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## **Evaluation of the efficiency of brush file as irrigation agitation technique versus passive ultrasonic irrigation on biofilm eradication and calcium hydroxide removal from straight root canals: A comparative *in-vitro* study**

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### **Abstract**

**Aim:** The study aimed to evaluate the efficiency of the Brush File and “passive ultrasonic” irrigation as irrigation agitation techniques compared to “conventional syringe irrigation” on “biofilm” eradication and “calcium hydroxide paste” removal from straight root canals.

**Materials and Methods:** Thirty-six extracted human single-rooted teeth with single canals were decoronated, mechanically prepared, and inoculated with “*E. faecalis*” for three weeks, and biofilm formation was verified using SEM (n = 3). According to the irrigation agitation method, teeth were divided into three equal groups (n = 11); Brush File group, PUI group, and “conventional syringe irrigation” group “control group”. Residual bacterial biofilm was taken by three sterile paper points, and CFUs were counted and calculated to CFUs/ml. For comparing calcium hydroxide removal efficacy, another thirty-three extracted single-rooted with single canals were instrumented, filled with Ca(OH)<sub>2</sub> paste for one week, and assigned randomly to one of three groups (n = 11), as previously mentioned. Then, Specimens were longitudinally split, and “Ca (OH)<sub>2</sub> remnants” were examined under 25x magnification using a stereomicroscope, calculated in mm<sup>2</sup>, and the percentage of Ca(OH)<sub>2</sub> in the specimen's “coronal, middle, and apical thirds” was recorded using image analysis software.

**Results:** Regarding “*E. faecalis*” biofilm eradication, there was a “significant difference” (p= 0.00) between the three groups; however, there was “no significant difference” between the Brush File and PUI. Regarding Ca(OH)<sub>2</sub> removal, the intergroup comparison revealed a “significant difference” across the three groups at “coronal, middle, and apical thirds” (p= 0.00), with “no significant difference” between the Brush File and PUI groups. The Brush File and CSI groups had “significantly higher “values of “Ca(OH)<sub>2</sub> remnants” in the apical third.

**Conclusion:** Brush File was as efficient as PUI in terms of “biofilm eradication” and “calcium hydroxide” removal, and both agitation techniques were superior to the “conventional irrigation” regimen.

**Keywords:** Brush file, PUI, irrigation activation, *E. faecalis*, biofilm, calcium hydroxide

### **Introduction**

Effective “chemo-mechanical” preparation of the “root canal system” is required for successful “endodontic treatment”. Pulp tissue remnants, dentin debris, microorganisms, and residual intracanal medication should be adequately removed from the root canal system to enable 3D hermetic obturation, promote periapical tissue healing, and prevent reinfection and “root canal treatment” failure. Data from studies show that at least 35% of the canal walls continue to be untouched after mechanical preparation [1-6]. This is probably due to variations in root canal system anatomy. In addition to the anatomical variations, there is also a geometrical asymmetry between the anatomy of the root canal and the instruments [7]. The areas that were not adequately prepared continue to harbor microorganisms, bacterial byproducts, tissue remnants, and debris, all of which are persistent infection sources [8]. This makes it clear why “root canal treatment” is a “chemo-mechanical” process; irrigation plays a significant part in complementing instrumentation.

Only chemical cleaning methods are suitable for these areas. "*Enterococcus faecalis* (*E. faecalis*)" is the most prevalent bacterial species responsible for primary and secondary "endodontic infections" [9]. It is a fact that microbes inside infected root canals exist in a "biofilm" instead of a planktonic form. "Biofilm" is an organized community of bacterial cells that are enclosed in a self-made matrix of "extracellular polymeric substances (EPS)". This EPS matrix increases survival in challenging conditions with nutrient deficiency and antimicrobial agent resistance [10]. Eradication of this infected "biofilm" is always a challenge in endodontics.

It is frequently required to use an "intracanal medicament", such as "calcium hydroxide paste", to disinfect the "root canal system" while improving endodontic treatment outcomes [11]. This intracanal dressing needs to be completely removed before root canal obturation to ensure a tight seal of the filling material. Instrumentation using a "master apical file" (MAF) and irrigation with "NaOCl" and "EDTA" is the most commonly employed technique for removing calcium hydroxide paste [12]. However, complete removal cannot be obtained, and remnants are frequently left on the root canal walls, particularly in the apical third. Moreover, this, in turn, might cause apical microleakage and negatively influence the success of "endodontic treatment" [13-15].

