Comparative evaluation of antimicrobial efficacy on *Enterococcus faecalis* and smear layer removal in curved canals by different irrigation techniques: An *in vitro* study

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**Abstract**

**Background:** Various irrigation techniques have been proposed to improve the effectiveness of root canal debridement.

**Aims:** The aim of the study was to compare the antimicrobial effect on *Enterococcus faecalis* and smear layer removal efficacy in curved canals by different irrigation techniques.

**Materials and Methods:** Eighty extracted permanent maxillary molars with curved mesiobuccal roots were inoculated with *E. faecalis*. The tooth samples were then divided into four groups: Group A – EndoVac, Group B – Passive Ultrasonic Irrigation (PUI), Group C – Photodynamic Therapy (PDT), and Group D – Laser Irrigation with Photon-Induced Photoacoustic Streaming (PIPS). The percentage of bacterial reduction was calculated. The presence of smear layer from coronal, middle, and apical sections was evaluated through scanning electron microscopy.

**Statistical Analysis:** Statistical analysis was performed using Kruskal–Wallis test. Intergroup comparison was made with Mann–Whitney U-test.

**Results:** Although statistically insignificant, the irrigation techniques have shown considerable reduction in *E. faecalis* biofilm (*P* > 0.05). EndoVac, PUI, and PIPS have shown significantly higher efficacy in removing smear layer from apical third than PDT (*P* < 0.001).

**Conclusion:** The newer PIPS technology can be used as an efficient tool in the decontamination of root canals. However, more clinical studies in this aspect are required to ensure more thorough debridement and disinfection of the root canal system.

**Keywords:** EndoVac; *Enterococcus faecalis*; laser irrigation with photon-induced photoacoustic streaming; passive ultrasonic irrigation; photodynamic therapy; scanning electron microscope

**INTRODUCTION**

One of the main causes of failure in endodontic treatment is incomplete removal of pulp tissue or microorganisms present within the root canals, especially *Enterococcus faecalis*.[1] Although chemomechanical procedures can lessen the number of *E. faecalis* from the root canal, it cannot eradicate completely.[2] Therefore, newer irrigation techniques have been proposed to effectively deliver the irrigant throughout the root canal system, even the narrowest spaces in the canal.

Very few studies have been done in curved root canals that compare the efficacy of different irrigation techniques.
Hence, the aim of this study was to assess and compare the antimicrobial effect on \textit{E. faecalis} and smear layer removal efficacy in curved mesiobuccal canals of maxillary first molars by EndoVac, Passive Ultrasonic Irrigation (PUI), Photodynamic Therapy (PDT), and Laser-Assisted Irrigation with Photon-Induced Photoacoustic Streaming (PIPS).

**MATERIALS AND METHODS**

The present study was an \textit{in vitro} study. Eighty extracted human permanent maxillary molars with curved mesiobuccal canals, with a curvature of 20°–40° were selected according to Schneider method.\(^{1(5)}\) After access preparation, tooth length was determined, the occlusal surface of the tooth was then trimmed to obtain a standardized tooth length of 16 mm. Working length (WL) was set 1 mm short of the tooth length. Apical foramen of the mesiobuccal root was sealed with flowable composite (Flow Plus, Medicept Dental) to simulate \textit{in vivo} condition. The rest of the root surfaces were then covered with bonding agent (Adper, Single Bond 2, 3M ESPE) to prevent lateral contamination. Mesiobuccal canals were then instrumented till 20 K-file to create space for culturing \textit{E. faecalis}.

The tooth samples were then placed in microtubes and autoclaved twice at 121°C for 20 min. After confirming root canal sterility, microbial inoculation in each sample was done.

