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ORIGINAL ARTICLE

Evaluation of Different Agitation Techniques on Smear Layer Formation and Dentine Erosions- An *In Vitro* Study

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ABSTRACT

Objective: This study was performed to assess smear layer formation and erosion after final irrigation protocols with metal and non-metal tips in the apical third of root canals.

Methods: Forty mandibular premolars were instrumented with ProTaper Gold files up to F3 and embedded in a closed silicone flask system. The teeth were subsequently cleaved and 4 sequential indentations (1 to 4 mm from the apical foramen) were prepared on the buccal root canal walls to standardize sites for environmental scanning electron microscopy (ESEM) imaging. The samples were cleaned in an ultrasonic bath and observed under ESEM (controls), reassembled and divided into four groups (n=10 each) and subjected to different final irrigation protocols; XPF Group (XP-endo Finisher) and PUI Group (passive ultrasonic irrigation) with metal tips; EA Group (EndoActivator) and MDA Group (Manual dynamic agitation) with non-metal tips. The smear layer formation and dentine erosion were evaluated using ESEM. The data were analyzed with Kruskal-Wallis test with Bonferroni correction.

Results: In comparison to the control groups, XPF group had significantly increased smear layer formation at 1 and 2 mm ($P<0.05$). PUI group had significantly higher smear layer ($P<0.05$) formation at 3mm while EA and MDA groups did not present with significantly higher smear layer at all levels. Erosion was significantly higher ($P<0.05$) in MDA, XPF and PUI groups at all levels when compared to controls while EA group presented with significantly more erosion only at 2 and 3 mm.

Conclusion: Final irrigation protocol using EA and MDA with non-metal tips did not result in significant smear layer formation. Dentine erosion was observed after all experimental irrigation protocols.

Keywords: Dentine erosion, EndoActivator, manual dynamic agitation, passive ultrasonic irrigation, smear layer, XP-endo Finisher

HIGHLIGHTS

- Final irrigation protocols with EndoActivator and Manual dynamic agitation did not present with significant smear layer formation.
- Non-metallic tips which are softer than dentin may prevent smear layer formation.
- All tested irrigation protocols resulted in dentin erosion.

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INTRODUCTION

Root canal instrumentation creates a residual smear layer comprising of contaminated and non-contaminated debris, which is distributed over the root canal dentine (1). Smear layer removal aids in the penetration of irrigants and intracanal medicaments into dentine, enhancing root canal disinfection and also improving adaptation of root filling materials to the radicular walls (2–4). Further, incomplete removal of the smear layer can interfere with the anti-microbial action of sealers (5).

The sequential use of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) is an extensively studied and preferred approach for effective root canal disinfection and removal of the smear layer (6, 7).

Irrigants must be in direct contact with the root canal wall for effective chemodebridment (8). Conventional needle irrigation is the most widely used technique (9). It allows acceptable irrigant control as the needle position and irrigant volume are known (10). However, it has been reported to be ineffective in clearing out tissue remnants and cleaning the most apical portions of the root canal system (11). Therefore, different irrigant agitation/activation protocols and devices have been introduced to improve intra-canal smear layer and debris removal, especially in the apical third (12).

Passive ultrasonic irrigation (PUI; Acteon, Merignac, France) can increase the flow and diffusion of irrigants, facilitating debris and smear layer removal (8, 12). XP-endo Finisher file (XPF; FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) a recently developed nickel titanium finishing system and EndoActivator (EA; Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) are also efficient in debris and smear layer removal (13, 14). Manual dynamic agitation (MDA) is a cost-effective and efficient technique for smear layer removal (15). However, none of the irrigation protocols completely removed the smear layer and debris in the apical region of the root canal (8, 13, 14, 16).

A recent publication has reported that irrigation coupled with ultrasonic activation, EasyClean or the EDDY agitation systems per se can lead to smear layer formation (1) necessitating further research with other irrigant agitation/activation protocols. Also, sequential irrigation with NaOCl and EDTA with or without activation can result in dentine erosion (17, 18). Hence, this *in vitro* study was designed to evaluate smear layer formation and dentine erosion after final irrigation protocols using NaOCl and EDTA coupled with irrigant activation using PUI and irrigant agitation using XPF, EA and MDA in the apical third of prepared root canals.