"Passive ultrasonic irrigation" (PUI) is a non-cutting irrigation approach that uses ultrasonic waves to transport acoustic energy from a smooth wire or an oscillating file to the irrigant solution within the canal space. This irrigation method induces two physical phenomena: stream of irrigation solution and cavitation, which disrupts the vapor lock. PUI outperforms "conventional needle irrigation" in eliminating any remaining pulpal tissue and "dentine debris" because it increases irrigant flow velocity and volume in the canal [16].

The Brush File Max is a newly designed irrigation agitation tool composed of six strands of stainless-steel wire twisted together with a latch-type head for use with rotating handpieces. When the metal bristles are closed, it has a diameter of 0.27 mm; upon rotation inside the root canal, it opens its bristles into a metal brush that, according to the manufacturer, activates the irrigation solution, enhances the cleaning of inaccessible areas from smear layer, debris, biofilm remains, and aids in removing filling paste and material residues from the canal walls. In addition, it is a cost-effective agitation technique.

Thus, this study aimed to evaluate the efficiency of the Brush File and "passive ultrasonic irrigation" as irrigation agitation techniques compared to "conventional syringe irrigation" on "biofilm" eradication and "calcium hydroxide" removal from straight root canals.

## Materials and Methods

A total of 69 extracted human permanent single-rooted teeth were selected in the present study after the ethics committee

(EC) approval of the "Faculty of Dentistry at Cairo University" (15-5-21). Teeth were assessed radiographically from mesio-distal and bucco-lingual directions to rule out immature, carious, cracked, resorbed, or calcified teeth. All procedures were performed by the main investigator.

## Specimen Preparation

Teeth were cleaned from any hard debris using hand scalers and then submerged in "5.25% NaOCl" for 30 mins enabling soft tissue removal. The occlusal surface of all teeth was flattened using a low-speed diamond stone under copious irrigation to obtain 16 mm uniform root lengths. After access cavity preparation, the canal's patency was evaluated with K-file size #10 "MANI, Matsutain Seisakusho Co., Tochigi-Ken, Japan", and a size 15 K-file 1 mm shorter than the tooth length was used to determine the working length. All canals were instrumented using ProTaper Next rotary files "Dentsply Maillefer" up to X4 (40/06) file using the E-connect S "Eighteenth, Changzhou Sifary Medical Technology Co., Ltd. China" wireless endo-motor.

Between every subsequent file, the canals were irrigated with 3 ml of a "2.6% NaOCl" solution. Following the mechanical preparation, the canals were irrigated with 3mL of normal saline, then 3 ml of 17% EDTA solution for 1 min for "smear layer removal" followed by 3 ml of "saline" and then, dried with F4 paper points "Dentsply Maillefer". The flowable composite "Filtek Supreme, 3M ESPE, St. Paul, Minnesota, USA" was used to seal the apical foramina. The root surface was sealed using two layers of adhesive resin "3M ESPE, St Paul MN, USA" to make it impermeable.

## Root Canal Biofilm Formation

### Specimen Sterilization

- The Specimens were autoclaved for 15 minutes at 121 °C to ensure proper sterilization.

### Culture and inoculum preparation

- In the microbiology laboratory, A suspension was made by adding 1 ml of a pure culture of "*E. faecalis*" (ATCC 29212) that had been grown for 24 hours in sterile brain-heart infusion broth (BHI) "Oxoid microbiology product, LTD, England". The turbidity of suspension was equivalent to  $\pm 0.5$  McFarland standard ( $1.5 \times 10^8$  CFUs/mL). All root canals were filled with 30 $\mu$  of "*E. faecalis*" suspension using a micropipette. The bacterial suspension was distributed to the root canal's whole length using sterile #15 K-type files.
- The infected specimens were placed inside a sterile Eppendorf tube and incubated aerobically for 21 days at 37 °C with refreshment by sterile BHI media every 48 hours.
- To verify the formation of the "*E. faecalis* biofilm", three random teeth were selected from among the specimens for "Scanning electron microscopy" (SEM) analysis at 1500x magnification.

