Effectiveness of Four Different Final Irrigation Activation Techniques on Smear Layer Removal in Curved Root Canals: A Scanning Electron Microscopy Study

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Abstract

Objective: The aim of this study was to assess the efficacy of apical negative pressure (ANP), manual dynamic agitation (MDA), passive ultrasonic irrigation (PUI) and needle irrigation (NI) as final irrigation activation techniques for smear layer removal in curved root canals.

Materials and Methods: Mesiobuccal root canals of 80 freshly extracted maxillary first molars with curvatures ranging between 25° and 35° were used. A glide path with #08-15 K files was established before cleaning and shaping with Mtwo rotary instruments (VDW, Munich, Germany) up to size 35/0.04 taper. During instrumentation, 1 ml of 2.5% NaOCl was used at each change of file. Samples were divided into 4 equal groups (n=20) according to the final irrigation activation technique: group 1, apical negative pressure (ANP) (EndoVac); group 2, manual dynamic agitation (MDA); group 3, passive ultrasonic irrigation (PUI); and group 4, needle irrigation (NI). Root canals were split longitudinally and subjected to scanning electron microscopy. The presence of smear layer at coronal, middle and apical levels was evaluated by superimposing 300-µm square grid over the obtained photomicrographs using a four-score scale with X1,000 magnification.

Results: Amongst all the groups tested, ANP showed the overall best smear layer removal efficacy (p < 0.05). Removal of smear layer was least effective with the NI technique.

Conclusion: ANP (EndoVac system) can be used as the final irrigation activation technique for effective smear layer removal in curved root canals.

Key Words: Root Canals; Sodium Hypochlorite; Smear Layer; Root Canal Preparation

INTRODUCTION

Smear layer is an amorphous, irregular entity formed during cleaning and shaping of the root canal space. It contains dentin debris and organic material such as vital or necrotic pulp tissue remnants, bacteria, and their metabolic...
by-products [1]. It prevents the penetration of intracanal medicaments into dentinal tubules and influences the adaptation of filling materials to canal walls [2]. A final irrigation with chemicals such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) is recommended to remove the inorganic and organic components of the smear layer [3-5].

Numerous techniques and irrigant delivery devices have been proposed to improve the distribution of irrigating solution within the root canal system [6]. But regardless of the techniques used, effectiveness of irrigating solutions remains limited in the apical third of a prepared canal [7, 8]. When canal curvatures are present, effective irrigant delivery becomes even more difficult, especially in the apical third [8]. Although conventional needle irrigation is one of the most commonly used techniques, its efficacy in the removal of the smear layer from the curved root canals especially in the apical third is still questionable [8-10]. Passive ultrasonic irrigation (PUI) refers to an irrigation protocol wherein a non-cutting ultrasonically activated file is agitated in the canal without contacting the walls [11]. It has been shown that sonic or ultrasonic activation of the irrigant in a curved canal does not improve the smear layer removal from the apical third of the canal [12]. Contradictory results were observed by Blank-Gonçalves et al. with the use of sonic or ultrasonic irrigant activation techniques in curved root canals. They showed that the smear layer was removed only up to 75-80% in the apical third of root canals with these agitation techniques [9]. The EndoVac system (Discus Dental, Culver City, CA, USA) uses apical negative pressure (ANP) to deliver the irrigating solution to the apical end of the canal system and to suction out the debris. This device consists of a master delivery/suction tip, a macrocannula, and a microcannula that are connected to a vacuum line [13]. Studies have shown that the use of apical negative pressure is effective in smear layer removal especially in the apical third of straight root canals [14, 15].

Manual dynamic activation (MDA) involves repeated insertion of a well-fitting gutta-percha cone to the working length (WL) of a previously shaped canal. The gutta-percha cones then applied in short strokes will activate the irrigant [16]. Gu et al. hypothesized that this technique might be useful in breaking the air bubbles located at the apical 0-2 mm of the canal with repeated gutta-percha insertion [6]. A recent clinical study conducted by Munoz and Camacho-Cuadra has shown that PUI and EndoVac are equally effective in delivering the irrigant to the full working length in curved canals [17]. But to date there are no studies evaluating the efficacy of apical negative pressure technique on smear layer removal in curved root canals.