**Inoculation of \textit{E. faecalis} in tooth samples**

\textit{E. faecalis} standard strain ATCC 29212 (American Type Culture Collection 29212) was cultivated in blood agar plates. An \textit{E. faecalis} suspension was prepared in sterile saline with a standard concentration of 0.5 McFarland corresponding to a density of \(3 \times 10^8\) cells/ml. About 1 ml of \textit{E. faecalis} suspension and 2 ml of sterile brain heart infusion broth was added to the microtubes such that the tooth was completely immersed in the solution. It was then incubated at 37°C for 4 weeks to simulate the resistant biofilm conditions.

Sterile paper points were used to absorb the canal contents, plated on blood agar, and incubated at 37°C for 24 h. The bacterial colonies on each plate were then counted to determine the initial colony-forming units (CFUs).

The tooth samples inoculated with \textit{E. faecalis} were then instrumented with X1 (17/0.04) and X2 (25/0.06) files of ProTaper Next rotary system (Dentsply Sirona, Switzerland) at a rotational speed of 300 rpm and 2N. cm torque, according to manufacturer’s instructions.

The 80 tooth samples were then randomly divided into four groups (20 samples in each group):

**Group A (EndoVac)**

Master delivery tip was used to deliver 2.5% NaOCl into the pulp chamber. Macro-irrigation was done initially for 30 s with macrocannula used in short up and down motion, while the irrigant was simultaneously delivered to the pulp chamber. This was followed by three cycles of micro irrigation where microcannula was placed at full WL of the canal. First microcycle consist of 2.5% NaOCl delivered to the canal for 10 s, irrigant flow stopped and then again continued for 10 s. Microcannula removed and NaOCl was left in the canal for 60 s to charge. Second microcycle involves the use of 17% ethylenediaminetetraacetic acid (EDTA) for 10 s and charging for 60 s. The third microcycle is the same as first microcycle.

**Group B (Passive Ultrasonic Irrigation)**

The root canals were flooded with 2.5% NaOCl. Ultrasonic Activator tip (18 mm/2%) (Eighteenth Ultra X, Changzhou Sifary Medical Technology, China) placed 2 mm from the WL and moved passively in up and down motion for 30 s. The activation was repeated again for two more cycles. This was followed by activation of 17% EDTA for two cycles of 30 s each. Finally, rinse with 2.5% NaOCl which was activated in the same way as done previously.

**Group C (Photodynamic Therapy)**

This involves using a photosensitizing compound which was activated at a specific wavelength in the presence of oxygen. This results in the formation of reactive oxygen species that cause microbial cell lysis.

15 µg/ml of toluidine blue O dye was the photosensitizer used in this study. The root canals were initially irrigated with 2.5% NaOCl for 1 min, followed by 17% EDTA for 1 min using syringe. The canals were dried with paper points. The dye was then injected into the canals and left for 1 min (preirradiation time). This time was necessary to allow photosensitizes to bind to the plasma membrane of microorganism and consequently cause cell damage.

Gallium aluminum arsenide diode laser (iLase; BIOLASE, CA, USA) with a flexible laser tip of diameter 200 µm (EZ-14 EZ tip) at a wavelength of 940 nm was delivered into the canal 1 mm short of WL and then withdrawn in a circumferential manner. Laser irradiation was performed in two cycles of 1 min each (total of 2 min) with an interval of 20 s between irradiations to prevent thermal damage. The dye was then removed from the canal by 2.5% NaOCl for 2 min.

**Group D (Laser-Activated Irrigation with Photon-Induced Photoacoustic Streaming)**

Er, Cr: YSGG laser (BIOLASE, Waterlase) with a tapered and stripped 600-µ tip was used. It utilizes extremely low
energy levels of laser light (20 mJ, 15 Hz, 0.3 W) at a low pulse duration of 50 µs.

The canal and pulp chamber were filled with 2.5% NaOCl using syringe. The laser tip was placed at the pulp chamber and activated for four cycles of 30s each. In between activations, hypochlorite was removed from the canal and replenished with fresh solution using syringe. The pulp chamber and canal were then filled with 17% EDTA and activated for two cycles of 30 sec each. Final irrigation was done with NaOCl which involves two cycles of 30 s each.