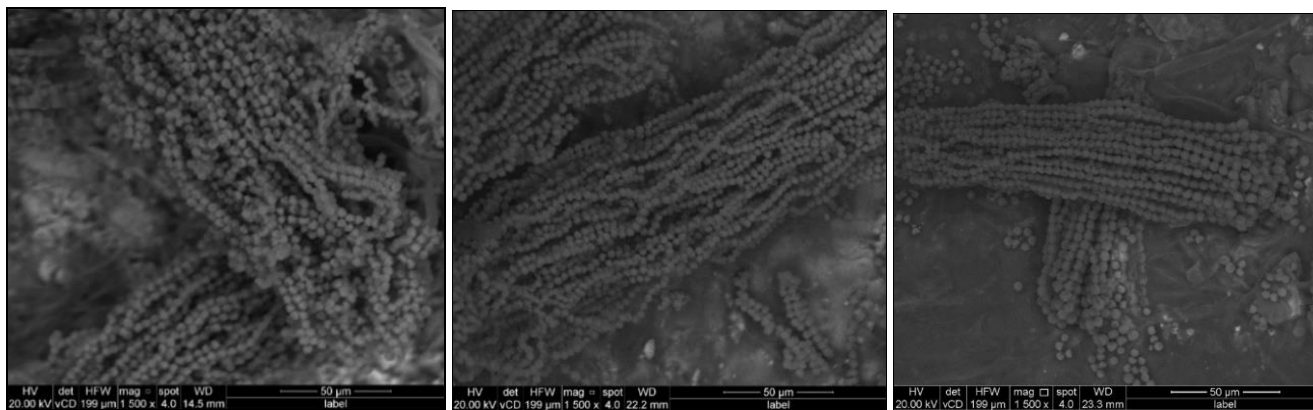


Fig 1: SEM images verifying the presence of 21-day-old *E. faecalis* biofilm.

### Experimental groups

The specimens were randomly allocated into three groups (n=11) based on the irrigation agitation regimen:

1. **Brush File group:** The canals were irrigated with 5 mL “2.6% NaOCl” that was activated for one minute with a Brush File rotating at a speed of 1000 rpm and torque value 0.5 N.cm. The Brush File was introduced 1 mm short of the WL, and the irrigation was activated for 1 min. The canals were then irrigated with 2.5 mL of “normal saline”, then 5 mL of “17% EDTA” that is activated for one minute with Brush File, as mentioned. After that, 2.5 mL of “normal saline” was used to flush the canals. One Brush File per specimen was used.
2. **PUI group:** The canals were irrigated with 5 mL “2.6% NaOCl” activated with a “Satalec P5 Newtron” ultrasonic system “Acteon® Group, Merignac, France Satalec” and an IrriSafe tip (25/0.0) “Acteon® Group, Merignac, France Satalec” in the sixth power setting. The IrriSafe tip was introduced 1 mm short of the WL, and the irrigation was activated for one minute. To keep the file from dampening its oscillatory motion, it was kept as centered as possible, away from the canal walls. The canals were then irrigated with 2.5 mL of “normal saline”, then 5 mL of “17% EDTA” that is activated with PUI for one minute. After that, 2.5 mL of “normal saline” was used to flush the canals. One IrriSafe tip per specimen was used.
3. **CSI group (Control group):** The canals were irrigated with 5 ml of “2.6% NaOCl” for one minute using a disposable plastic syringe with a “30-gauge-max-i-probe” side vented needle reaching 1-2 mm short of the WL. The canals were then irrigated with 2.5 mL of “normal saline”, then 5 mL of “17% EDTA” for an additional one minute. After that, 2.5 mL of “normal saline” was used to flush the canals.

