ($p < 0.05$).

** Indicates a significant difference from the value for group PIPS ($p < 0.05$).

Figures

a**b**

Figure 1

SWEEPS and PIPS fiber tips. a The fiber tip of SWEEPS has a diameter of 0.60 mm, a length of 12 mm, and a flat end. b The fiber tip of PIPS has a diameter of 0.60 mm, a length of 9 mm, and a tapered end.

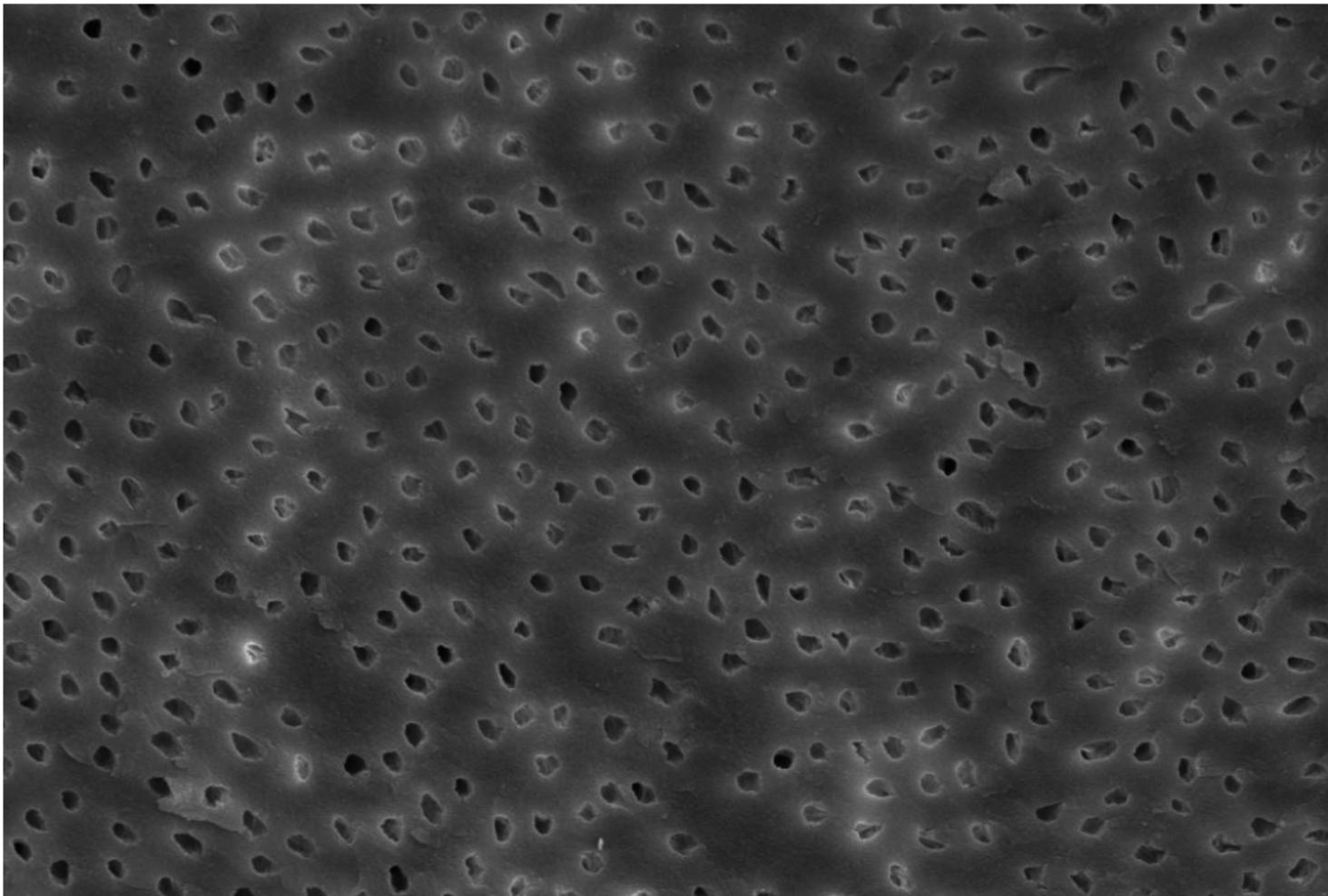


Figure 2

A scanning electron microscopy image shows elimination of the smear layer in the middle canal ($\times 1000$ magnification).