In the present study design, concentration and contact time of the irrigants were standardized. About 2.5% NaOCl for a total of 3 min and 17% EDTA for 1 min were used. Volume cannot be standardized, as different delivery systems have different mechanisms of action and different volumes delivered at a given time.

Finally, second microbiological analysis was performed to determine the final bacterial count [Figure 1]. The percentage reduction in bacterial count was calculated using the following formula:

\[
\text{Percentage reduction in } E. \text{ faecalis colony count} = \frac{\text{Initial count} - \text{Final count}}{\text{Initial count}} \times 100
\]

**Scanning electron microscopy**

Samples were split longitudinally into two halves using a chisel; the tooth half showing most of the canal was selected for scanning electron microscopy. The samples were mounted on metallic stubs, gold-sputtered and examined under a scanning electron microscope (JEOL, Model-JSM 7610 F PLUS, Japan). The presence of smear layer was evaluated from coronal, middle, and apical sections (3 mm, 6 mm, and 9 mm from the apex, respectively) at ×2000 [Figure 2].

The digital images obtained were analyzed individually by two observers in a blind scoring manner based on Hülsmann scoring system.[6] The mean score of the two observers were calculated.

**Statistical analysis**

The data were subjected to statistical analysis using IBM SPSS Software version 18 (IBM Inc, NY, USA). The data were assessed for normality by Shapiro–Wilk test.

The bacterial reduction and the mean smear layer removal between four groups were compared using Kruskal–Wallis test. Intergroup comparison was made with Mann–Whitney U-test. The significance level for all statistical analysis was set at \( \alpha = 0.05 \).

**RESULTS**

- There was considerable reduction in *E. faecalis* CFUs in all the groups. However, there was no statistically significant difference between the four irrigation techniques in eliminating *E. faecalis* biofilm from the root canals \( (P > 0.05) \) [Table 1].

![Figure 1](image.png)

*Figure 1:* (1a and 1b) Initial and final colony count after EndoVac irrigation, respectively. (2a and b) Initial and final colony count after ultrasonic irrigation, respectively. (3a and b) Initial and final colony count after photodynamic therapy, respectively. (4a and b) Initial and final colony count after laser irrigation with PIPS, respectively. PIPS: Photon-induced photoacoustic streaming
- Smear layer removal was found to be greater in coronal third than middle and apical third of root canals ($P < 0.001$)
- There was no statistically significant difference between the four groups in terms of smear layer removal from coronal and middle third of root canals ($P > 0.05$)
- However, in the apical third, statistically significant reduction in smear layer was found between the groups ($P < 0.001$). Intergroup analysis revealed that EndoVac, ultrasonic, and laser irrigation with PIPS exhibited higher efficacy in removing smear layer from the apical third of root canals than PDT ($P < 0.05$) [Table 2].

### DISCUSSION

*E. faecalis* has been widely used as a valuable microbiological marker for *in vitro* studies because it has been able to colonize the root canal wall in biofilm manner and penetrate dentinal tubules. It is also able to survive in extreme conditions and are resistant to phagocytosis and antimicrobial agents.[9]

Various concentrations of sodium hypochlorite have been shown to be effective against *E. faecalis*. Siqueira *et al.* suggested that 2.5% NaOCl exhibits similar antibacterial efficacy when compared to 5%, when modifications such as using larger volumes or combining with other irrigants or agitation were done to increase its efficiency.[8] Therefore, in the present study, a combination of 2.5% NaOCl and 17% EDTA were used which were activated by different irrigation techniques.

