Original Article

Assessment of different irrigation techniques on the penetration depth of different sealers into dentinal tubules by confocal laser scanning microscope: An *in vitro* comparative study

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Abstract

Background: The effective disinfection of the entire root canal system aids in the penetration of irrigants into the dentinal tubules further improving sealer penetration and achieving a three-dimensional seal in endodontically treated teeth. Various final irrigation techniques can be employed to achieve this goal. Therefore, this study intended to assess and compare the efficacy of three final irrigation techniques on the depth of penetration of two root canal sealers into dentinal tubules using confocal laser scanning microscope (CLSM).

Methods: Forty-eight single-rooted mandibular premolars were selected and decoronated to a length of 12 mm. All the samples were prepared using ProTaper Gold rotary files and divided into three groups: Group 1 – Conventional syringe irrigation (CSI), Group 2 – passive ultrasonic irrigation (PUI), and Group 3 – Pro-agitator tip system (PATS). Each group was divided into two subgroups: Subgroup A – AH Plus and Subgroup B – GuttaFlow Bioseal (GFB). Then, sealers were mixed with 0.1% rhodamine B dye and the samples were obturated. All the samples were sectioned at 2 mm and 5 mm from the apex and visualized under confocal laser scanning microscope (CLSM) (10×) for maximum mean penetration depth and percentage of sealer penetration. Statistical analysis was done using the independent *t*-test and one-way analysis of variance test, followed by Tukey's *Post hoc* analysis.

Results: PUI performed better in the apical third, whereas PUI and PATS showed comparable results in the middle third for both depth and percentage of sealer penetration. Among the two sealers, GFB performed better than AH Plus in both the apical and middle third. These values were statistically significant. ($P < 0.05$).

Conclusion: Final irrigation activation with PUI or PATS can significantly improve sealer penetration. The average depth of penetration of GFB both at the middle and apical third of the root was significantly superior to AH Plus.

Keywords: AH Plus; GuttaFlow Bioseal; passive ultrasonic irrigation; Pro-agitator tips system; pro-agitator system

INTRODUCTION

The successful outcome of endodontic treatment depends on the complete extirpation of infected pulp, adequate disinfection of the root canal system, and achieving a three-dimensional apical seal.[1] Challenges faced during instrumentation are the cleaning of the isthmus, the apical

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delta, and the smear layer which may consist of bacteria and their byproducts.[2] This layer further poses a challenge for the penetration of irrigants into the dentinal tubules.

At present, a single irrigant cannot act against both inorganic and organic debris. Therefore, a combination of irrigants with different properties and modes of action is used.[3] The recommended irrigation protocol is a combination of a deproteinizing agent, sodium hypochlorite (NaOCl), and a calcium chelating agent, ethylenediaminetetraacetic acid (EDTA) in conjunction with normal saline.[4] To maximize the efficacy of irrigants used, various techniques have replaced the conventional syringe irrigation (CSI).

One such technique includes passive ultrasonic irrigation (PUI), proven to be a highly effective system in the market.[5] The Pro-agitator Tips System (PATS) (Innovations EndoLtd., India), launched in 2017, is an activation system with sparse studies.[6] Root canal sealers such as the epoxy-based sealer AH Plus (Dentsply DeTrey, Konstanz, Germany) have been considered the gold standard in endodontics.[7] GuttaFlow Bioseal (GFB) (Coltène/Whaledent AG, Altstatten, Switzerland) is a new bioactive sealer that has a limited number of studies.[8]

Given the importance of proper irrigation and providing a three-dimensional seal after instrumentation during obturation, this study was undertaken to assess and compare the depth and percentage of sealer penetration of two different sealers into the dentinal tubules after using three different irrigation techniques using a Confocal Laser Scanning Microscope (CLSM).

METHODS

This study was approved by the Institutional Ethics Committee (KIMS/IEC/D004/2019).

Sample preparation

Forty-eight single-rooted human mandibular premolars extracted for orthodontic purposes were selected for the study. Samples were stored in 0.5% chloramine-T solution. Radiographs were taken to examine the specimens for root curvature and morphological similarity. Only Vertucci type I configuration, uncurved teeth, and mature apices were included in the study. The teeth were decoronated to standardize the root length to 12 mm. The working length (WL) determination was done using the visual technique by subtracting 1 mm from the recorded length of the canal when the tips of the #10K file were visible at the apical foramina. The canals were then prepared using a ProTaper Gold with a torque-controlled motor to a #F3 apical preparation (tip size 30 with a 0.09 taper). 5 mL of 5.25% NaOCl was administered between each subsequent set size in all groups using a passively positioned 30-gauge side vent needle.