The null hypotheses tested were:

1. None of the final irrigation protocols tested would lead to the formation of a smear layer.

2. Final irrigation protocols evaluated will not result in erosion of dentinal tubules.

MATERIALS AND METHODS

This *in vitro* study was approved by the Institutional ethical review board of Dr. M.G.R. Educational and Research Institute, Chennai (Dr. MGRDU/TMDCH/2020-2021/14102001). Sample size calculation was done using G*power 3.1.9.7 software (19), based on the results of a pilot study done with 5 samples per group. By fixing the power of the study at 80% and allowing 5% alpha error, with an effect size of 0.53, the sample size was calculated as 10 per group.

Sample Collection

This study included 40 freshly extracted human mandibular premolars from patients aged between 25 and 50 years. The teeth were radiographed and viewed under an operating microscope with 16x magnification to ensure that the inclusion and exclusion criteria were met. Only teeth with fully formed root apices and single canal with a curvature of less than 15° were included. Teeth with caries, cracks, signs of internal/external resorption, and prior root canal treatment were excluded. The selected teeth were stored in 0.1 % thymol solution till the experiment.

Access Opening and Standardization of Working Length

Access preparation was done and the samples were standardized to a length of 12mm by sectioning the crown with a diamond disc. A #10 K file (Mani Inc., Utsunomiya, Japan) was introduced into the root canal till the tip was seen at the apical foramen. A rubber stopper was adjusted to the reference point (occlusal edge of the access cavity) and the true length of the tooth was established, from which 1 mm was subtracted to establish the working length (WL). All the samples were initially instrumented to the WL using a #15 K file followed by root canal preparation with rotary files (ProTaper Gold, Dentsply Maillefer, Switzerland) from S1 to F3. Irrigation (20) between instruments was performed with 3% NaOCl using a 30-gauge side vented needle (NaviTip; Ultradent, South Jordan, UT) attached to a disposable 5ml plastic syringe resulting in 15 ml irrigation of NaOCl per sample. Canal patency was achieved with a #15 K file between instrumentation.

Standardization of Closed Irrigation Flask System

A closed flask system was setup in silicone impression material similar to the model described by Kato et al. (21). Longitudinal grooves were created on the mesial and distal aspects of each root with a diamond disc without reaching the canal lumen. To prevent accidental invasion into the root canal space, a gutta-percha point was placed inside the canal. Next, the root apices were sealed with wax and the roots were embedded into 1.5 mL plastic tubes containing a silicone-based impression material (Zetaplus, Zhermack, BadiaPolesine, Italy) upto the level of cemento-enamel junction. Once the silicone material had set, the roots were split open with a chisel (GDC, New Delhi, India) to facilitate assembly and reassembly of the samples.

Standardization of Indentations in the Apical Third

The buccal halves of the roots were then taken out of the flask system using hemostatic forceps. Only the apical thirds were evaluated for standardization purposes. Four sequential indentations measuring about 0.3 mm in height, width and depth were prepared on the inner wall of the root canal with a 0.15 mm-thick diamond disc1 mm from each other, 1 to 4mm from the apical foramen which helped to further standardize (Fig. 1) the sites to be observed in environmental scanning electron microscopy (ESEM) imaging (1).

Sample Cleaning and ESEM Analysis

The samples were next cleaned in an ultrasonic bath initially with 3 % NaOCl and then with 17% EDTA for 1 minute each following which the samples were washed in running water for 1 minute. Subsequently, the samples were dried in an incubator at 38°C for 24 hours and kept in sealed plastic containers to guarantee absolute absence of debris.

