ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
March2022 • Vol. 25

Effect of Eighteeth Ultra X Ultrasonic activator device versus Syringe irrigation technique on reduction of Enterococcus Faecalis count inside the root canal space (An in-vitro study)

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Aim: This in-vitro study aimed to compare the effect of Eighteeth Ulta X Ultrasonic activator device and syringe irrigation using sodium hypochlorite (NaOCI) (5.25%) on reducing Enterococcus faecalis (E. faecalis) count in the root canal space.
Materials and Methods: Thirty single rooted mandibular premolars were selected for this study. Root canals were prepared using Edge File-X7 (by EDGE ENDO) and enlarged to size 40/04 taper. After sterilization, all teeth were contaminated using E. faecalis suspension. The teeth were divided into two groups: Group (I) (non-activation group, syringe irrigation technique using 3 ml syringe with 30 gauge needle) and Group (II) (activation group using Ultra X Ultrasonic activator device). Three samples of the Colony Forming Units (CFU) were taken for both groups. First sample before any intervention and named (Group I, Sample 1(GIS1) and Group II, Sample 1 (GIIS1). Second sample after chemo-mechanical preparation and named (Group I, Sample 2 (GIS2) and Group II, Sample 2 (GIIS2). Third sample with no activation of irrigant and named (Group I, Sample 3 (GIS3) and with activation of irrigant and named (Group II, Sample 3 (GIIS3). Data were statistically analyzed using Shapiro-Wilk's test, Levene's test and independent t-test ($p<0.05$).
Results: There was a significant reduction of E.faecalis count at Sample 3 in Group II in comparison to Group I $p<0.001$).
Conclusions: It was concluded that the use of Ultra X Ultrasonic activator device significantly eliminated bacterial count in the root canal space in comparison to non-activation, irrigating syringe technique.

Keywords: Enterococcus faecalis; Irrigation; Root Canal Debridement; Sodium Hypochlorite; Ultrasonic Irrigation

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Introduction

Treatment of apical periodontitis depends mainly on removal of huge amounts of micro-organisms and their by-products inside the root canal space.[1] Bacterial species mainly the persistent ones proved to be the main reason of failure in endodontics.[2] The most common type of bacteria detected in asymptomatic persistent infection is Enterococcus faecalis (E. faecalis) due to its various survival and virulent factors.[3] Persistent intra-radicular infection was found to be higher than untreated chronic apical periodontitis.[4] Hence, continued research to eliminate E. faecalis out of the root canal space was carried out.[3]

The most commonly used irrigating solution in endodontics is sodium hypochlorite (NaOCl) (Household cleaning products of Egypt).This solution proved to have an antibacterial action as well as dissolution efficacy on organic tissue.[5] However, it was found that basic chemo-mechanical preparation using NaOCl leaves 40%-60% of initial bacterial counts inside the root canal space.[6] Hence, using a combination of Ethylene Diamine Tetra Acetic Acid (EDTA) (MD-Chelcream, Meta Biomed Co Ltd, Korea) and NaOCl could eliminate inorganic tissues and smear layer from the root canal and might increase negative cultures in some cases.[7]

Therefore, the need of maximizing the efficiency of irrigating solutions for better disinfecting the root canal space before obturation should be highlighted.[8] This could be achieved by activating the irrigating solutions using delivery systems like sonic activation, Photon Induced Photo-acoustic Streaming (PIPS) and most recently newly introduced Ultra X Ultrasonic activator device (Eighteeth, Changzhou Sifary Medical Technology, China). Ultra X Ultrasonic activator device is a portable light weight device with autoclavable, flexible and smooth surface finish tips. It has a contra-angle design, two power modes with LED indicator and 1500 mAh powerful battery. According to manufacturer's instructions, this device works at ultrasonic frequencies of 45kHz. These frequencies utilize the principle of acoustic streaming, agitation and cavitation to reach difficult to instrument areas in complex root canal system, hence, disrupting smear layer and biofilm, open up plugged dentinal tubules, remove gross dentinal debris and amplify the efficiency of irrigating solutions. Therefore, the purpose of this study was to investigate the effect of Ultra X ultrasonic activator device compared to syringe irrigation technique on the reduction of E. faecalis count in root canal space.

Materials and Methods

Thirty single rooted recently extracted mandibular premolars with intact crowns were used in this study. Teeth were divided into 2 groups, 15 teeth per each group, group (I): non-activation group, syringe irrigation technique using 3 mL syringe with 30 gauge needle and group (II): activation group using Ultra X Ultrasonic activator device (Eighteeth, Changzhou Sifary Medical Technology, China). The teeth were then kept in 0.9% saline solution at 40C for one day, then rinsed with distilled water.