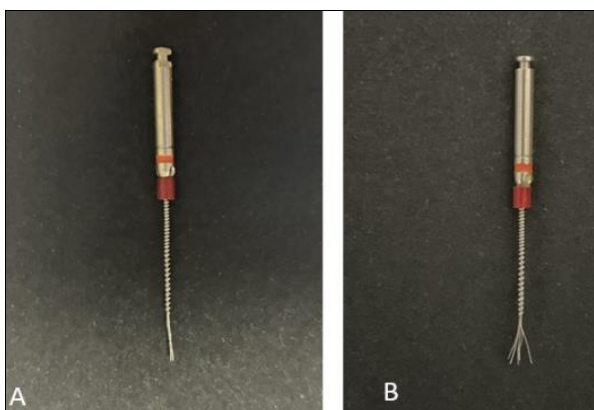


Fig 2: (A) Brush File with its metal bristles closed. (B) Brush File with its metal bristles open

### Root Canal Sampling and Colony Counting

To collect the samples, each canal was filled with a sterile 0.9% saline solution, and three sterile paper points (F4) were inserted into the full WL and saturated for 1 min. After that, paper points were transferred to sterile eppendorf tubes containing 1 ml of phosphate-buffered saline (PBS), and they were vortexed for 1 min. The solution was then diluted to 1/10 concentration by placing 100 μl aliquots of the vortexed samples into a sterile eppendorf tube with 900 μl of PBS. Sterile micropipettes with yellow tips were used to collect 20 μl from tubes and then smeared on brain-heart infusion agar plates “TM MEDIA®, TITAN BIOTECH LTD. Rajasthan, India” using a sterile L-shaped glass rod, then the plates were placed in the incubator at 37 °C for 48 hours. Each Plate was examined for bacterial growth; visible colonies of “*E. faecalis*” were counted and calculated to CFU/ml.

### Sample Preparation for “Ca(OH)<sub>2</sub> paste” placement

Thirty-three human single-rooted teeth with single canals were recruited and prepared, as previously mentioned. The canals were filled with “calcium hydroxide paste”, Metapaste “Meta Biomed Products, Chungju, Korea”, using disposable applicator tips positioned 1 mm from the apex. The tip was slowly retracted from the canal as the paste was injected until the paste could be seen extruding from the canal's apex and packed to the whole WL of the canal. Periapical radiographs were taken to confirm the density and length of Ca(OH)<sub>2</sub> paste filling. Then, the canals were sealed apically with flowable composite. To seal the access cavities, a tiny cotton pellet and temporary filling “ORAFIL G TEMPORARY FILLING, Prevest Direct, India” were used.

The specimens were stored at 37 °C in 100% humidity for seven days and assigned randomly to one of three groups (n = 11), as previously mentioned following this time, the temporary filling was removed. The irrigation agitation steps were done as previously mentioned regarding each group. After that, the canals were dried using paper points.

### Specimen preparation for stereomicroscope assessment of “Ca(OH)<sub>2</sub> paste” removal

Following different irrigation regimens, the paper points were left inside the root canals to prevent dentin dust from seeping into the areas of root evaluation during splitting. Then, roots were separated longitudinally using a diamond disc at low speed and copious irrigation. Later, the specimens were split longitudinally with a chisel and a mallet allowing for the subsequent Stereomicroscope analysis for residual “Ca(OH)<sub>2</sub> paste”. The intact half was chosen to evaluate residual “Ca(OH)<sub>2</sub> paste”. All specimens in each group were evaluated through a stereomicroscope “TECHNIVAL 2, CARLA

ZEISS, GERMANY". The image of the whole specimen was taken at 10x magnification. "The coronal, middle, and apical thirds" of the root canal were examined individually in each specimen at 25x magnification.

**Evaluation of "Ca(OH)<sub>2</sub> paste" removal**

Using the image analysis software "Image ware, IMAGEJ, version 1.6.0-20, USA", the remaining Ca(OH)<sub>2</sub> on each third of the canal walls was computed in mm<sup>2</sup>. The procedure for calculating "Ca(OH)<sub>2</sub> remnants" on canal walls was as follows: Initially, the canal boundaries were manually traced on the program, allowing the canal surface area to be calculated. The total area covered by "Ca(OH)<sub>2</sub> remnants" was calculated by darkening all Ca(OH)<sub>2</sub> parts. Then, the percentage of the canal surface area occupied by "Ca(OH)<sub>2</sub> remnants" was calculated.