After instrumentation, the samples were randomly divided into three groups according to the final irrigation protocol.

Group 1: Conventional syringe irrigation

The final irrigation was done using 2 cycles of 5 mL 5.25% NaOCl and left in the canal for 30 s, followed by 2 cycles of 5 mL 17% EDTA with a needle tip 1 mm short of WL for 30 s. Normal saline was used as a buffer between two irrigants.

Group 2: Passive ultrasonic irrigation

Final irrigation was performed with a P5 booster (ACTEON) modified with a #25 Irrisafe tip placed in the canal 1 mm short of the WL and activated at a power setting of 4 without touching the root canal wall. 5 ml of 5.25% NaOCl with two cycles of 30 s of activation, followed by 5 ml of 17% EDTA with two cycles of 30 s of activation. Normal saline was used as a buffer between two irrigants.

Group 3: Pro-agitator tips system

Final irrigation was performed with PATS, where the polymer tip was placed 1 mm short of the WL and activated with 5 ml of 5.25% NaOCl for 2 activation cycles of 30 s each, followed by 5 ml of 17% EDTA for 2 cycles of 30 s. Normal saline was used as a buffer between the two irrigants.

Obturation of samples

Finally, each sample was rinsed with 3 mL of normal saline and dried using sterile paper points. Samples in each experimental group were subdivided into two groups:

- Subgroups A: AH Plus (Dentsply DeTrey, Konstanz, Germany)
- Subgroups B: GFB (Coltène/Whaledent AG, Altstatten, Switzerland).

To facilitate fluorescence under CLSM, both sealers were mixed with rhodamine B dye and applied to the root canal walls using a #20 Lentulo spiral, and a single-cone obturation technique was performed using #F3 master cone coated with sealer. The cone was condensed vertically using a plugger to evenly spread the sealer into the root canal. The teeth were sealed with Cavit (Coltène/Whaledent AG, Altstatten, Switzerland) at the coronal end. Radiographs were taken postobturation to ensure a void-free obturation. The samples were stored at 37°C and 100% humidity for 7 days to allow the sealer to set completely.

Sectioning

Specimens were cut using a slow-speed microtome saw at 2 and 5 mm from the root apex with water coolant to represent the middle and apical thirds, respectively.

Analysis for sealer penetration using CLSM

Sections were observed under CLSM (Leica Microsystems) at ×10 magnification in fluorescence mode. Image analysis was done using LASX software to measure the maximum sealer penetration depth and the percent sealer penetration into the dentinal tubules. Measurements were performed by one observer.

The maximum mean depth of penetration was obtained by calculating the depth of penetration at 12, 3, 6, and 9 o'clock corresponding to the buccal, mesial, lingual, and distal directions, respectively. Sealer penetration depths were calculated for each direction and averaged.

The percentage of sealer penetration was calculated using the following formula:

$$\text{Dentin area} = \text{Total area - root canal area \% of sealer penetration into dentinal tubule}$$

$$= \frac{\text{area filled by sealer} - \text{root canal area}}{\text{Dentin area}} \times 100$$

Statistical analysis

Data were analyzed using the SPSS 22.0 statistical package (Chicago, IL, USA) and the level of significance was set at $P < 0.05$. Statistical analysis was performed using an independent *t*-test and one-way analysis of variance, followed by Tukey's *post hoc* analysis to determine the difference between any two groups.

RESULTS

The mean penetration depth in Group 1 (CSI), Group 2 (PUI), and Group 3 (PATS) in the middle third was significantly greater than in the apical third in both subgroups – AH plus and GFB. ($P = 0.0001$).

When subgroups were compared, GFB showed better penetration depth compared to AH Plus and the difference was statistically significant ($P = 0.0001$) in all three groups [Table 1 and Figure 1].

When an intergroup comparison was made to evaluate the depth of penetration, it was maximal in PUI followed by PATS, and least in CSI in both apical and middle portions ($P = 0.0001$). However, in the middle third, there was no significant difference between the PUI and PATS groups ($P = 0.27$). While PUI performed better in the apical third compared to PATS and CSI.

The percentage of sealer penetration in both AH plus and GFB groups showed superior penetration in the PUI group followed by the PATS group and least in the CSI group in both middle and apical sections.

Compared to the CSI group, the other two groups were better in terms of percentage of sealer penetration.