Access cavity was carried out in all teeth using round diamond bur (Dentsply, Tulsa Dental, Dentsply Maillefer, USA) and canal was established using K-file #10. De-coronation of the crowns was performed and working length was standardized to be 16 mm for all teeth. The apex of the teeth was sealed with epoxy-resin to avoid any bacterial contamination. The teeth were mounted in silicone impression material blocks. Samples were sterilized at 121 °C for 20 minutes.

E. faecalis derived from ATCC 29212 was obtained from the Department of

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Microbiology, Faculty of Medicine, Cairo University. The bacteria were aerobically cultured on blood agar for 48 hours at 35 °C. Colonies were grown in Brain Heart Infusion (BHI) broth for 24 hours at 37°C. In sterile BHI broth, inoculum was prepared with turbidity set to 0.5 McFarland which corresponds nearly to 1.5 × 108 colony forming units per milliliter (CFU/mL). 10 µl of the culture was instantly inoculated inside root canals using sterile micro-pipette. The teeth were kept in sterile cups, then incubated at 37°C for 72 hours. First bacterial sample was taken from root canals using #15 sterile paper points and then cultured to record the bacterial count before any intervention and named as follows GIS1(Group I, Sample 1) (Figure 1) and GIIS1 (Group II, Sample 1) (Figure 2).

Chemo-mechanical preparation was then performed using Edge File-X7 (by EDGE ENDO) and enlarged to size 40/04 taper using 5.25% NaOCl in a 3 mL syringe throughout the whole shaping procedure followed by 5% sodium thiosulphate to inactivate NaOCl followed by 3 mL volume of saline as a final flush in-order to remove any remaining solution. EDTA gel (17%) (MD-Chelcream, Meta Biomed Co Ltd, Korea) was applied to each rotary file before being introduced in root canal. Second bacterial sample was taken using #40 sterile paper points and kept in sterile test tube containing Phosphate Buffered Saline (PBS) with pH 7 to prevent any contamination. The specimens were then cultured to record bacterial count after chemo-mechanical preparation and named as follows GIS2 (Group I, Sample 2) (Figure 3) and GIIS2 (Group II, Sample 2) (Figure 4).

The teeth in Group 1 (non-activation group, syringe irrigation technique) were then irrigated with a 2 mL/min of EDTA, then 3 mL/min of saline to stop the action of EDTA, then 3 mL/min NaOCl followed by 5% sodium thiosulphate to inactivate NaOCl and a final flush with 3 mL/min saline to remove any remaining solution using 3 mL syringe shorter than the working length by 2 mm. Third bacterial sample named GIS3 (Group I, Sample 3) was taken with #40 sterile paper points and placed in sterile test tubes containing PBS. While in Group II (activation group) the same procedures were carried out but with using Ultra X Ultrasonic activator device #20 tip size with 2% taper (i.e. 2 mL/min EDTA with activation for 30 seconds shorter than the working length by 2 mm as mentioned by the manufacturer, 3 mL/min saline as a wash, 3 mL/min NaOCl with activation for 30 seconds shorter than the working length by 2 mm as mentioned by the manufacturer followed by 5% sodium thiosulphate to inactivate NaOCl and then a final flush using 3 mL/min saline). The third sample of Group II (Group II, Sample 3) was also taken with #40 sterile paper points and placed as well in sterile test tubes containing PBS. The samples were then cultured to determine the bacterial count in Group I (non- activation group, syringe irrigation technique) (Figure 5) and Group II (activation group) (Figure 6).