**Statistical analysis**

"IBM SPSS Statistics version 20" for Windows was used for managing data and statistical analysis. The terms "mean", "standard deviation", "median", and "range" were used to summarize numerical data. The "Kolmogorov-Smirnov" and "Shapiro-Wilk" tests and the data distribution were used to

determine the normality of the data. The one-way analysis of variance "ANOVA test" was used to compare groups in terms of normally distributed numeric variables (CFUs), followed by the "Bonferroni post hoc test" for pairwise comparison. The "Kruskal-Wallis test" was used to compare groups in terms of non-parametric numeric variables and "calcium hydroxide paste" removal. To study the interaction of the group and segment variables, the two ways "ANOVA test" was performed. All "p-values" are two-sided. "P-values ≤0.05" were considered significant.

**Results**

**1. Bacterial counts (x10<sup>4</sup> CFU/mL)**

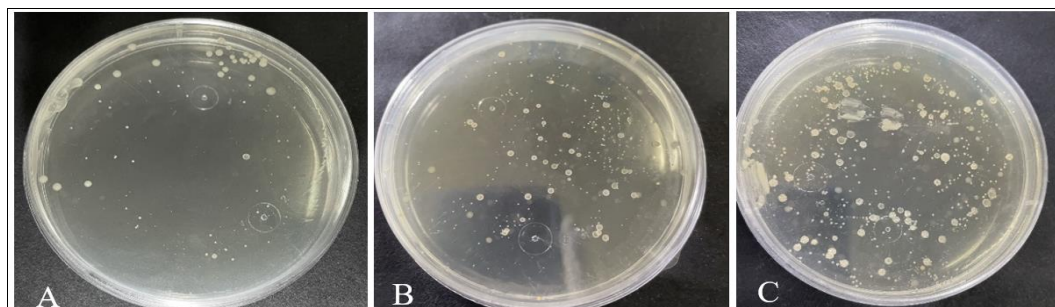
- The highest "mean" value was recorded in group 3 (CSI) [218.64±38.8], followed by group 2 (PUI) [80.73±8.72], with the least value recorded in group 1 (Brush File) [72.64±72.64]. The difference between groups was "statistically significant" (p= 0.00). "The post hoc test" revealed "no significant difference" between the Brush File and PUI groups.
- The results also showed that ...none of the experimental irrigation protocols obtained 100% eradication of "*E. faecalis*" biofilm.

**Table 1:** Descriptive statistics and comparison of CFUs/mL (X104) and comparison between groups "ANOVA test"

	Mean	SD	"95% Confidence Interval for Mean"	P - Value
Group 1 (Brush File)	72.64 <sup>b</sup>	12.31	(64.37-80.90)	0.000*
Group 2 (PUI)	80.73 <sup>b</sup>	8.72	(74.87-86.58)	
Group 3 (CSI)	218.64 <sup>a</sup>	38.81	(192.56-244.71)	

"Significance level ≤0.05, \*significant

Means sharing the same superscript letter are not significantly different."



**Fig 3:** Remaining bacterial colonies after using (A) Brush File, (B) PUI, (C) CSI

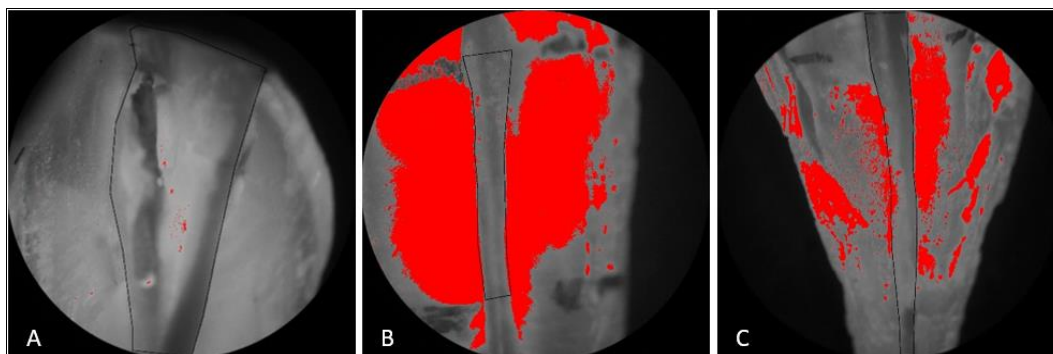
- Regarding "calcium hydroxide paste" removal... the result showed "no significant difference" between the brush file group and the PUI group. The highest values of "Ca(OH)<sub>2</sub> remnants" were recorded in the CSI group with a "significant difference" from the other groups in all thirds (p= 0.00).
- While comparing Ca(OH)<sub>2</sub> remnants within the same group, the apical third showed a significantly higher

value of Ca(OH)<sub>2</sub> remnants in the Brush File and control groups with no significant difference between "the coronal and middle thirds". Within the PUI group, the "middle third" showed a higher value of Ca(OH)<sub>2</sub> remnants than "the coronal and apical thirds". The difference between segments was "not statistically significant."

