ORIGINAL ARTICLE

Turk Endod J 2022;7(3):85–90
doi: 10.14744/TEJ.2022.40412

Evaluation of the effectiveness of different irrigation activation techniques in the removal of *Enterococcus faecalis* from oval-shaped root canals

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Purpose: To compare the efficacy of EDDY, XP-Endo Finisher R (XPFR), and EndoUltra in removing *Enterococcus faecalis* (*E. faecalis*) from oval root canals with standard needle irrigation (SNI).

Methods: A total of 72 teeth with single-rooted and single oval-shaped root canals were selected. Teeth were sterilized in an autoclave, infected with *E. faecalis*, and inoculated in an incubator at 37°C for 7 days. Four teeth were processed for scanning electron microscopy analysis to control biofilm formation. The remaining 68 teeth were prepared with Reciproc Blue 25.08 and 40.06 files and then divided into four groups according to the irrigation activation techniques used: EDDY, EndoUltra, XPFR, and SNI (n = 17). Before (S1) and after (S2) irrigation techniques, the bacterial samples were taken from the root canals and colony-forming units (CFUs) were counted and calculated (CFU/mL). The data were statistically analyzed using the Shapiro–Wilk, Kruskal–Wallis, and Wilcoxon tests (p = 0.05).

Results: It was observed that CFU counts decreased significantly from S1 to S2 ($p< 0.05$). However, there was no significant difference among the irrigation activation techniques in terms of removing *E. faecalis* ($p> 0.05$).

Conclusion: The efficiency of EDDY, EndoUltra, XPFR, and SNI in the removal of *E. faecalis* was similar.

Keywords: EDDY, EndoUltra, *Enterococcus faecalis*, irrigation activation, XP-Endo Finisher R.

Introduction

Chemomechanical preparation plays an important role in removing bacteria from the root canal system. However, anatomical variations in the complex root canal system, such as oval-shaped root canals, make these procedures difficult. Several studies showed that necrotic pulp tissue and bacterial biofilm can exist after the preparation of oval-shaped root canals due to the untouched areas (1–3). In addition, due to the complex morphology of the root canal system, irrigation solutions cannot penetrate sufficiently into the dentinal tubules with the standard needle irrigation (SNI) technique. Therefore, several irrigation activation methods, such as manual dynamic activation, sonic and passive ultrasonic irrigation (PUI), and laser-assisted irrigation, have been developed to assist irrigation solutions in penetrating the structural irregularities in the root canal system.

Cite this article as: Yurdagül FZ, Aktemur Türker S, Cömert F. Evaluation of the effectiveness of different irrigation activation techniques in the removal of *Enterococcus faecalis* from oval-shaped root canals. Turk Endod J 2022;7:00-00.
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Submitted: July 19, 2022 Revised: September 13, 2022 Accepted: October 07, 2022

PUI and sonic activation techniques create a three-dimensional motion, which induces acoustic streaming and cavitation. The main difference between them is the frequency range in which they are being used. The frequency range of PUI is 30–45 kHz (4,5) and that of sonic activation is 1–6 kHz (6). In PUI, a straight wire or an oscillating file creates acoustic streaming through ultrasonic waves, and it is transmitted to the irrigation solution. It was shown in previous studies that the amount of intracanal bacteria decreased significantly after chemomechanical preparation using PUI (7). EndoUltra Ultrasonic Activator (Vista, Racine, WI, USA) is a cordless device produced for PUI at a frequency of 40 kHz (4). Recently, EDDY (VDW, Munich, Germany), a sonic-powered device that operates at a frequency of 5–6 kHz has been introduced. Its tip is made of flexible polyamide to prevent damage to the canal wall when in use (8). The EDDY sonic activation device was reported to be effective in removing bacteria from the root canal system (8,9).

The recently produced XP-Endo Finisher R (XPFR) (FKG, La Chaux-de-Fonds, Switzerland) is specially designed with MaxWire (MaxWire, MartensiteAustenite Electropolish Flex, FKG Dentaire) technology. It removes root canal filling materials, especially in the curvature or oval areas, after conventional techniques. It can be applied as a supplementary final step with any file system of diameter #30 or larger (10). It has also been used as a supplementary approach to irrigation procedures to clean the hard-to-reach areas in teeth with complex anatomy such as oval-shaped root canals (11,12). The file expands at body temperature acquiring a spoon shape over the apical few millimeters (13). To date, the bacteria removal efficiency of XPFR has not been evaluated. Therefore, in this *in vitro* study, it was aimed to compare the efficacy of sonic activation (EDDY), PUI (EndoUltra), and XPFR with SNI in oval-shaped root canals infected with *E. faecalis*. The null hypothesis of this study was that there would be no difference between the bacteria removal efficiency of these irrigation activation techniques.