Control Group

Each sample was then submitted to ESEM (Thermo Scientific Quattro S, Wilmington, DE) at 1200x to evaluate for the pres-

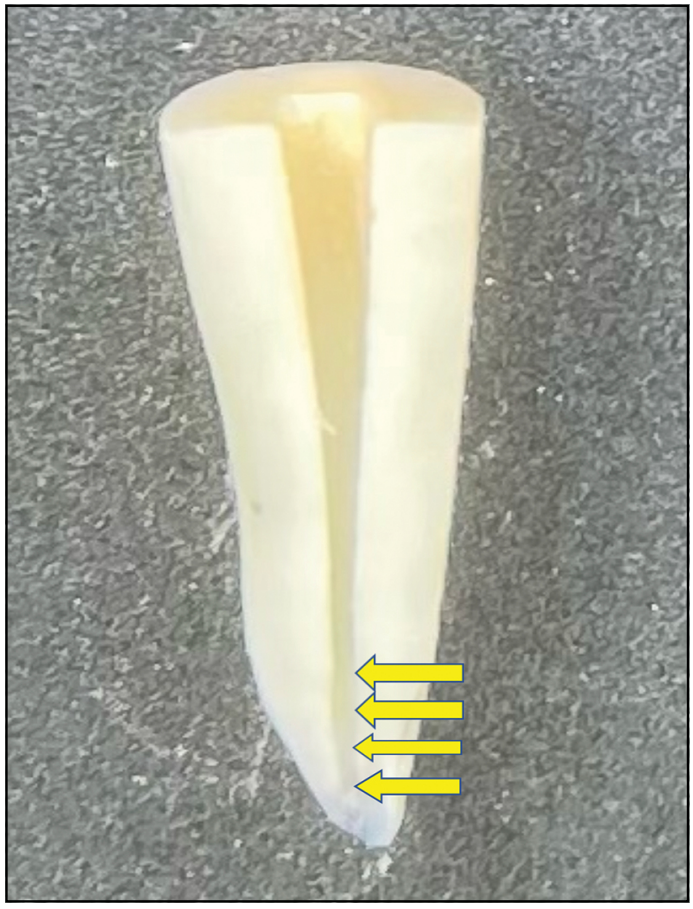

Figure 1. Standardization of the locations evaluated at 1, 2, 3 and 4mm from the apical foramen

ence of a smear layer and 5000x for evaluation of root dentine erosion. The areas to be observed were immediately above the previously placed indentations. For each site, 10 images were captured and the mean scores were analyzed. Initial images of all four observation sites prior to the final irrigation protocol were captured and digitally stored representing the control group. The specimens were reassembled in the flask and subjected to experimental final irrigation protocols.

Since ESEM does not necessitate any prior sample preparation, the same samples were used for both control and experimental groups ensuring standardization (1).

Final Irrigation Protocol for Experimental Groups

The 40 samples were randomly allocated to 4 groups (n=10 each); XP-endo Finisher (XPF), Passive ultrasonic irrigation (PUI) with metal tips; EndoActivator (EA) and Manual Dynamic Agitation (MDA) groups with non-metal tips.

XPF Group (Metal Tip)

The canal was filled with 3% NaOCl using a 30-gauge side vented needle attached to a disposable 2mL plastic syringe placed 1 mm from the working length, and agitation was performed using an XPF instrument (25/.00) (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland). The file was used at a speed of 800 rpm and torque of 1 Ncm, in an Endodontic motor (X-S-mart, DentsplyMaillefer, Ballaigues, Switzerland) (22) using mild up and down strokes, 1mm short of the WL. NaOCl agitation was done in 3 cycles of 20 seconds each, and 2mL of NaOCl was renewed after each cycle. The irrigant was aspirated and a similar sequence was performed with 17% EDTA solution followed by aspiration and 3 more cycles with 3% NaOCl. Overall, 12 mL of 3% NaOCl and 6 mL of 17% EDTA (20) were used in approximately 3 minutes. Finally, the root canal was flushed with double distilled water to enable complete removal of the irrigating solutions (23).

PUI Group

Irrigant activation was performed using an ultrasonic tip (20/.01) (Acteon, Merignac, France) at 1 mm short of the WL at a power setting of 1 (18). The procedures of activation, aspiration, and irrigant renewal were similar to steps performed for the XPF group.

EA Group

Irrigant agitation was done with EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK), using the red tip (25/0.04) at 1 mm from the WL at 10,000 cycles per minute (20). The procedures of agitation, aspiration, and irrigant renewal were similar to steps performed for the XPF group.