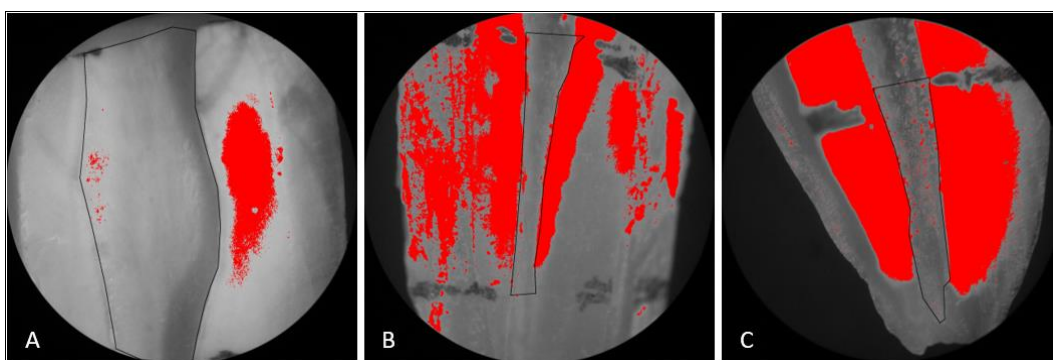
**Table 2:** Descriptive statistics and Intragroup comparison of calcium hydroxide paste percentage in different segments within the same group "Kruskal Wallis test"

Groups		Median	Mean	SD	"95% Confidence Interval for Mean"	P - Value
Group 1 (Brush File)	Apical	5.49 <sup>a</sup>	6.16	4.90	(2.88-9.45)	0.047*
	Middle	2.59 <sup>a, b</sup>	4.44	5.12	(1.11-7.98)	
	Coronal	0.54 <sup>b</sup>	2.19	3.43	(-0.12-4.49)	
Group 2 (PUI)	Apical	4.24	4.39	3.19	(2.84-7.13)	0.056 ns
	Middle	4.58	4.47	3.43	(2.17-6.77)	
	Coronal	1.57	2.38	3.48	(0.04-4.71)	
Group 3 (CSI)	Apical	33.58 <sup>a</sup>	39.18	15.94	(28.47-49.89)	0.001*
	Middle	13.37 <sup>b</sup>	19.88	20.38	(6.19-33.57)	
	Coronal	13.57 <sup>b</sup>	15.33	8.94	(9.32-21.34)	

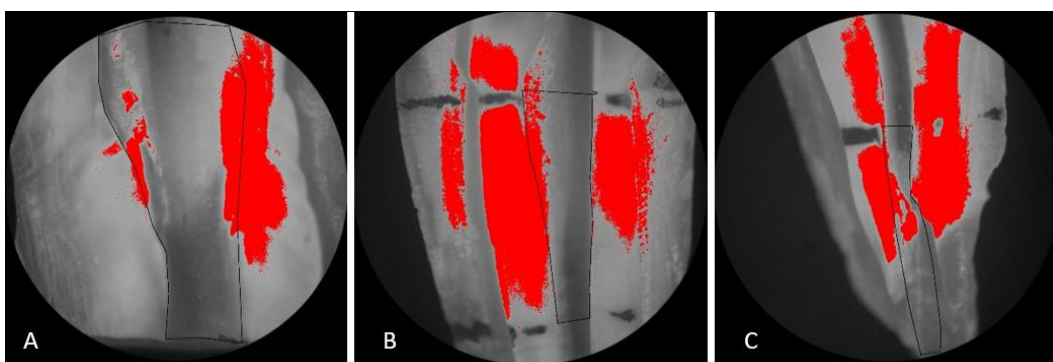
"Significance level ≤0.05, \*significant, ns=non-significant...medians sharing the same superscript letter are not significantly different."