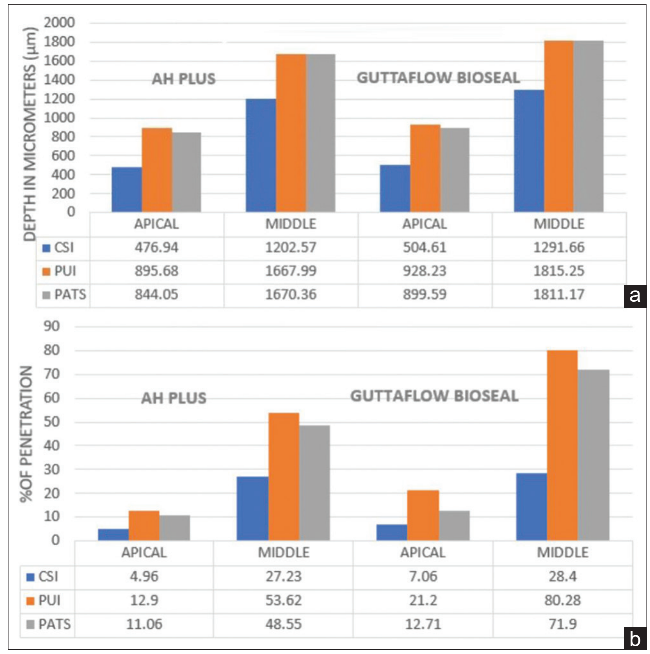

Figure 1: (a) Maximum mean penetration depth of AH Plus and GuttaFlow Bioseal (GFB) at 5 and 2 mm; (b) Percentage of sealer penetration of AH Plus and GFB at 5 and 2 mm

Table 1: Maximum mean penetration depth into dentinal tubules in different groups

Groups	Mean±SD (mm)	
	Apical 2	Middle 5
AH plus		
1A (syringe)	476.96±24.7	1202.57±8.54
2A (PUI)	895.68±2.96	1669.99±21.21*
3A (PATS)	884.05±8.25	1670.36±10.79*
GFB		
1B (syringe)	504.61±5.10	1291.66±3.46
2B (PUI)	928.23±5.08*	1815.24±17.32*
3B (PATS)	899.59±4.46	1811.17±7.33*

SD: Standard deviation, GFB: Guttaflow Bioseal, PUI: Passive ultrasonic irrigation, PATS: Pro-agitator tips system

PATS worked similarly to PUIs in the middle third but lacked efficacy in the apical third ($P < 0.05$) [Table 2 and Figures 1 and 2].

DISCUSSION

The outcome of endodontic therapy is dictated by complete canal debridement and three-dimensional obturation of the root canal system.[9] To achieve this goal, in addition to routine treatment options, various techniques such as irrigation activation ensure improved debridement and depth of sealer penetration into the dentinal tubules, which subsequently increases the quality of the obturation.

In this study, CLSM was used to assess the penetration of dentinal tubules using rhodamine B dye because it does not require sample processing. This ensures that observations

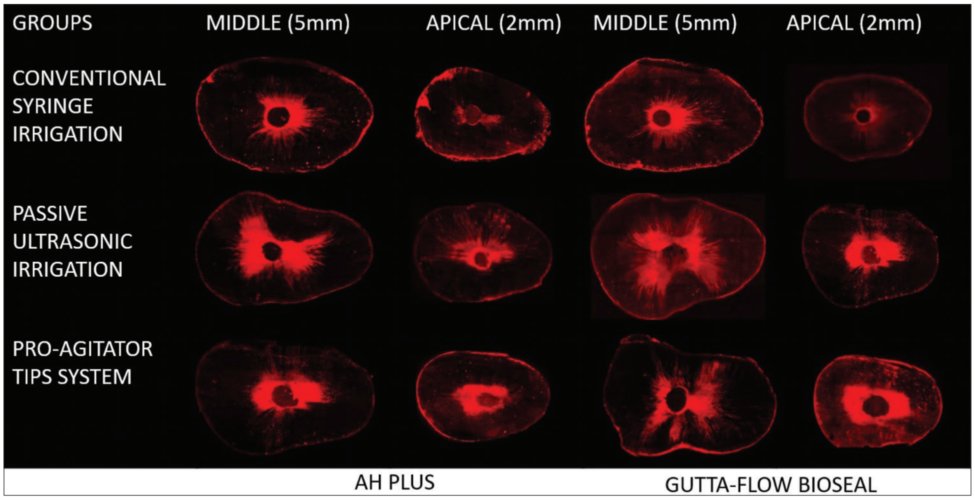

Figure 2: A representative confocal laser scanning microscope image of a sample from each group at 5 mm, and 2 mm levels as middle, and apical third regions of a root canal, respectively

Table 2: Percentage of sealer penetration into dentinal tubules in different groups