MDA Group

Push-pull strokes were performed manually up to 1mm short of the WL by using a size 30/09 gutta-percha cone (Dentsply Maillefer, Ballaigues, Switzerland) at an approximate rate of 100 strokes per minute. The procedures of agitation, aspiration and irrigant renewal were similar to steps performed for the XPF group.

After performing the final irrigation protocol, the buccal portion of the root was removed from the flask, dried in an incubator at 37°C and evaluated again under ESEM at 1200x to evaluate for the presence of a smear layer and 5000x for evaluation of root dentine erosion at the previously standardized locations.

Image Evaluation

The experimental and control group images at all 4 levels were placed side by side using a presentation software (PowerPoint; Microsoft, Redmond, WA) and classified by 4 calibrated examiners who were blinded to the study for smear layer formation and dentine erosion.

Smear Layer Formation (21, 24):

Score 1: Open dentinal tubules without smear layer.

Score 2: Open dentinal tubules with less than 50% of the examined area covered with smear layer.

Score 3: Open dentinal tubules with more than 50% of the examined area covered with smear layer.

Score 4: Dentinal tubules with 100% of the examined area covered with smear layer.

Dentine Erosion (25):

Score 1: No erosion was present and all tubules looked normal in appearance and size.

Score 2: Moderate erosion was present. The peritubular dentine was eroded.

Score 3: Severe erosion was present and the intertubular dentine was destroyed, connecting the tubules with each other.

Statistical Analysis

Statistical analysis of data was done using SPSS (Version 26.0, IBM, Chicago, IL). The level of inter-examiner agreement was determined using the kappa test. Shapiro-Wilk test was used to assess normality in data distribution pattern and it revealed that data deviated significantly from normal distribution. Therefore, Kruskal-Wallis test (non-parametric test) was used. The data pertaining to smear layer formation and erosion was described in terms of Mean Ranks and Median values. Pairwise comparisons with Bonferroni correction was used to assess the statistical significance between the groups which was set at 0.05 (P<0.05).

RESULTS

Inter-examiner agreement was found to be high (kappa=0.92). All control samples presented with no smear layer at all four levels evaluated. When compared with controls; XPF (1 and 2mm) and PUI groups (3mm) had significantly higher smear layer formation (Table 1); EA and MDA groups did not present with significantly more smear layer formation at all four levels (Fig. 2). On inter-group analysis, XPF had significantly higher smear layer formation than MDA group at 1 mm. At 2, 3 and 4 mm intergroup comparison of experimental samples did not present significant differences in smear layer formation. When compared to controls, XPF, PUI and MDA groups had significantly higher erosion scores at all 4 levels while EA group had significantly higher erosion scores only at 2 and 3 mm. Inter group comparison of erosion scores at all 4 apical levels did not present with significant differences (Table 2 and Fig. 3).

TABLE 1. Mean Rank and Median smear layer formation scores (in Parentheses) attributed to experimental groups in comparison to control groups at the 4 apical levels assessed

	1 mm	2 mm	3mm	4mm
Control	18.10 (1.0)	19.00 (1.0)	19.45 (1.0)	20.35 (1.0)
XPF	38.15 (2.5)*	33.25 (1.5)*	26.40 (1.0)	27.20 (1.0)
PUI	26.45 (1.0)	30.90 (1.0)	33.75 (1.5)*	29.90 (1.0)
EA	24.80 (1.0)	23.35 (1.0)	21.50 (1.0)	25.20 (1.0)
MDA	20.00 (1.0)	21.00 (1.0)	26.40 (1.0)	24.85 (1.0)
Kruskal-Wallis test	P=0.001	P=0.007	P=0.018	P=0.222

*: Statistically significant difference (Kruskal-Wallis test with Bonferroni correction, P<0.05). XPF: XP-endo Finisher, PUI: Passive ultrasonic irrigation, EA: EndoActivator, MDA: Manual dynamic agitation

TABLE 2. Mean Rank and Median erosion scores (in Parentheses) attributed to experimental groups in comparison to control groups at the 4 apical levels assessed