**Fig 4:** Stereomicroscope images of (A) coronal, (B) middle, and (C) apical thirds of the Brush File group



**Fig 5:** Stereomicroscope images of (A) coronal, (B) middle, and (C) apical thirds of the PUI group



**Fig 6:** Stereomicroscope images of (A) coronal, (B) middle, and (C) apical thirds of the CSI group

## Discussion

“Conventional syringe irrigation” is the most often used irrigation technique. Although the “apical third” of the root canal has a smaller diameter than “the coronal and middle thirds” of the root canal, this causes inadequate irrigant circulation, making the apical third challenging to clean. Since the needle point is typically positioned in the coronal segment of narrow canals and the middle third of large canals<sup>[17]</sup>, irrigation only extends 1 mm past the needle tip<sup>[18]</sup>, preventing the solution from reaching the “apical third”. It is critical to dynamically deliver the irrigant apically to ensure proper cleansing and disinfection, as the irrigant can only disinfect when in direct contact with the surface. The mechanical flushing effect of passive needle irrigation is minimal, as described by Guerreiro-Tanomaru *et al.*, 2013<sup>[19]</sup>. Also, the vapor lock effect, or the entrapment of air around the needle's tip, is a problematic phenomenon that can arise during “passive needle irrigation” and potentially prevents the irrigant from making contact with or disinfecting the apical region<sup>[20-23]</sup>.

Activation irrigation, either by passive ultrasonic irrigation, canal brushes, or any other agitation technique, appears to be an essential method of increasing the antibacterial and

antibiofilm activity of root canal irrigants, not only within the root canal but also within the anatomical complexities of the root canal system and dentinal tubules. Activation of irrigants may further improve the removal of the intracanal dressing. After reviewing the available literature, we determined that the Brush File had yet to be evaluated as an irrigation agitation technique for biofilm eradication and calcium hydroxide removal from straight root canals. Hence, this *in vitro* study aimed to evaluate the efficiency of the Brush File and “passive ultrasonic irrigation” as irrigation agitation techniques compared to “conventional syringe irrigation” on “biofilm eradication” and “calcium hydroxide paste” removal from straight root canals.

Regarding the results of the current study, higher CFUs values were encountered in the control group, where conventional syringe irrigation without activation was implemented. This finding came in accordance with several studies that attributed this result to the limited hydrodynamic action of the irrigation solutions with this technique, which is something incompatible with the complexity of root canal morphology, including multiple recesses, ramifications, isthmuses, and lateral canals<sup>[17, 19]</sup>.

On the contrary, the other groups that used PUI and Brush

File as irrigation agitation methods achieved maximum disinfection by considerably reducing intraradicular bacterial biofilm compared to CSI. These findings are congruent with those of Abusrewil *et al.*, 2020 [24], who determined that irrigation activation through agitation causes a greater reduction in microbial load than CSI.

The efficiency of PUI in the removal of biofilm was supported by many authors [25–28], who demonstrated superior antibiofilm efficacy of PUI over syringe irrigation. Also, Nagendrababu *et al.*, 2018 [29] conducted a systematic review that compared the effectiveness of “ultrasonically activated irrigation” (UAI) in root canal disinfection to other types of irrigant activation and conventional syringe irrigation. It concluded that root canal microbes are reduced more effectively when UAI systems are used.

However, these findings contradicted the findings of Guerreiro-Tanomaru *et al.*, 2015 [30], who compared the efficacy of the PUI with CSI protocol and did not find a significant difference between PUI and CSI. This may be advocated by the lower concentration of NaOCl 1%, and the activation time was 40sec implemented in their study. Also, their study used only 5 ml of irrigation, either NaOCl or saline solution, rather than the 15 ml used in our study “5 ml of 2.6% NaOCl, 5 ml of 17% EDTA, and 5 ml saline solution”. Meanwhile, the results are in contrast to those shown by Orozco *et al.*, 2020 [31] and Gründling *et al.*, 2011 [32], who found “no significant difference” between PUI and CSI. This possibly is related to differences in experimental design.

No previous study assessed the effectiveness of the Brush File as an irrigation agitation technique. However, Neelakantan *et al.*, 2018 [33] evaluated a similar instrument called the Gentlefile-Brush and compared its performance to that of CSI in removing pulp tissue remnants following root canal instrumentation and concluded that using such an instrument as an irrigation agitation technique decreased the pulp tissue remnants more than CSI.

