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# THE EFFECT OF COMBINED PASSIVE ULTRASONIC IRRIGATION AND XP–ENDO FINISHER ON BACTERIAL BIOFILM: A COMPARATIVE STUDY

Merhan Mohammed Elgharbawi*, Ahmed Abdel Rahman Hashem** *and* Tarek Mostafa Abdel Aziz***

## *ABSTRACT*

**Introduction:** This study was established to compare the effect of combining agitation techniques on bacterial biofilm eradication to their effect separately.

**Methods and Materials:** Cleaning and shaping was performed on human premolars then divided randomly to four groups: passive syringe irrigation, passive ultrasonic irrigation (PUI) , XP- endo Finisher (XPF) (FKG Dentaire, Swiss Endo, Switzerland) and combined (PUI and XPF). Bacterial biofilm was evaluated by means of Confocal Laser Scanning Microscope (CLSM). Two-way mixed model ANOVA was used to evaluate different tested variables along with interactions . Pairwise t-tests with Bonferroni correction compared main and simple effects. The statistical significance level was set at $P < 0.05$.

**Results:** There was no significant difference on combining agitation techniques (PUI and XPF) in comparison to single technique agitation. PUI showed the highest significance in comparison to other irrigation techniques. Regardless of agitation technique apical third showed highest percentage of dead bacterial followed by middle and coronal thirds.

**Conclusion:** Complete eradication of bacterial biofilm is impossible. Regardless of the agitation technique used bacterial elimination is better in comparison to passive syringe irrigation.

**KEYWORDS:** XP-Endo Finisher, Passive Ultrasonic Irrigation, Bacterial Biofilm, Confocal Laser Scanning Microscope.

## INTRODUCTION

Failure of root canal treatment is a major problem in endodontics due to complexity of root canal system[1] that harbors tissue remnants and microorganisms that causes persistent infection[2]. RCT aims to ensure proper instrumentation as well as to reduce the bacterial biofilm to attain successful treatment. Studies proved that about 30%-50%

* MSc in Endodontics-Ain Shams University, Cairo, Egypt.
** Professor of Endodontics- Head of Department of Endodontics, Ain Shams University, Cairo, Egypt.
*** Lecturer of Endodontics- Ain Shams University, Cairo, Egypt.

of canal walls remain untouched[3-6] after using standard NiTi endodontic files, thus protecting the microbiota which causes reinfection and failure of treatment. Mechanical instrumentation must be complemented by proper chemical irrigation to optimize disinfection; as the configuration of files never corresponds to the canal geometry[7]. Although the wide range of chemicals present, NaOCl remains the irrigant of choice in endodontics[8]. Passive syringe irrigation is not efficient as irrigant reaches only 1-2mm beyond tip[9] in addition to the vapor lock effect[10] that impinges proper distribution. Recently agitation techniques are used to overcome drawbacks of passive syringe irrigation; ultrasonic activation initiates acoustic streaming and cavitation within the fluid which improves fluid distribution within the root canal system [9],[11] .XP-endo Finisher a non-cutting , single use NiTi file used for irrigation agitation for better cleanliness [12],[13]

### Aim of the study

It was proposed that combining agitation techniques will enhance bacterial elimination; so, the current study aimed to compare effect of combined agitation techniques on bacterial biofilm eradication to their effect separately.

## MATERIALS AND METHODS

### Specimens selection and preparation

Ethical Committee of Ain Shams University approved the current study (Approval Number : FDASU-Rec EM 021706). Forty-four single rooted mandibular human premolars were selected upon an inclusion criteria: straight roots with single root canal;free from root caries, cracks, fractures and apical resorption. Confirmation of single root canals was done by radiographs. Calculus and other soft tissue debris were removed using ultrasonic scaler. Samples were autoclaved for 20 minutes at 121°C and then stored in distilled water until use to avoid dehydration.

Low speed diamond saw was used to decoronate teeth under copious amount of water to standardize root length (15mm); St. st. K-files#10 were used to negotiate canals and confirm patency. All canals were instrumented using ProTaper NEXT (Dentsply Maillefer, Ballaigues, Switzerland) X1,X2 and X3 at speed of 300 (rmp) and torque of 3 Ncm in crown down manner. Irrigation at each file exchange using 5ml of 2.5% NaOCl delivered was by 30 G needle and then patency was checked using #10 St.st. K-file. Final flush of 5ml of 2.5% NaOCl followed by 5ml of 17%EDTA solution was done over 1 minute each. 3ml of saline after instrumentation was completed then dried with paper points (#30, Dentsply Maillefer, Ballaigues, Switzerland). All samples were autoclaved at 121°C for 20 minutes to ensure proper sterilization of samples.