Figure (1): Bacterial colonies in Group I before any intervention

Figure (2): Bacterial colonies in Group II before any intervention

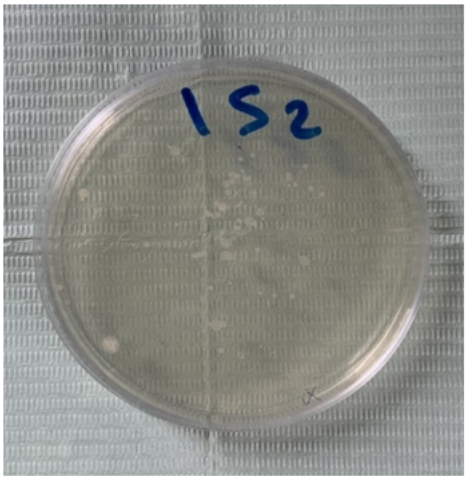

Figure (3): Bacterial colonies in Group I after mechanical preparation

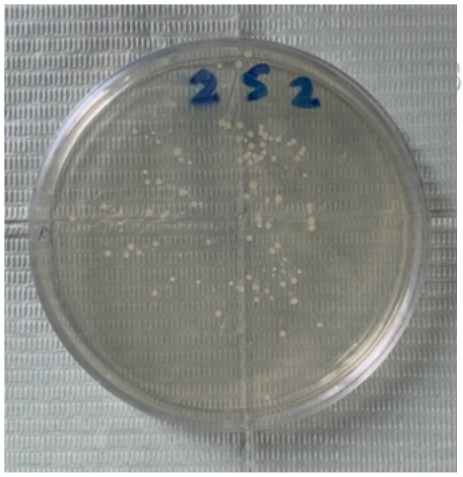

Figure (4): Bacterial colonies in Group II after mechanical preparation

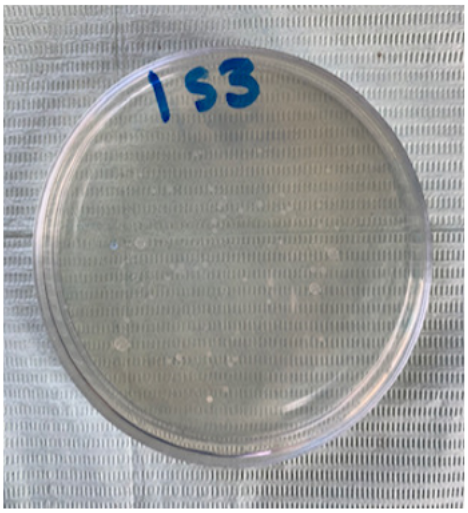

Figure (5): Bacterial colonies after syringe irrigation technique

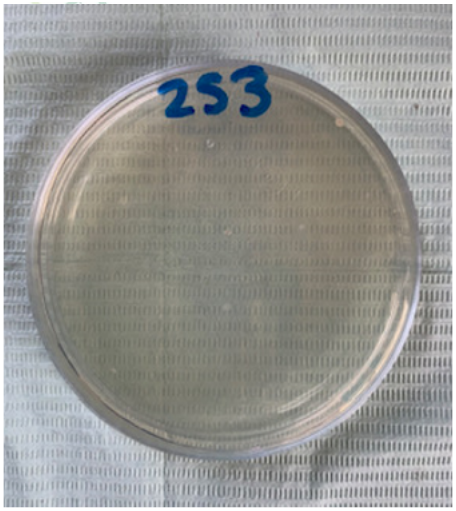

Figure (6): Bacterial colonies after using Ultra X Ultrasonic activation device

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. Data were parametric and showed variance homogeneity so independent t-test was used to analyze intergroup comparisons. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.2 for Windows1.

Results

In this study, results showed that at (S1), the bacterial count in samples of both groups was 1000 CFU. At (S2), the bacterial count in Group (I) was (30.13±5.02) and in Group (II) it was (30.87±4.23) with a non-statistically significant difference between both groups (t=-0.44, p=0.666). At (S3), Group (II) (12.40±3.12) had significantly lower bacterial count than Group (I) (22.27±5.82) (t=5.79, p<0.001). Results of intergroup comparisons are presented in (Table 1). Mean and standard deviation values of the two groups are shown in (Figure 7).

Table (1): Intergroup comparison between bacterial count (CFU) values

Time	*Bacterial count (CFU) (Mean±SD)*		*Mean difference[95%CI]*	*t-value*	*p-value*
	Group (I)	*Group (II)*			
S2	30.13±5.02	30.87±4.23	-0.74 [-4.21:2.73]	-0.44	**0.666**
S3	22.27±5.82	12.40±3.12	9.87 [6.33:13.41]	5.79	**<0.001***

95% Confidence Interval= 95%CI; *significant (p<0.05)

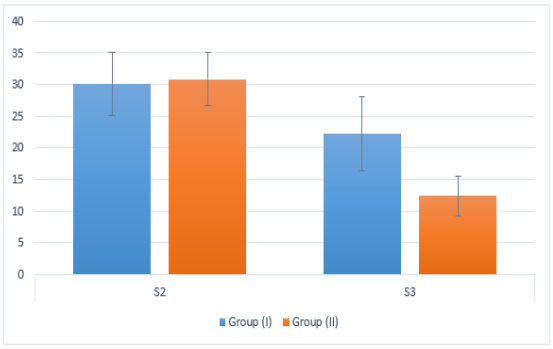

Figure (7): Bar chart showing mean and standard deviation values of bacterial count (CFU)