In terms of “Calcium hydroxide paste” removal, the CSI group exhibited the highest value of “Ca(OH)<sub>2</sub> remnants”, especially at the apical part. The findings of Gawdat & Elkhodary (2017) and Shi *et al.* (2021) [34, 35] were in accordance with these results. This is maybe anticipated due to the lack of activation regimen with the control group with the implemented CSI that had a limited efficacy regarding apical debridement and overcoming the vapor lock effect with a subsequent negative impact on Ca(OH)<sub>2</sub> removal capability of the irrigation solution [36,37].

In contrast, the other groups wherein the PUI and Brush File were utilized as irrigation agitation methods exhibited lower “Ca(OH)<sub>2</sub> remnants” values in all segments with “no statistically significant difference”. This was consistent with the findings of Van Der Sluis *et al.*, 2007 [38] and Taşdemir *et al.*, 2011 [39], who determined that mechanical agitation of the irrigant was more successful than irrigation without agitation in eliminating Ca(OH)<sub>2</sub>. This agitation boosted irrigant penetration into the irregular canal areas.

However, Balvedi *et al.*, 2010 [40] investigated the efficiency of PUI over CSI and found “no significant difference” in “Ca(OH)<sub>2</sub> paste” removal in the “apical third” of root canals within these two techniques. This could be due to a difference in experimental design.

The results of the (PUI) group revealed no statistically significant difference in the value of Ca(OH)<sub>2</sub> remnants between segments; this part of the findings came in agreement with Gawdat & Elkhodary, 2017 [34].

The results of the Brush File group agreed with the study of

Koprowicz & Koprowicz, 2021 [41], which reported the use of the Gentlefile-Brush for improving Ca(OH)<sub>2</sub> removal.

Regardless of technique, Ca(OH)<sub>2</sub> elimination was more efficient in “the coronal” and “middle thirds” of the canal than in the apical third. This could be due to the large dimensions of the root canal at these levels, which expose the root canal dentin to larger volumes of irrigation solutions with better hydrodynamic flow and higher shear stresses than the apical dentin [42, 43]. This can also be linked to the vapor lock effect, which occurs along the canal's apical 0.5–1 mm, causing fluid stagnation in this area, a dead water zone, and compromising proper irrigant replacement [20]. As a result, “calcium hydroxide paste” is removed more effectively from the coronal third and middle third. These findings were congruent with those of Gokturk *et al.*, 2017 [44], who detected more “Ca(OH)<sub>2</sub> remnants” in the apical segment of root canals with simulated irregularities after using XP and PUI.

The comparable ability of Brush File to PUI might be explained by the similar efficiency of both techniques in activating the irrigant solution inside the root canal, removing the adherent biofilm, and flushing “Ca(OH)<sub>2</sub> remnants”. However, the irrigation activation mechanisms of both approaches were different. The removal of the biofilm and Ca(OH)<sub>2</sub> by Brush File is a result of physical contact between the brush file and the canal walls, as the Brush File functions by opening the six stainless-steel strands when rotated. This may scrape the canal walls more efficiently than syringe irrigation to eliminate adherent “microbial biofilms” and Ca(OH)<sub>2</sub>. It is also a result of the activation of the irrigation solution due to its rotation at 1000 rpm. PUI's mechanism of action depends on the acoustic microstreaming and cavitation from an oscillating file or smooth wire, which causes shear stress, causing the solution to stream from the apical to the coronal direction [38]. This unique characteristic may be responsible for the irrigant activation, permitting the disruption and elimination of biofilm and “calcium hydroxide paste”.

## Conclusions

Within the limitations of the present study, no irrigation/irrigation agitation technique could completely remove “*E. faecalis* biofilm” and “calcium hydroxide paste”. However, Brush File was as efficient as PUI in terms of “biofilm eradication” and “calcium hydroxide removal”, and both agitation techniques were superior to the CSI.

## Recommendations

We recommend Further investigations with larger and more representative sample sizes to form an evidence-based conclusion on the efficacy of the Brush File as an irrigation agitation regimen. Additionally, Further investigations are recommended to compare Brush File with other recent methods of irrigant activation.

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This study is self-funded.

## Conflict of Interest

The authors declare no conflict of interest.

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