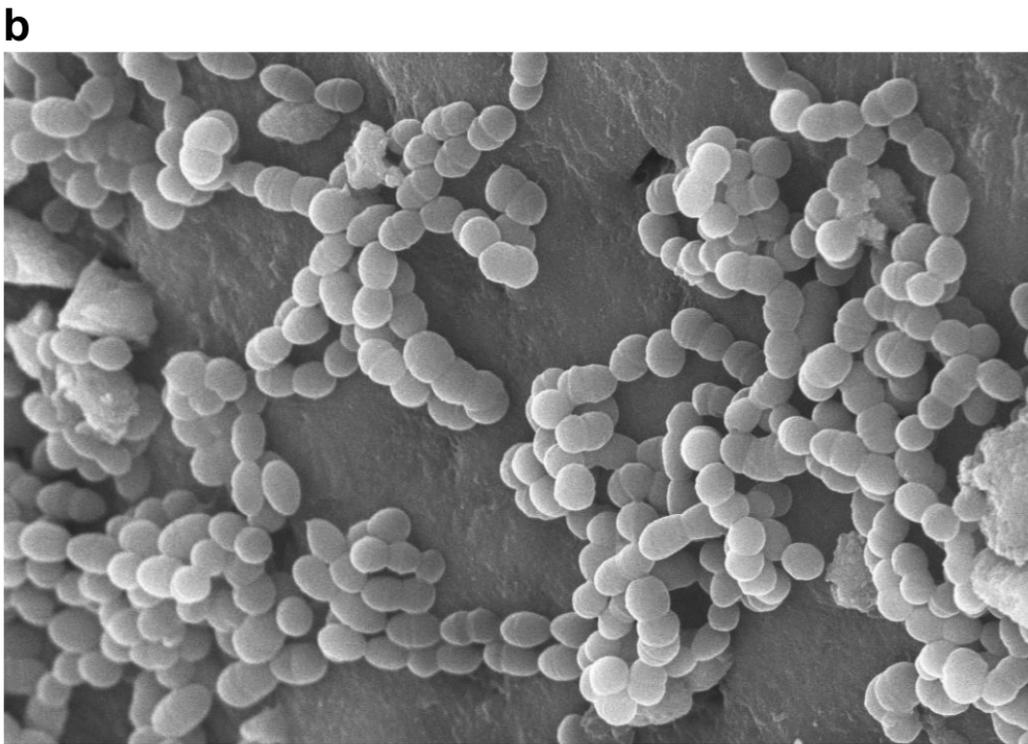
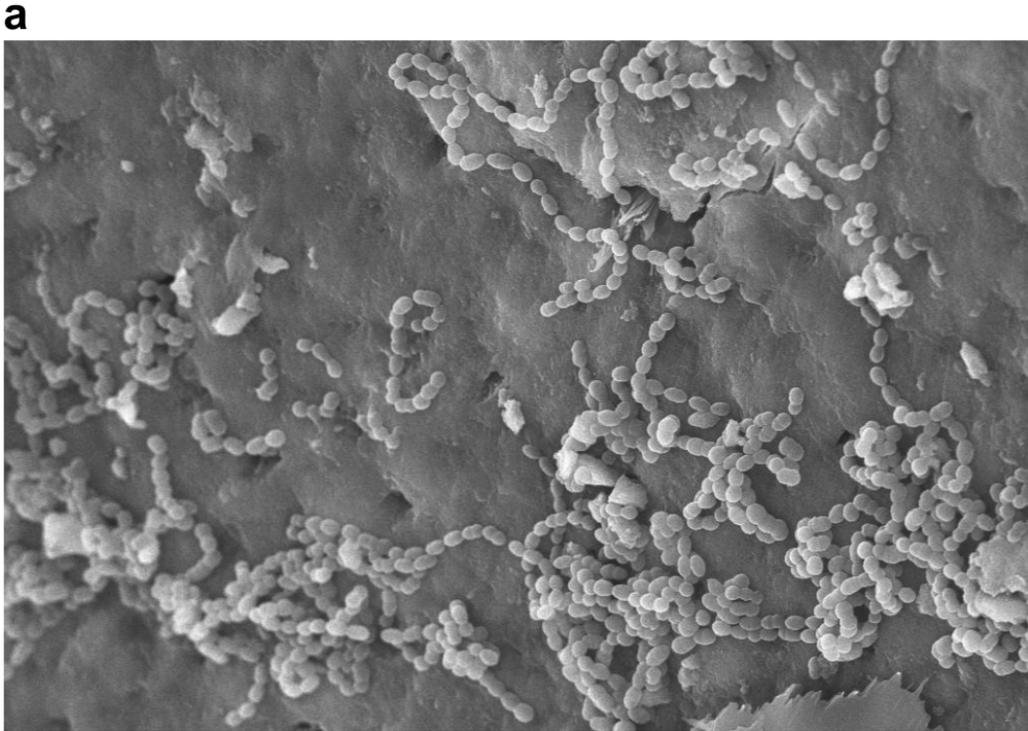


Figure 3

Scanning electron microscopy images show a successfully established *Enterococcus faecalis* biofilm at a $\times 2000$ magnification and b $\times 5000$ magnification.