MDA is significantly better in smear layer removal in the apical third for curved canals as compared to syringe irrigation [18]. Hence, the purpose of this study was to compare smear layer removal after final irrigation with apical negative pressure (ANP), manual dynamic agitation (MDA), passive ultrasonic irrigation (PUI) and needle irrigation (NI) techniques in curved root canals.

**MATERIALS AND METHODS**

**Selection and preparation of the specimens**

A total of 80 curved (25°-35°) single canal mesiobuccal roots from fully developed freshly extracted maxillary first molars were used. The teeth were stored at 4°C in 0.9% sodium chloride solution supplemented with 0.02% sodium azide to prevent bacterial growth until use. The degree of curvature was determined according to Schneider’s method [19]. Teeth that had received restorative or endodontic treatment before extraction were excluded. To standardize canal instrumentation, the crowns were removed and the roots were adjusted to a standardized length of 14 mm. To simulate the clinical situation, the apex was sealed with sticky wax [20].
Working length (WL) was set at 13 mm and a glide path was established using #08-15 K-files. Canals were instrumented with Mtwo rotary nickel-titanium (NiTi) instruments (VDW, Munich, Germany) up to size 35/0.04 taper with sizes 10/0.04, 15/0.05, 20/0.06, 25/0.06, 30/0.05 and 35/0.04. During the entire preparation, 1 mL 2.5% NaOCl was used as irrigant between each file.

**Experimental Groups**
Specimens were randomly divided into 4 experimental groups (n=20) according to the technique used during final irrigation.

**ANP group [n=20]:** With the ANP technique, macro-irrigation was done during instrumentation. Following this, a modified protocol described by Saber and Hashem was used in our study [15]; wherein only 2 micro-irrigation cycles were used instead of 3 micro-irrigation cycles as originally suggested by Neilson and Craig Baumgartner [21]. A total of 5 mL of 2.5% NaOCl and 5 mL of 17% EDTA were used at a flow rate of 1.8 mL min⁻¹ through the microcannula.

**MDA group [n=20]:** The canal was flooded with 2.5% NaOCl and push-pull strokes were performed manually to the WL by using a size ISO #35, 4% taper gutta-percha cone at an approximate rate of 100 strokes per minute for 30 seconds. This was followed by irrigation with 2.5 mL of 2.5% NaOCl and again repeating the same procedure for next 30 seconds using a new gutta-percha cone with 2.5% NaOCl.

The above cycle was repeated with 17% EDTA, thus activating each irrigant for 1 minute with a total volume of 5 mL per irrigant [15].

**PUI group [n=20]:** In this group, 2.5% NaOCl and 17% EDTA were each activated for 1 minute by using #20/0.00 taper stainless steel non-cutting pre-bent ultrasonic tip (IrriSafe; Satelec, Acteon, Merignac, France) at 1 mm from the WL. The tip was operated by a piezoelectronic unit (P5 Newton; Satelec) at power setting 5. The canal was irrigated with 2.5 mL after 30 seconds of ultrasonic activation, with a total volume of 5 mL per irrigant [15].

**NI group [n=20]:** A final irrigation sequence of 5 mL 2.5% NaOCl, followed by 5 mL 17% EDTA, was used to remove the smear layer. Solutions were delivered by a syringe and a 30-gauge needle (NaviTip; Ultradent, South Jordan, UT). The tip was placed as deep as possible into the root canal without wedging to permit backflow of the irrigant.

Finally, the specimens were irrigated with 5 mL sterile distilled water, dried, temporarily sealed, and stored separately in labelled bottles containing 10% formaldehyde as a fixative for any remaining soft tissue debris.

**Scanning Electron Microscopy Examination**
The root canals were dried with paper points. A longitudinal groove in the buccolingual direction was made using a diamond disk. Colored gutta-percha cones were used to prevent the intrusion of the cutting disk into the canals [18].