Materials and Methods

This *in vitro* study was approved by the Non-Interventional Clinical Research Ethics Committee of the Zonguldak Bülent Ecevit University (2020/3).

Sample Size Calculation

The sample size was calculated using sample-size calculating software G*Power version V3.1.9.6 based on the data obtained from a previous study (14). It was calculated that there should be at least 17 specimens in each group with the following parameters: $\alpha = 0.05$; power = 0.95; and effect size, f = 0.52.

A total of 72 extracted single-rooted teeth were collected. Roots with cracks, fractures, multiple canals, and calcifications were excluded. All teeth were measured 5 mm from the apex to confirm that they had an oval-shaped ratio of greater than 2.5/1 ratio between the buccolingual and mesiodistal dimensions. The tooth surfaces were cleared of any remnant tissues, using a universal periodontal curette and cleaned with gauze soaked in 5% sodium hypochlorite.Then the selected teeth were kept in distilled water until use.

The crowns of teeth were removed with a sterile diamond fissure bur under water-cooling to obtain a total length of 18 mm. After access cavity preparation, the canal patency was checked with a #10 K-file (Dentsply Maillefer, Ballaigues, Switzerland). The working length was determined by subtracting 1 mm from the length determined by seeing the tip of the file through the apical foramen. To avoid microbial leakage, all root surfaces of the teeth were covered with two layers of nail polish. The apices of teeth were covered with a flowable composite to prevent the extrusion of irrigation solutions from the apex. All samples were sterilized in the autoclave at 121°C and 1 atm pressure for 20 min before bacteria inoculation.

Inoculation of Enterococcus faecalis

A 1 mL pure culture of *E. faecalis* strain (ATCC 29212) was added to brain–heart infusion broth at 37°C for 24 h. A 0.5 McFarland suspension (a bacterial concentration of 1.5 x 10^8 colony-forming units (CFU)/mL) was prepared in sterile broth. After this procedure, root canals were filled with *E. faecalis* suspension using sterile micropipettes. The bacterial suspension was transferred to the entire canal length with size #10 K-files. Teeth were incubated at 37°C for 1 week. A fresh culture medium was added to the root canal every 48 h. Gram staining and colony morphology were used to assess the growth of *E. faecalis*. Four roots were fixed in 10% buffered formalin and processed for scanning electron microscopy (SEM) to confirm bacterial colonization (Fig. 1).

Before root canal preparation, the amount of initial bacterial load (S1) in 68 roots was counted. Initially, the root canals were flushed with 1 mL distilled water to remove unattached bacteria, and then three sterile size #15 paper points were used for 1 min to soak up the canal contents. These paper points were inserted into Eppendorf tubes containing 1 mL of brain–heart infusion broth and vortexed for 1 min, and 0.1 mL aliquots were plated onto blood agar and incubated at 37°C for 48 h. After 48 h, the CFUs were recorded in CFU/mL.

Root canals were prepared with Reciproc Blue 25.08 and 40.06 files in reciprocal motion using X-Smart Plus en-

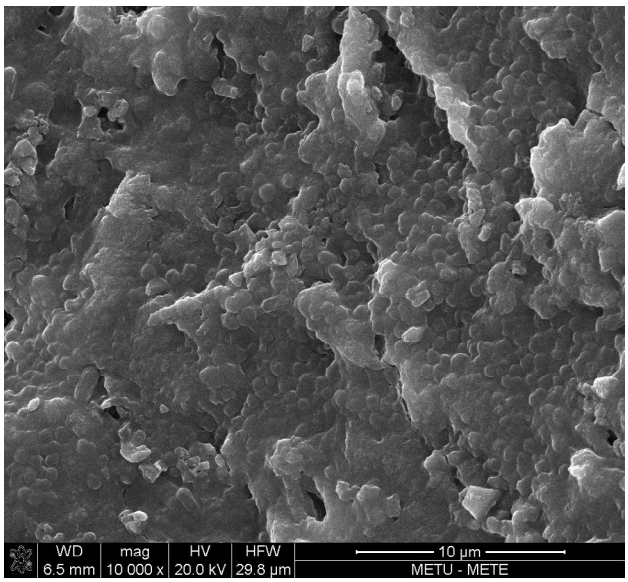

Fig. 1. Positive control group displaying *Enterococcus faecalis* growth.

domotor (Dentsply-Sirona, Germany). During the preparation of the root canals, 2 mL of 0.85% sterile saline was used after each file change. Thereafter, 68 roots were divided into four experimental groups using a randomization method (www.randomizer.org) according to the irrigation activation technique used (n = 17). Specimens were irrigated with the respective technique as detailed in the following sections.