Discussion

Endodontic microorganisms and their by-products are the causative factor of periapical diseases. Hence, the main goal of endodontic procedure is disinfecting root canal space and preventing the recurrence of any microbial infection.[9] However, there are some challenges that can occur during treatment due to anatomical complexities in root canal space and limitations in root canal shaping devices which can render some parts of root canal walls remaining unprepared.[10]

E. faecalis was found to survive many challenges inside the root canal space due to its virulence factors such as biofilm, lytic enzymes, pheromones, aggregation substance and lipoteichoic acid which can suppress lymphocytes resulting in endodontic failure.[11] Hence, such bacteria was selected and used in this study.

Although in-vivo studies are the best to study antibacterial properties, ethical considerations and difficulties in standardization made extracted teeth, ex-vivo and animal studies more convenient.[12] Thus, single rooted with single canalled extracted mandibular premolars human teeth were used in this study to mimic the clinical situation excluding any anatomical complexities.[13]

Instrumentation of root canal is considered an important endodontic procedure in eliminating bacteria. It was found that Rotary nickel titanium instruments (Ni-Ti) used in continuous rotation results in significant microbial reduction.[14] Therefore, Edge File-X7 rotary instrument was used in this study for preparing the root canal space.

It has been reported that, sodium hypochlorite (NaOCl) is considered one of the most commonly used irrigating solution due to its potent antibacterial effect. Warming the solution was found to increase its efficacy. Therefore, activation of irrigation warm up the solution and hence might increase its antimicrobial effectiveness.[15]

Therefore, chemo-mechanical debridement in addition to activated irrigation techniques play an important role in disinfecting the root canal space.[16]

Activation of irrigation was found to increase the efficiency of irrigating solutions.[17] Hence, Ultra X Ultrasonic activator device was used in this study in comparison to syringe irrigation technique.

In the present study, it was found that activation of irrigation using Ultra X Ultrasonic activator device resulted in significantly better bacterial eradication compared to non-activation, syringe irrigation technique. For better root canal debridement, irrigant activation is indicated as it eliminates pulp tissues and debris as a result of higher flow of irrigant in root canal where the chemical products easily reach the isthmuses and accessory canals resulting in better killing of bacteria than syringe irrigation.[18]

This comes in agreement with Van der Sluis et al (2007)[19], Ghoddusi et al (2019)[20] and Nakamura et al (2018)[9] who significantly demonstrated much better microbial reduction with Ultrasonic and Sonic activation of NaOCl in comparison to non-activation. This is due to the fact that activation of irrigation allows deeper penetration of the irrigating solution where shear stresses allow biofilm detachment of bacteria from dentinal walls leading to their killing.[21]

On the other hand, Beus et al (2012)[22] reported no significant difference between Ultrasonic activation and syringe irrigation in elimination of bacteria. This might be due to the absence of standardization before taking any sample in both groups. In their study, the samples were taken immediately after ultrasonic activation, while in syringe irrigation, a hand file equivalent in size to master apical file was placed in root canal, moved against the walls in order to eliminate any debris or bacteria and then the sample was taken.

Also, Forghani et al (2017)[23] concluded that there was no significant difference using sonic activation compared to syringe irrigation technique in reducing E. faecalis count inside the root canal. This can be explained by the difference in study design and methods used where primary teeth rather than permanent teeth was used in such study.

In the present study, it was found out that chemo-mechanical preparation resulted in reduction of bacterial counts but not its complete eradication. This could be attributed to the presence of positive cultures resulting from root canal complexities.[24] Hence, it is better to use activated devices to allow for the activation of root canal irrigating solutions for better elimination of bacterial counts from root canal space.[25]

Conclusions

Within the limits of this in-vitro study, conclusions revealed the following

1-Chemo-mechanical preparation plays an important role in reduction of bacterial counts in root canal space.

2-Ultra X ultrasonic activator device significantly resulted in bacterial elimination from root canal space in comparison to non-activation, irrigating syringe technique.

Further in-vivo studies should be carried out to evaluate the clinical signs and symptoms accompanied with bacterial reduction before and after root canal preparation as well as the use of activated irrigation devices.

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