### Biofilm preparation

*E.faecalis* (ATCC 29212) obtained from the American Type Culture Collection was used to prepare the bacterial inoculum. In Microbiology laboratory, isolated colonies of pure cultures of *E.faecalis* grown aerobically on BHI agar plates were suspended in 3 ml brain heart infusion (BHI) and incubated at 37°C for 24 hours in 100% humidity to allow bacterial colonization. Then suspensions were prepared on surface of BHI plates under similar incubation conditions; bacterial cells were suspended in 4ml of sterile phosphate buffered saline. Each root sample was placed in sterile Eppendorf cone and mixture of 5ml of sterilized BHI and 5ml bacterial inoculum. Samples were then inoculated with *E.faecalis*. Aliquots culture medium 5ml was replaced with fresh medium every 3 days . After 4 weeks samples were removed from Eppendorf cones and apices were sealed with fast set epoxy resin to mimic the closed system in clinical condition.

### Final agitation techniques

Samples were assigned randomly to four experimental groups (n=10) **Group I:** samples were

irrigated with 2ml of 2.5%NaOCl by a 30-gauge NaviTip (Ultradent, South Jordan, UT, USA) mounted on disposable plastic syringe with a gentle in and out movement over a period of **1 minute**. Smear layer was removed using 2ml of 17% EDTA for another **1 minute** followed by 2ml of saline as a final flush, **Group II:** Samples were irrigated with 2ml of 2.5% of NaOCl followed by 2ml of EDTA. Each solution was activated by using #25 withIrriSafe tip (Satelec, Acteon, France) mounted on a piezoelectric unit Suprasson P5 Booster unit-set at power of (5) for **1minute** 1mm of WL followed by a final flush of 2ml saline, **Group III:** Samples were irrigated 2ml of 2.5% NaOCl activated using XP-endo Finisher (mounted to Saechin endodontic motor at speed of 800 rpm and torque of 1 N. cm with slow up and down 7-8 mm long movements up to working length) for **1 minute**. Smear layer was removed by 2ml of 17% EDTA activated by XP-endo Finisher for an additional **1 minute** in the same manner followed by a final flush of 2ml saline, **Group IV:** Samples were first irrigated with 1ml of 2.5%NaOCl then by 1ml of 17% EDTA activated by IrriSafe tip #25 for **30 seconds** each solution. All samples received a final flush of 2ml saline; Followed by 2ml of 2.5%NaOCl followed by 2ml of 17% EDTA solutions activated by XP-ENDO Finisher each for **30 seconds**. All samples received a final flush of 2ml saline. All groups received 2ml sodium thiosulfate to neutralize the effect of NaOCl. **Positive control** (n=2) samples were inoculated with bacteria and didn't receive any treatment; to confirm presence of *E. faecalis* alive. **Negative control** (n=2) samples were only prepared and autoclaved without inoculation of *E.faecalis*; to confirm absence of bacteria. All samples were split using Isomet 4000 under copious amount of coolant.

### Specimens evaluation

For examination of bacterial biofilm under CLSM 1mm thickness sections were obtained from each half. Samples were stained using propidium iodide and acridine orange. Samples were imaged using Confocal Laser Scanning Microscope and analyzed by ZEN Lite 2012 software (Carl Zeiss) at a resolution of 1024 x 1024 pixels and image stacks were viewed using LSM browser.

### Statistical analysis

Numerical data were explored for normality by checking the data distribution and using normality tests (Kolmogorov-Smirnov and Shapiro-Wilk tests). Data showed parametric distribution which were represented as mean and standard deviation (SD) values. Two-way mixed model ANOVA was used to study the effect of different tested variables and their interaction. Comparison of main and simple effects were done utilizing pairwise t-test with Bonferroni correction. The significance level was set at $P \leq 0.05$ within all tests. Statistical analysis was performed with IBM SPSS Statistics Version 26 for Windows.