	1 mm	2 mm	3mm	4mm
Control	10.50 (1.0)	9.70 (1.0)	9.80 (1.0)	11.65 (1.0)
XPF	29.60 (2.0)*	26.90 (2.0)*	29.00 (2.0)*	28.00 (2.0)*
PUI	35.30 (2.0)*	34.50 (2.0)*	30.85 (2.0)*	33.95 (2.5)*
EA	22.50 (2.0)	29.50 (2.0)*	28.85 (2.0)*	25.90 (2.0)
MDA	29.60 (2.0)*	26.90 (2.0)*	29.00 (2.0)*	28.00 (2.0)*
Kruskal-Wallis test	P<0.000	P<0.000	P<0.000	P<0.002

*: Statistically significant difference (Kruskal-Wallis test with Bonferroni correction, P<0.05). XPF: XP-endo Finisher, PUI: Passive ultrasonic irrigation, EA: EndoActivator, MDA: Manual dynamic agitation

DISCUSSION

The ability of irrigant agitation/activation protocols to thoroughly debride the apical third of the root canal is challenging (14). Smear layer removal depends on the direct contact of irrigants with the root canal walls. However, metal or non-metal tips used to agitate/activate irrigants can come into contact with root dentine and cause undesirable effects (26). A recent study concluded that the final irrigation step with ultrasonic activation, EasyClean and EDDY led to the formation of a smear layer (1). Further, irrigation with PUI and EasyClean has been reported to lead to both smear layer formation and root dentine erosion (18). Therefore, the present study was formulated to evaluate whether other commonly used irrigant agitation/activation protocols can lead to the formation of a smear layer and dentine erosion in the apical third of the root canal.

In the present study, the MDA and EA groups with non-metal tips presented with minimal smear layer at all 4 apical levels which was not significant when compared to the controls. However, the XPF group at 1 and 2 mm and the PUI group at 3 mm presented with significantly more smear layer when compared to the controls. Therefore, the first null hypothesis was rejected. XPF and PUI techniques utilize metal tips which are harder than dentine. The inadvertent contact of these tips with dentine could have led to the formation of smear layer in apical root canals (26).

EA system used disposable flexible polymer tips of different sizes that do not cut root dentine and cannot create a smear layer (27, 28). Results of the present study were in accordance with a previous report (14) where EA performed better in smear layer removal at 3, 5 and 8 mm from the apex and with Elnaghy et al. (13) where EA was seen to remove significant smear layer from the root canal.

MDA group samples presented with minimal smear layer which was not significantly higher when compared to the controls at all 4 apical levels and was significantly lower in comparison with XPF at1 mm. MDA has been reported to perform better than PUI in removal of the smear layer (15). The efficacy of MDA in smear layer removal might be attributed to the following reasons. MDA was done with a gutta-percha cone which is softer than dentine and corresponded to the canal taper and preparation size, preventing air bubble formation in the apical third (29). The tapered canal preparation gave reflux space, allowing the irrigating solution to flow up and down along the cone with solution being displaced outward when the cone is inserted at length and flowing inward when it is removed (27).

In the present study, all 4 groups (including controls) demonstrated dentine erosion at all 4 levels. Samples in all the test