Groups	Mean±SD (mm)	
	Apical 2	Middle 5
AH plus		
1A (syringe)	4.96±0.34	27.23±1.71
2A (PUI)	12.9±0.77*	53.62±1.59*
3A (PATS)	11.06±1.28	48.55±1.66*
GFB		
1B (syringe)	7.06±0.21	28.47±0.58
2B (PUI)	21.12±1.04*	81.28±2.07*
3B (PATS)	12.71±0.77	71.9±2.86*

*Statistically significant, SD: Standard deviation, GFB: Guttaflow Bioseal, PUI: Passive ultrasonic irrigation, PATS: Pro-agitator tips system

are made under normal conditions. In addition, CLSM produces no image artifacts and is a non-destructive approach.[10] Rhodamine B helps to accurately determine the depth and percentage of penetration at a relatively lower magnification without disturbing the properties of the sealer.[11]

This study evaluated irrigation system efficiency and sealer penetration depth at 2 levels; 2 and 5 mm from the apex. This was done because the root apex has 98% of the apical branches and 93% of the lateral canals, which are in the apical 3 mm of the root canal.[12]

Complete debridement of the root canal system is possible only with the right combination of irrigants. NaOCl is the most recommended irrigant, which deproteinizes the tissue and has antimicrobial properties, making it an ideal solution for use during instrumentation.[6] The chelating agent EDTA is used in conjunction with NaOCl to effectively eliminate the smear layer.[13]

The results of this study confirmed the results of previous studies with the syringe technique, which has the least effectiveness in removing the smear layer.[14-16]

The PUI group had better results in both depth and percentage of sealer penetration with the AH plus as well as the GFB subgroups. This can be attributed to the complete shaping of the canal allowing free movement of the ultrasonic tip and penetration of the irrigant and cleaning of the apical area as well. In addition, PUI creates cavitation and acoustic microstreaming inside the root canal that effectively removes the smear layer from the dentinal walls. However, the flow intensity decreases when the instrument contacts the canal wall as in curved canals.[17] Therefore, only teeth presenting straight canals were included in the study.

The PATS uses a polymer tip operating at 30 psi and a sonic frequency of 6000 Hz.[6] In this study, PATS performed similarly to the PUI system in the middle thirds but lacked the same effectiveness in the apical thirds. The probable reason may be that the free movement in the middle third is more compared to the apical third and another reason may be the vertical movement of the file. However, many more studies are needed to confirm this hypothesis.[6] Another study concluded that EDDY (VDW GmbH, Munich, Germany), a sonically activated device performed similarly to PUI when used in straight and curved canals.[18]

AH Plus is a commonly used epoxy resin-based sealer with good physicochemical properties and adaptability. They form tags that penetrate the dentinal tubule and create a mechanical and chemical bond. A chemical bond is formed when the amino groups of dentin collagens bind with the AH Plus epoxy rings.[8]

GFB (Coltene/Whaledent, Altstatten/Switzerland) is a newer generation bioactive sealer developed in 2015 with an increased ability to penetrate dentinal tubules and bond instead of simply adhering to the dentinal surface and core material. GFB consists of gutta-percha powder and bioactive glass, which form hydroxyapatite crystals on the surface. There is an increased rate of sodium release due to the presence of bioactive glass, which stimulates the formation of mineralized tissues, which in turn provides better seal penetration and a better bond to the root dentin.[19]

In this study, AH Plus performed inferiorly to GFB in all three groups. This could be due to the hydrolysis of proteins or peptides by NaOCl on collagen, which reduces the chemical interaction and increases the gaps between the AH Plus and dentin interface.[8] In addition, AH Plus requires a fluid-free environment for solidification, while GFB is a bioactive sealer when exposed to dentinal canals that have approximately 20% wt. water. Furthermore, the characteristics of calcium silicates in GFB induce dentin remineralization and allow a slight expansion of the material, which is beneficial as a sealer. In addition, another reason for better penetration of GFB could be its particle size of approximately 2–10 μm when compared to AH Plus, which has a larger particle size of approximately 8–10 μm.[14]

Despite using irrigation activation techniques reduced, sealer penetration was observed in the apical portion of the root canal system which could be attributed to the reduced diameter of the dentinal tubules from the coronal to the apical region. In addition, the apical part of the root canal forms various ramifications, and dentinal sclerosis may also be present, which may be the causative factor for less penetration of the dentinal tubular canals compared to the middle third.[20]

CONCLUSION

Within the limitation of the present study, it can be concluded that the depth, as well as percentage of sealer penetration for both the sealers using CSI, PUI, and PATS in the middle and apical thirds, were statistically significant, with PUI performing better in the apical third. The PATS performed on par with PUI at the middle third but lacked efficacy in the apical sections. GFB performed better in comparison to AH Plus at both apical and middle third in all three groups.

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Conflicts of interest
There are no conflicts of interest.

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