In the present study, there was no statistically significant difference between the four irrigation techniques in eliminating *E. faecalis* biofilm from the root canals ($P > 0.05$). This was similar to the studies done by Brito *et al.* and Fernandes *et al.*, where the irrigant activation techniques showed insignificant difference in microbial reduction.[9,10]

In this study, laser irrigation with PIPS was found to be more effective in reducing *E. faecalis* biofilm from the root canals than PDT. This finding was similar to the study done by Durmazpinar *et al.*, The profound photoacoustic and photomechanical phenomenon generates faster streaming

| Table 1: Intragroup comparison of percentage reduction in *Enterococcus faecalis* colony-forming units using Kruskal–Wallis test |
|---------------------------------------------------------------|
| **Mean percentage reduction in *Enterococcus faecalis* colony-forming units** |
| **n** | **Mean** | **SD** | **Minimum** | **Maximum** |
| Group 1 – EndoVac | 20 | 98.52 | 3.227 | 88 | 100 |
| Group 2 – Ultrasonic | 20 | 99.52 | 1.778 | 92 | 100 |
| Group 3 – Photodynamic therapy | 20 | 97.75 | 3.320 | 90 | 100 |
| Group 4 – Laser with PIPS | 20 | 99.57 | 1.829 | 92 | 100 |
| $P$ | | | | | 0.0573 |
| Inference | | | | | There is no significant difference |

*Number of samples in each group. SD: Standard deviation.*
of fluids distant from the source of irradiation, which was sufficient to penetrate and disrupt the biofilm created by the *E. faecalis*. Moreover, erbium lasers has the highest absorption in water and therefore was readily absorbed by the biofilms resulting in microbial killing.\(^{[11]}\)

Due to the high absorption of water by the erbium lasers, the cavitation process generates vapor-containing bubbles, whose implosion produces photoacoustic shockwaves within the irrigant that will be strong enough to disrupt the smear layer.\(^{[13]}\) This may be the reason for the statistically significant reduction in smear layer from the apical third of canals when compared to PDT.

In the present study, EndoVac also showed better antimicrobial efficacy than PDT (\(P > 0.05\)). This finding was similar to the study done by Miranda et al.\(^{[14]}\) EndoVac also exhibited statistically significant reduction in smear layer in the apical third than PDT (\(P < 0.001\)). The ability of EndoVac in eliminating apical vapor lock and the increased volume of irrigant delivered along with its continuous replenishment with fresh solution may be responsible for its improved antimicrobial and smear layer removal efficacy.\(^{[15]}\)

PUI exhibited better antimicrobial efficacy than PDT (\(P > 0.05\)). This result was in accordance with the study done by Bilgin et al.\(^{[16]}\) The formation and implosion of bubbles during the ultrasonic activation results in the formation of powerful shockwaves that produces shear stress along the canal walls that help in the detachment of biofilm from the root canal.\(^{[17]}\)

However, PUI exhibited reduced efficacy in removing smear layer from the apical third of curved root canals than EndoVac. This may be because of the curvature which increases the possibility of the ultrasonic tip coming in contact with the canal walls. This results in decreased amplitude and oscillation of the ultrasonic tip along the constricted part of the canal, thus reducing its efficacy.\(^{[18]}\) This contact may also result in indirect accumulation of debris due to the activation process itself.\(^{[19]}\)

In the present study, PDT was found to be least effective in eliminating *E. faecalis* as well as smear layer from the curved root canals. The presence of dead cells and cell remnants in the biofilm structure neutralizes the PDT-mediated killing, so that the photosensitizer, oxygen, and the light may not reach bacteria in the deeper layers of biofilm.\(^{[20]}\)

The low antimicrobial efficacy of PDT was also due to low concentration of oxygen available in the canals, especially in irregularities and in dentinal tubules.\(^{[21]}\)

Hence, in the present study, the adjunct use of NaOCl and EDTA may be responsible for the effectiveness of PDT.

**CONCLUSION**

Within the limitations of this study, it can be concluded that:

- The newer PIPS technology can be used effectively for root canal debridement
- The improved cleaning ability of EndoVac would result in remarkable improvements in treating canal complexities.

However, more clinical studies in this aspect are required to ensure more thorough debridement and disinfection of the root canal system.

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**Conflicts of interest**

There are no conflicts of interest.

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