## RESULTS

Results of this study showed that combining PUI and XPF did not show significant difference ($P>0.05$) in their ability to eliminate bacterial biofilm in comparison to single agitation technique. Regardless of the root level, all groups showed higher mean percentage of dead bacteria in comparison to conventional syringe irrigation (38.76±9.28) as seen in ***table (1)***. Regardless of irrigation technique, apical third showed statistically significant highest percentage of dead bacteria (49.66±11.47) as in ***table (2).*** Average of dead bacteria (%)for different root sections within each irrigation technique is demonstrated in ***table (2).*** CLSM images from coronal, middle and apical thirds of each technique demonstrating live bacteria (green) and dead bacteria (red) are shown in ***figure (1)***.

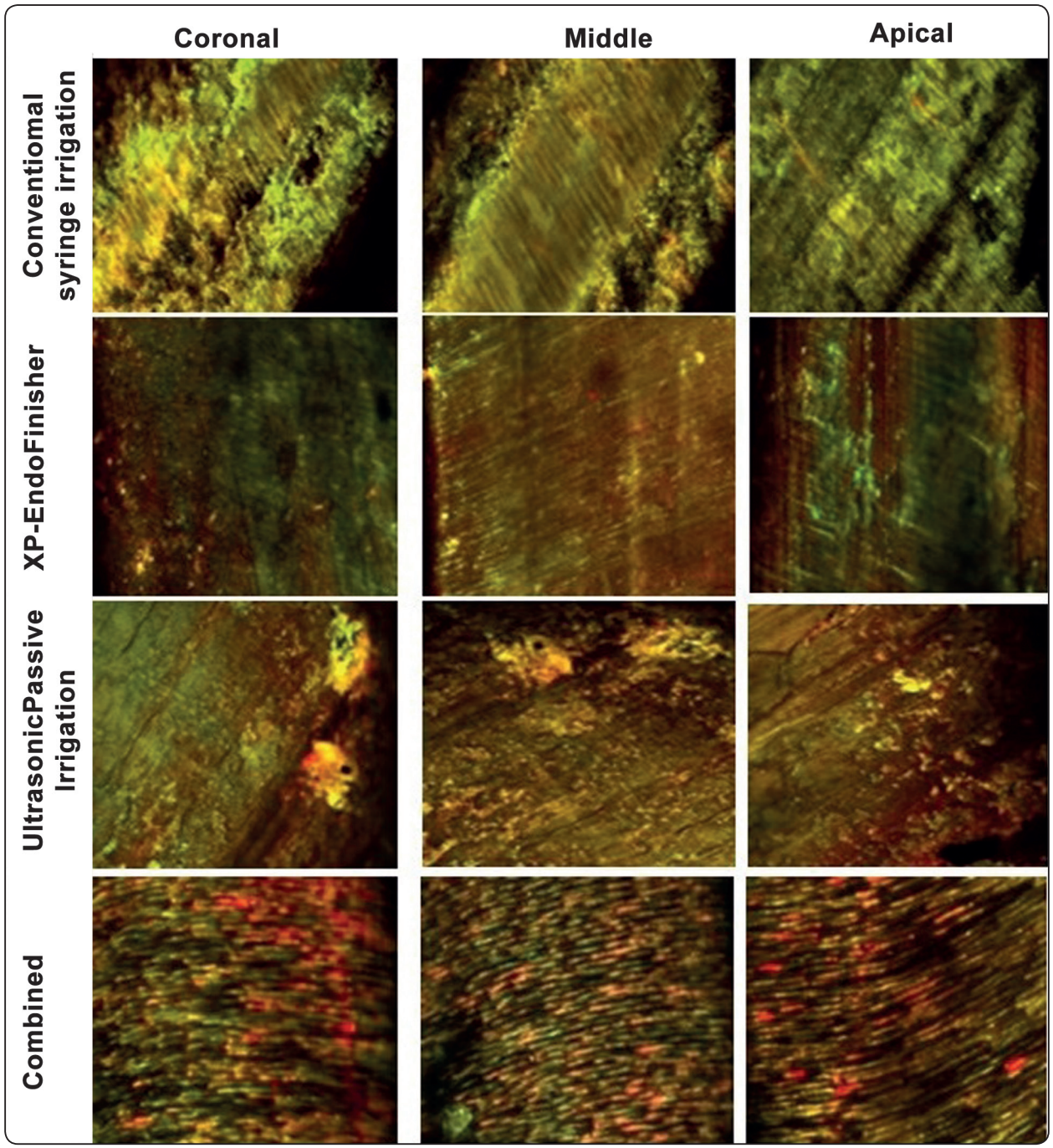
Coronal
Middle
Apical
Conventiomal syringe irrigation
XP-EndoFinisher
UltrasonicPassive Irrigation
Combined