Specimens were split by applying slight pressure to an enamel chisel into the longitudinal groove and one half of the specimen was randomly chosen for scanning electron microscope (SEM) evaluation. Each specimen was dehydrated in graded series of ethanol solutions, mounted on stubs, gold-sputtered, and examined under scanning electron microscope (Hitachi S-3400N SEM, Tokyo, Japan) at 15 kV. SEM photomicrographs were taken at x 1,000 magnification of each canal third of all the specimens for analysis.

A 300-µm square grid was superimposed on the photomicrographs obtained, from which smear layer scores were evaluated.
Each square was considered as an assessment unit, and the amount of smear layer present in each of the “assessment units” was assessed and recorded in accordance with the criteria proposed by Sadr-Lahijani et al. [22].

Score 1- Dentinal tubules completely open
Score 2- >50% dentinal tubules open
Score 3- <50% dentinal tubules open
Score 4- Nearly all of the dentinal tubules covered with smear layer

Samples were scored independently by two blinded evaluators. The scores were then compared, and if a difference was found, the two evaluators jointly examined the sample.

For each photomicrograph, the average score for the smear layer was calculated from the raw data by dividing the sum of all the individual scores by the number of assessment units. The code was broken at the end of the study and samples were assigned to the respective groups. The mean scores for smear layer were finally recorded at all three levels.

**Statistical Analysis**

Statistical analysis was performed with the Kruskal-Wallis test to compare final irrigation techniques. Dunn’s post-hoc test was used for pairwise comparisons between techniques.
Within each of the four groups, Friedman test was used to compare between root canal thirds. Further, pairwise comparison of the thirds was done using the Wilcoxon signed rank test. The significance of the pairwise comparisons were interpreted at a p value of 0.017, obtained after adjusting for multiple hypothesis testing by Bonferroni’s correction. Statistical analysis was performed with SPSS (Statistical Package for Social Sciences) 20.0 statistical package for Windows (Chicago, IL).

RESULTS

Comparison between overall groups
On observing the overall mean score for smear layer removal amongst all the groups (Table 1), ANP had the highest smear layer removal efficacy. This was followed by MDA and PUI, which were statistically not significant. Removal of the smear layer was least effective with NI technique.

Intergroup comparison between various irrigant techniques for smear layer removal in the apical, middle and coronal thirds
It was observed that NI was least effective in smear layer removal at all three levels of the root canal amongst all groups (Table 2). At the apical third, smear layer removal efficacy was comparable between ANP and MDA, with no significant differences between them. This was followed by PUI that showed statistically significant higher smear layer scores as compared to ANP and MDA. At the middle third, ANP had the least smear layer scores and highest smear layer removal efficacy as compared to MDA and PUI. MDA and PUI showed no statistically significant difference in smear scores. At the coronal third, smear layer removal efficacy was comparable between ANP, MDA and PUI with no statistically significant difference between them. Values in mean with different superscript letters (uppercase-for different activation techniques; lowercase-at the three segments with each irrigant) are statistically different (significant at p < 0.017).

Comparison between canal thirds
Within the ANP group, there was no statistically significant difference in the apical and the middle third for smear layer removal. The coronal third had the highest smear layer removal efficacy and was statistically significant from the apical third. Within the MDA group, coronal third had significantly lower smear scores compared to the middle and the apical third. However, the efficacy of the middle and apical third were not significantly different from each other. Within the PUI group, smear layer removal was least effective in the apical third, followed by the middle third and then the coronal third. Similar results were also obtained in NI group.

Table 1. Comparison of Mean Overall Scores for Smear Layer Removal Using Various Irrigation Techniques

| Groups | Mean ± SD | Minimum | Maximum | Pairwise Comparison | P-Values |
|--------|-----------|---------|---------|---------------------|----------|
| ANP    | 1.49 ± 0.535 | 1.00    | 3.00    | ANP vs MDA          | 0.013*   |
|        |            |         |         | ANP vs PUI          | <0.001*  |
|        |            |         |         | ANP vs NI           | <0.001*  |
| MDA    | 1.83 ± 0.656 | 1.00    | 3.00    | MDA vs PUI          | 0.019    |
|        |            |         |         | MDA vs NI           | <0.001*  |
| PUI    | 2.19 ± 0.754 | 1.00    | 3.25    |                     |          |
| NI     | 2.73 ± 0.840 | 1.00    | 4.00    | PUI vs NI           | 0.001*   |