Standard Needle Irrigation

A 2 mL 0.85% sterile saline was placed in the root canal via a 27-gauge needle within 1 mm from the working length. To ensure length control, a stopper was placed on the needle at the required length. The irrigation needle was moved in the root canal with up and down movements throughout for 30 s.

EndoUltra

A 2 mL 0.85% sterile saline was placed in the root canal. EndoUltra (Vista Apex, Wisconsin, United States) ultrasonic tip (20.02) was attached to the device and powered 1 mm shorter than the working length at 40 kHz frequency for 30 s.

EDDY

A 2 mL 0.85% sterile saline was inserted into the root canals. EDDY (VDW, Munich, Germany) irrigation tip (25.04) was attached to SONICflex 2003 (Kavo, Genova, Italy) air scaler and powered 1 mm shorter than the working length at 6 kHz frequency for 30 s.

XP-Endo Finisher R

A 2 mL 0.85% sterile saline, heated to 37°C, was inserted into the root canals. The XPFR file (FKG Dentaire, La Chaux de Fonds, Switzerland) was used 1 mm shorter than the working length with X-Smart Plus (Dentsply-Sirona, Germany) at 1000 rpm and 1 N cm torque for 30 s.

In each group, irrigation activation procedures were repeated two more times as described earlier. S2 samples were taken from the root canals with three #40 paper points, as described previously. CFUs were counted as described in S1 samples. A total of 6 mL of 0.85% sterile saline was used for each tooth during the irrigation activation procedures in each group.

Statistical Analysis

The data were analyzed using IBM SPSS V23. Normality was assessed using the Shapiro–Wilk test. The Kruskal–Wallis test was used to compare the number of colonies and reduction percentage that were not normally distributed. The Wilcoxon test was used to compare the number of colonies before and after the treatment within the groups. Analysis results were presented as median (min–max) for quantitative data. The significance level was taken as $p< 0.05$.

Results

Table 1 shows the amount of the bacterial load and bacterial reduction values. In the intergroup analysis, in terms of bacterial load in S1 and S2 samples, there was no sig-

Table 1. Bacterial load and reduction values for groups (CFU/mL)

Groups	S1, Median (min–max)	S2, Median (min–max)	p[a]	Percentage decrease
SNI	510 (2–2080)	17 (0–164)	<0.001	93.75 (40–100)
EndoUltra	280 (20–1550)	10 (0–93)	<0.001	96.91 (66.67–100)
EDDY	24 (3–2020)	1 (0–285)	<0.001	97.48 (61.96–100)
XPFR	95 (4–1740)	3 (0–42)	<0.001	96.84 (0–100)
p[b]	0.100	0.06		0.996

[a]The Wilcoxon test. [b]The Kruskal–Wallis test.

nificant difference between the groups (p = 0.100 and p = 0.060, respectively). However, in intragroup analysis, a significant difference was found between S1 and S2 samples in all groups (p< 0.001). In group SNI, CFU counts were 510 and 17 in the S1 and S2 samples, respectively (p< 0.001). In group EndoUltra, CFU counts were 280 and 10 in the S1 and S2 samples, respectively (p< 0.001). In group EDDY, CFU counts were 24 and 1 in the S1 and S2 samples, respectively. In group XPFR, CFU counts were 95 and 3 in S1 and S2 samples, respectively. No significant difference was found among groups in terms of bacterial reduction (p = 0.996). Bacterial reduction percentages were 93.75%, 96.91%, 97.48%, and 96.84% for SNI, EndoUltra, EDDY, and XPFR, respectively.

Discussion

The complex and irregular anatomy of root canals may prevent the removal of bacteria from the root canal system. With agitation of irrigation, the solutions are provided to contact the root canal walls more, in consequence of a more effective cleaning can be made (15). In this *in vitro* study, the bacteria removal efficacy of EDDY, EndoUltra, and XPFR were compared to SNI.

This study was based on single-rooted teeth with an oval-shaped single root canal. The samples were standardized to reduce the bias of the study. Roots with a ratio of 2.5 or greater (buccolingual diameter to mesiodistal diameter) were selected, and the working lengths were set at 17 mm. Teeth were contaminated with *E. faecalis* and prepared with Reciproc Blue 25.08 and Reciproc Blue 40.06 files before irrigation activation techniques were applied. During irrigation procedures, sterile saline solution was used as an irrigant to avoid any antimicrobial effect and to test whether the respective activation techniques were able to provide a satisfactory bacteria removal without the influence of any chemical substance, as some previous studies have used (16–18).