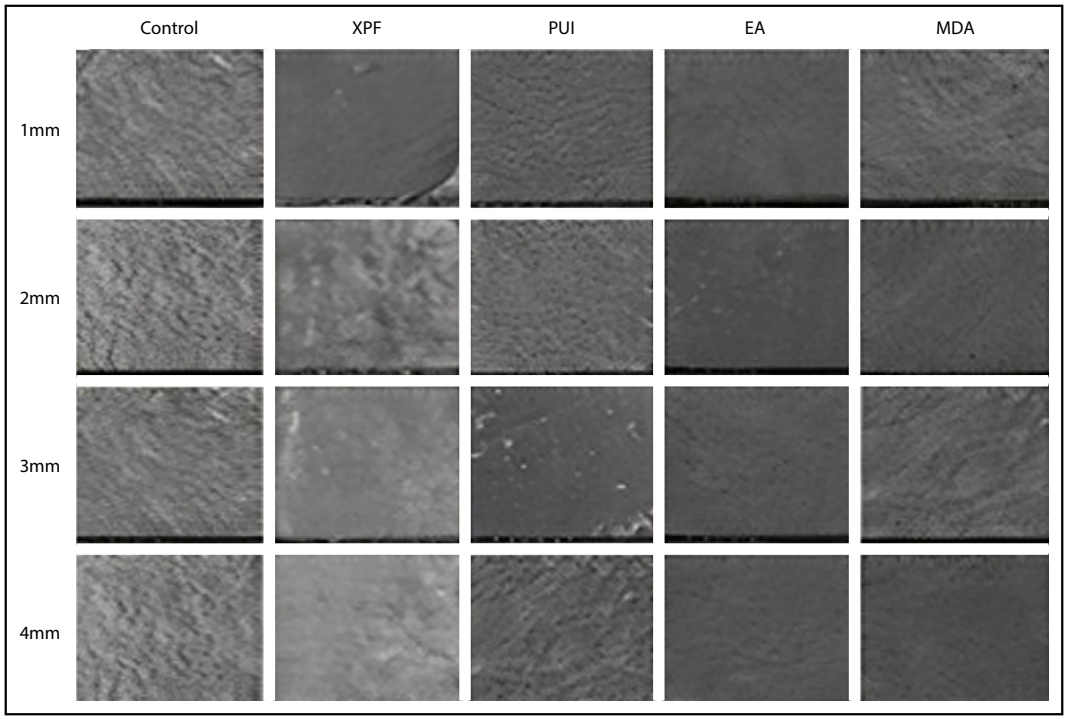

Figure 2. Representative ESEM images evaluating smear layer formation at 4 apical levels in XPF, PUI, EA and MDA groups

ESEM: Environmental scanning electron microscopy, XPF: XP-endo Finisher, PUI: Passive ultrasonic irrigation, EA: EndoActivator, MDA: Manual Dynamic Agitation

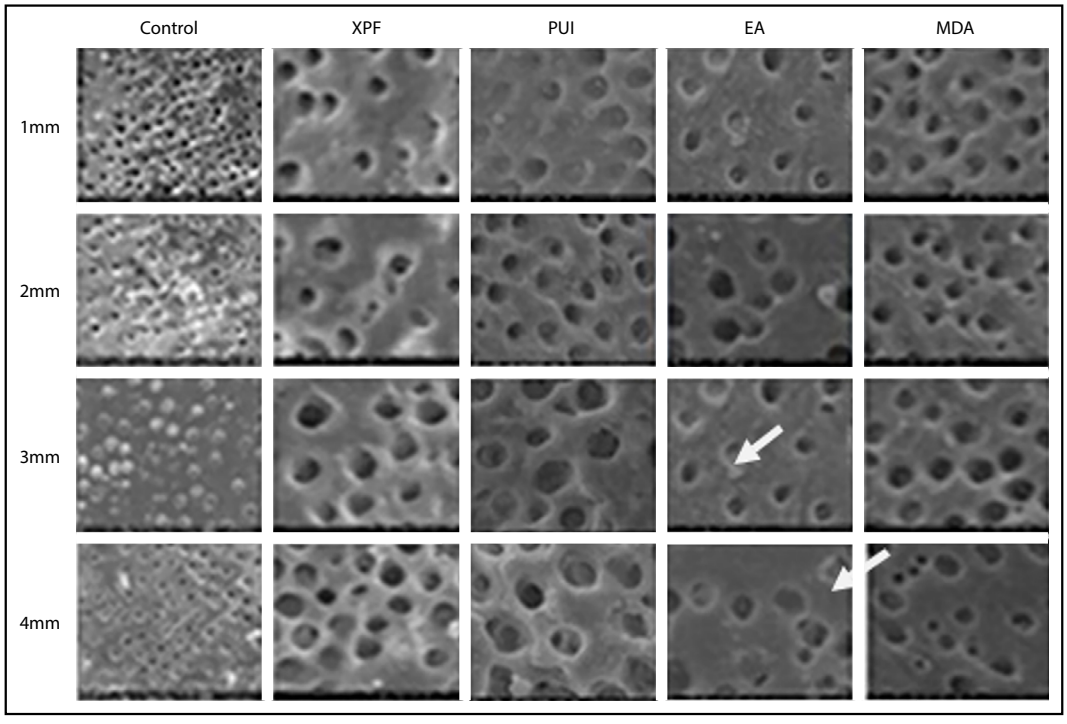

Figure 3. Representative ESEM images evaluating dentine erosion at 4 apical levels in XPF, PUI, EA and MDA groups. Arrows in the image indicate dentine erosion