Fig. (1)

TABLE (1): Mean ± standard deviation (SD) of dead bacteria (%) for different irrigation techniques.

| Conventional syringe irrigation | PUI | XPF | Combined |
|---|---|---|---|
| $38.76\pm9.28^{B}$ | $50.68\pm13.86^{A}$ | $48.64\pm9.58^{A}$ | $48.56\pm5.78^{A}$ |

***Different superscript letters indicate a statistically significant difference within the same horizontal row*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).***

TABLE (2): Mean ± standard deviation (SD) of dead bacteria (%) for different root sections within each irrigation technique.

| Irrigation technique | Coronal | Middle | Apical | P-value |
|---|---|---|---|---|
| **CSI** | $43.71\pm8.83^{A}$ | $41.40\pm5.66^{A}$ | $31.12\pm9.30^{B}$ | **0.026*** |
| **PUI** | $41.06\pm7.62$ | $49.46\pm7.34^{B}$ | $71.88\pm6.58^{A}$ | **<0.001*** |
| **XPF** | $48.65\pm6.82^{A}$ | $49.78\pm12.58^{A}$ | $47.27\pm8.51^{A}$ | **0.855ns** |
| **Combined** | $47.24\pm6.68^{A}$ | $48.39\pm3.16^{A}$ | $49.95\pm6.40^{A}$ | **0.297ns** |
| **Total** | $45.61\pm9.20^{b}$ | $47.86\pm8.63^{b}$ | $49.66\pm11.47^{a}$ | **<0.001*** |

***Different superscript letters indicate a statistically significant difference within the same horizontal row*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$).***

## DISCUSSION

To obtain a successful endodontic treatment microorganisms should be properly eliminated from root canal system to allow a 3D hermetic obturation which will promote healing of periapical tissues, prevent reinfection and failure of RCT[14] Because of the variations of root canal system anatomy, studies proved that at least 35% or more of root canal walls were left un-instrumented after mechanical preparation[3],[4],[5],[6].Not only due to the anatomical variations but also due to the geometrical dissymmetry between root canal anatomy and instruments [7].The left unprepared areas remain a harbor for bacteria, bacterial byproducts, tissue remnants and debris which remain as a source for persistent infection[2]. These areas can only be cleaned chemically which clarifies why RCT is a chemo-mechanical procedure; irrigation has a major role to complement instrumentation. Irrigation has mechanical (flushing), chemical and micro (biological) functions which make it a key factor in endodontic success. Passive syringe irrigation is a common technique used to irrigate root canals; however, it is not effective in cleaning the apical third of the root due to the smaller diameter when compared to coronal and middle thirds thus compromises the circulation of the irrigant. Irrigation only progress 1mm beyond needle tip[15] that never allows the solution to reach the apical third as the needle tip is usually located at the coronal third in narrow canals and middle thirds of wide canals[16]. So, it is of great importance to dynamically deliver the irrigant apically to ensure proper disinfection; as the irrigant can only disinfect when in direct contact with surface. It is not only that conventional syringe irrigation have a weak