According to the results of this study, CFU counts decreased significantly from S1 to S2. It means that all irrigation techniques removed bacteria significantly. However, no difference was found among them. Therefore, the null hypothesis tested was accepted. Irrigation agitation techniques did not show superior performance in removing bacteria compared with SNI. This finding may be due to a wide apical preparation size in root canals which facilitates insertion of the irrigation devices short of WL as well as distribution and exchange of the irrigant in the root canal. In this study, the root canals were enlarged to size 40 with a taper of 0.06. It can be speculated that this apical preparation size may permit unimpeded needle and tip penetration of the devices to the full length of each canal, resulting in similar bacteria removal. Many studies show similar results as this study, comparing SNI and PUI together (19,20). In a study by Bhuva et al. (19), no significant difference was observed between SNI with 1% sodium hypochlorite and PUI with 1% sodium hypochlorite. However, they reported that both SNI and PUI with 1% sodium hypochlorite were more effective in removing the biofilm than SNI with sterile saline solution. Therefore, it can be concluded that the use of NaOCI as an irrigation solution was more important concerning bacterial load reduction than the mechanical agitation of the irrigation solution, as previously stated by Siqueira et al. (21). No statistically significant difference was found among SNI, PUI, and EndoActivator in a previous study by Tardivo et al. (20). In contrast to the results of the present study, some studies found superior performance for EndoUltra, EndoActivator (4,8,22), and EDDY (8,22) in removing bacteria compared with SNI. The different results could be attributed to the differences in study designs. Unlike these studies, in the present study, the infected teeth were prepared up to size 40.06 before irrigation activation procedures were performed, and to avoid any antibacterial activity, NaOCI was not used.

The present study demonstrated that irrigation activation techniques showed comparable results in antibacterial efficacy. This result is consistent with a previous study in which EDDY and EndoActivator sonic-powered irrigant agitation systems have equivalent intracanal bacteria reduction efficacy (8). In contrast to the present study results, Al-Obaida et al. (22) reported that the EndoActivator system was superior to EDDY and EndoUltra in reducing live bacteria within the root canal.

The XPFR has been developed for retreatment cases and has similar features to the XP-Endo Finisher (XPF) with the main differences being a larger tip (size 30 for XPFR and size 25 for XPF) and the semi-active tip, which makes it stiffer and therefore more aggressive than XPF file (13). To the authors' knowledge, the effect of XPFR on the removal of bacteria from infected oval-shaped root canals has not been compared yet. However, the bacterial removal efficiency of XPF was shown in a few recent studies (23–25). Teves et al. (23) reported that the multispecies biofilm removal was significantly improved using XPF and PUI when compared with SNI. Carvalho et al. (24) stated that when XPF is used as a method of agitation of the irrigation solution, it improves the bacteria removal efficiency of XP-Endo Shaper and Reciproc Blue files. Furthermore, Pedrinha et al. (25) reported that XPF enhanced the intratubular antibacterial activity of irrigation solutions.

In the present study, bacterial reduction by irrigation activation was demonstrated in *in vitro* conditions using the

culture method. The culture method is easy and widely used method and allows us to obtain data related to the microbial load on the surface of the root canals. However, there are some limitations to this technique. It is insufficient for the quantitative analysis of the microorganisms remaining in the dentinal tubules. Additionally, microorganisms that are viable but cannot colonize due to low metabolic activities may not be detected by this method. Therefore, the results of the present study should be interpreted with caution.

Conclusions

Within the limitation of this study, the results revealed that sonic, ultrasonic, or XPFR irrigation activation techniques did not increase the *E. faecalis* removal compared with SNI.

Authorship Contributions: Concept: F.Z.Y., S.A.T; Design: S.A.T, F.C.; Supervision: S.A.T; Materials: S.A.T., F.C.; Data: F.Z.Y., F.C.; Analysis: S.A.T.; Literature search: F.Z.Y., S.A.T.; Writing: F.Z.Y.; Critical revision: S.A.T.

Acknowledgements: We thank Bio, Msc. Gülhan Avcı for all her assistance in microbiology laboratory.

Source of Funding: This study was supported by the Scientific Research and Development Department of Zonguldak Bülent Ecevit University (Grant number: 2020-27194235-02).

Conflict of Interest: None declared.

Ethical Approval: The study protocol was approved by the Zonguldak Bülent Ecevit University Clinical Research Ethics Commitee (date: 05.02.2020, protocol no: 2020/03).

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