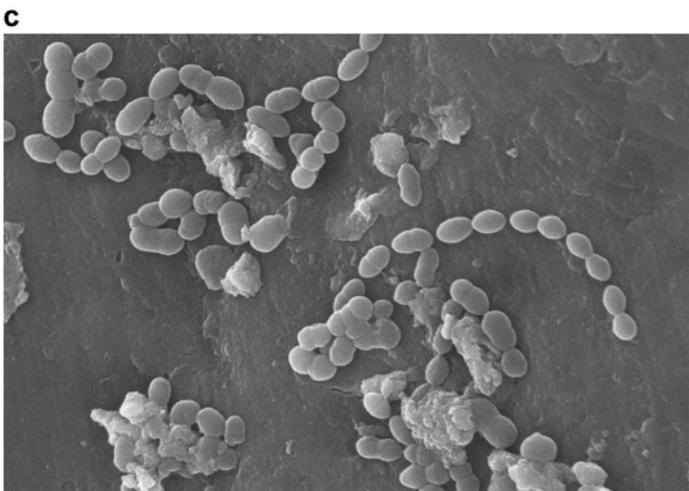
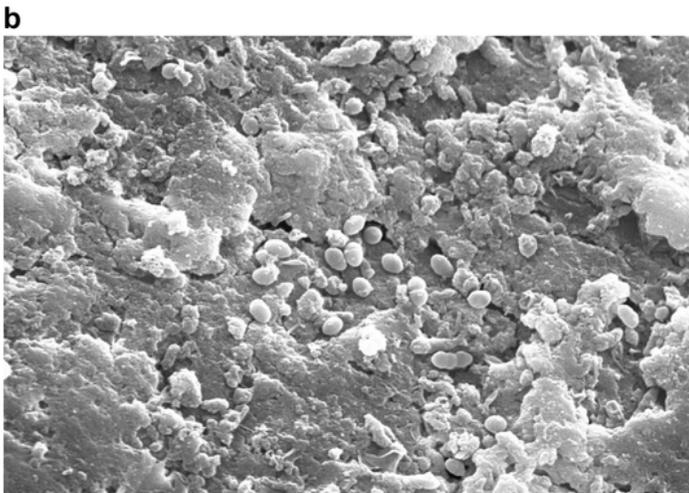
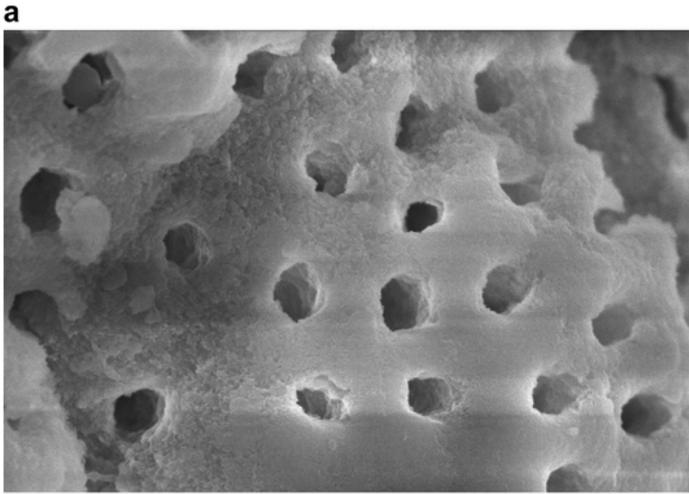


Figure 4

Scanning electron microscopy images of the middle root canals show the bacterial clearance effects with three modes. a Er:YAG laser activation using the SWEEPS mode ($\times 5000$ magnification). The *Enterococcus faecalis* biofilm is almost completely removed. b Er:YAG laser activation using the PIPS mode ($\times 5000$ magnification). Some residual bacteria are present in the root canal. c No laser activation ($\times 5000$ magnification). A large number of bacteria can be seen in the root canal.