mechanical flushing action[9],[16-18] ,but also vapor lock effect. Studies proved that vapor lock (air entrapment in apical third when a liquid solution advances into a closed end microchannels) hinders the delivery of irrigation apically which negatively affects disinfection[18],[19],[20]. Each agitation technique is considered to have limitations so it is proposed that combining agitation techniques will improve penetration of irrigant to eradicate bacterial biofilm. Facultative anaerobes mainly E. *faecalis* noticed as the most common microorganism isolated from failing obturated teeth with chronic periapical pathology[21],[22]. Over time these bacteria colonize and develop a biofilm which makes it more challenging to eradicate. Oral bacteria mainly *E. faecalis* has the ability to obtain nutrition from the fluids within the periodontal ligaments and alveolar bone to remain viable for long periods[23]. Different procedures of detecting bacteria are available such as **microbiological sampling, histological sections**, **transmission electron microscope** and **high-resolution SEM**; each technique have its limitations to properly evaluate bacterial biofilm [24]. To properly detect and study bacteria viability **Confocal Laser Scanning Microscope** was used to image samples of this study to overcome the 2D imaging of a 3D biofilm and understand its multilayered environment of bacteria embedded in an extracellular matrix[24]. CLSM has the ability to detect the viability of bacterial cells, architecture of biofilm and spatial distribution of bacteria within biofilm[24].Most importantly is its ability to eliminate out-of-focus light thus no image haziness which provides high quality images[24] In addition, it has the ability to image thin optical sections (slicing technique) of thick bacterial biofilms which provides detailed and precise analysis of biofilm[25].We found that complete eradication of *Enterococcus faecalis* was impossible regardless of the activation techniques and irrigant used; results of this study are in accordance with results of previous studies done by *Mancini et al.*[11], *Capar and Aydinbelge* [26] and *Sanghamitra et al.*[27]Passive ultrasonic irrigation operates in a transverse manner to produce nodes and antinodes which results in high turbulence intensity (>96%)[28] in solution associated with better disinfection of apical area, fins and isthmuses which agrees with the results of our study. In addition to the higher wall shear stress (875 Pa) compared to syringe irrigation (open-ended: 185 Pa) & (Side-vented: 425 Pa) that results in acoustic streaming[28],[29]. Better removal of bacterial biofilm using PUI is due to the high power that results causing de-agglomeration and cavitational streaming (by creating expansion and contraction of air bubbles in a solution). This results in weakening of bacterial cell membrane thus increasing NaOCl permeability [31]. Results of this study were in accordance with previous studies that concluded that PUI showed better biofilm removal. Previous studies concluded that PUI showed better penetration of solution into lateral canals, tissue dissolution and smear layer elimination in comparison to syringe irrigation.

The resultant acoustic microwaves, cavitation and heat generation justifies these results[9],[32]. In accordance with the results of the current study that denotes that PUI group showed the highest percentage of dead bacterial in comparison to other experimental groups, as well as the apical third of PUI showed significantly higher percentage of dead bacteria among all groups. *Bao et al.*[33] quantitively evaluated efficacy of three agitation techniques (CNI, PUI and XPF) on biofilm removal using SEM. They concluded that XPF showed highest biofilm elimination followed by PUI. No statistical differences when results of apical, middle and coronal thirds were compared. The difference in results between this study and our study maybe due to the different ultrasonic tips used in each study as Bao used E12 endodontic tip and a U- file # 20 while we used Irrisafe Tip size #20/21mm. According to the results of recent study, Xp-Endo Finisher showed no statistically significant

difference between coronal and middle thirds when compared with those of conventional syringe and PUI. On the other hand, XPF showed significantly higher percentage of dead bacteria apically when compared with conventional syringe irrigation this could be due to the gentle scraping effect by XPF, ability to transform shape at different temperatures (Max-Wire Alloy), its helical movement and better distribution of solution. Although it was expected that combining two irrigation techniques will give a synergistic effect on eradication of biofilm; results of this group did n't show higher percentage of dead bacteria when compared to single technique. This may be due to the fact that combining two techniques required greater time to show greater number of dead bacteria but in this study irrigation time was the same in all experimental groups to achieve standardization. Regardless of root level PUI, Xp-Endofinisher and combination of both techniques showed statistically significant higher mean percentage of dead bacteria than conventional syringe irrigation. Regarding the root level no matter, the technique used, apical third showed the highest mean percentage of dead bacteria; while the lowest percentage was in the coronal third. Which may be due to the greater mechanical action of both PUI and XP-endofinisher apically than coronally. Considering limitations of the current study, the null hypothesis that combining two activation techniques (PUI & XPF) will increase bacterial biofilm eradication has been rejected.

## CONCLUSION

The current study concluded that passive syringe irrigation is not a suitable method to eliminate bacterial biofilm so agitation of irrigant is a must. Regardless of agitation technique complete eradication of biofilm is impossible. Combining two agitation techniques did not show the expected eradication of bacterial biofilm. CLSM is a precise evaluation tool in detecting live and dead bacteria.

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