ESEM: Environmental scanning electron microscopy, XPF: XP-endo Finisher, PUI: Passive ultrasonic irrigation, EA: EndoActivator, MDA: Manual Dynamic Agitation

groups (both metal and non-metal) demonstrated higher erosion after the final irrigation protocol when compared to the controls which was significant at all four levels except at 1mm and 4 mm in the EA group. Hence, the second null hypothesis was also rejected. However, intergroup comparisons between experimental groups were not significant indicating that erosion was similar in all experimental groups at all 4 levels.

The irrigation sequence used in the present study (NaOCl-EDTA-NaOCl) has been reported to result in improved disinfection of dentinal tubules compared to NaOCl-EDTA (27). Root canal dentine erosion has been reported to be significant when NaOCl irrigation was done after EDTA but not when NaOCl irrigation was done prior to EDTA (30). A similar irrigation sequence coupled with the EndoVac, PUI, EasyClean or Self-Adjusting file led to decreased accumulation of hard-tissue debris (31) in root canals. Also, NaOCl during instrumentation and a final irrigation of EDTA- NaOCl with or without PUI can lead to higher success rates of root canal treatment (32).

Severity of erosion is based on the contact time of NaOCl and EDTA with dentine (33). In the present study, exposure of dentine to EDTA was limited to 1 minute in controls to minimize erosion (34). However, the prolonged use of NaOCl prior to EDTA can also lead to erosion (17). Dentine erosion has been reported with irrigation protocols using PUI and EasyClean when NaOCl was used before and after EDTA (18). Therefore, the irrigation sequence used in this study (NaOCl-EDTA-NaOCl) coupled with the irrigant agitation/activation protocols used could have led to dentinal erosion. Though erosion may help in achieving a clean canal surface (33), extensive erosion can lead to a decrease in dentine mechanical properties (17). The clinical implications of different irrigation sequences are not known (35) and further research is necessary to identify irrigation protocols which minimize erosion.

The results of this study indicate that irrigant agitation with gutta-percha points or polymer-based tips which are non-metallic and softer than dentine may prevent smear layer formation. This is not in correspondence with a previous paper (1) where smear layer formed after irrigant agitation with the EasyClean and EDDY systems which utilize plastic tips. This could be explained by differences in plastic tip cross-sections and type of motion between irrigant agitation systems. Further studies are necessary to develop adequate final irrigation protocols which do not result in smear layer formation and also prevent dentine erosion without compromising their efficacy in cleansing the root canal walls.

The apical third segment usually encounters the vapor lock effect which can be disrupted by irrigant agitation (36). Ultrasonic cavitation may not be effective if the tip vibrates in the vapor zone in the absence of fluid (37). However, MDA has been reported to disrupt the vapor lock (29, 38), The present study evaluated the effectiveness of different final irrigation protocols in the apical third for removal of the smear layer using a closed flask system (21) which can prevent irrigant extrusion and can better replicate clinical conditions (39).

In the present study, ESEM was used mainly because it does not require any dehydration procedure or metal coating of the samples, thereby allowing reuse of a non-damaged specimen after evaluation of control images (1). The characteristics of this method coupled with the placement of indentations in the apical third of the root canal allowed standardization and more reliable data (21). The closed silicone flask system used in this study enabled reassembly of the cleaved samples while preventing extrusion of irrigant (21).

The limitations of the present study include the lack of investigation of other irrigant types including continuous chelation (35), concentrations, and sequences. Also, further research is necessary to evaluate the effect of final irrigant agitation/activation protocols using Micro-CT (40).

CONCLUSION

Within the limitations of this study, it can be concluded that smear layer was formed by XPF and PUI protocols in the apical root canal. Irrigation agitation with MDA and EA did not result in the formation of significant smear layer. All final irrigation protocols evaluated presented with dentine erosion.

Disclosures

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by The Dr. M.G.R. Educational and Research Institute, Chennai Ethics Committee (Date: 14/10/2020, Number: Dr. MGRDU/TMDCH/2020-2021/14102001).

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