*Significant at p < 0.017
DISCUSSION
The irrigation of the root canal is an essential procedure for the removal of the smear layer. SEM is one of the most commonly used techniques to assess the removal of the smear layer [3, 9, 23]. Literature search has revealed that few studies exist regarding smear layer removal in curved canals of molars [9, 10, 12, 18]. In this study, the apex was sealed with sticky wax to create a closed-end canal model [20]. The instrumentation was performed up ISO #35, 4% size with the Mtwo rotary system to facilitate better irrigant penetration and to allow the agitation devices/irrigant tips to reach the appropriate working length [24]. According to Brunson et al. [25], regarding the minimal apical preparation size, instrumentation up to size ISO #35 results in clinically adequate irrigant volumes for negative pressure systems. Moreover, this apical diameter also allowed penetration of the EndoVac microcannula up to the working length, because it has a 0.32 mm tip diameter [13, 21]. The Irrisafe tips used in our study were pre-bent to facilitate acoustic streaming within the canal and to avoid severe file-wall contact. In an earlier study, Ahmad et al. proved that pre-curving K-files ensured maximum efficiency for ultrasonic irrigation in curved root canals [26]. Al-Jadaa et al. have also showed that it minimized the risk of canal transportation [27].

The grid scoring method followed by Goel and Tewari [28] and Wu et al. [29] was used in this study. This scoring method may be a more accurate method of representation of the sample area being photographed. Results of this study showed that smear layer removal in the apical third of the root canal was superior with the ANP and MDA groups in comparison with the other groups. Previous studies have also reported that EndoVac system is more effective for the removal of the smear layer in the apical third of the straight canals [14, 15].

The probable reason could be because EndoVac disrupts the vapour lock and hence enables more volume of the irrigant to reach the apical third of the root canal. In an earlier study, Boutsioukis et al. stated that constant replenishment of the irrigant throughout the canal facilitates better removal of the smear layer [30]. It has also been observed in a previous study that the orifices of the EndoVac microcannula aids in the removal of the debris in closed end canal systems by aiding as a portal of exit [31]. In the present study, MDA showed better smear layer removal efficacy in the apical third in comparison to PUI and NI. Bronnec et al. stated that MDA allows the irrigating solution to flow up and down along the master gutta-percha cone [32].

|                      | APICAL   | MIDDLE   | CORONAL  |
|----------------------|----------|----------|----------|
| **Group 1-ANP**      | 20 Mean±SD Median | 1.77±0.6861.625 | 1.42±0.4021.250 | 1.19±0.3621.205 |
| **Group 2-MDA**      | 20 Mean±SD Median | 2.12±0.6952.040 | 2.00±0.6282.000 | 1.37±0.3511.250 |
| **Group 3-PUI**      | 20 Mean±SD Median | 2.93±0.2823.000 | 2.18±0.6472.125 | 1.48±0.4151.410 |
| **Group 4-NI**       | 20 Mean±SD Median | 3.56±0.3963.455 | 2.83±0.3673.000 | 1.98±0.5392.000 |

Table 2. Median, Mean and SD Values of Smear Layer Scores of Various Groups

Values in mean with different superscript letters (uppercase for different activation techniques; lowercase at the three segments with each irrigant) are statistically different (significant at p < 0.017).
When this cone is inserted into the working length, the solution will be displaced outward whereas when gutta-percha cone is removed from the canal, it allows inward movement of the irrigating solution. Saber et al. mentioned that the use of a gutta-percha cone that corresponds to the canal size and taper, ensured the displacement of air in the apical portion of the root canal when it is kept to full working length [15]. Caron et al. compared the smear layer removal efficacy of the three different activation systems in the apical third of curved canals and thus observed that MDA and sonic activation systems performed better than the RinseEndo technique [18]. In this study, the results showed that PUI removed more smear layer than conventional irrigation in all thirds of root canals. In a study carried out by Blank-Gonçalves et al., it has been proved that PUI removed more smear layer than conventional irrigation in the apical third of curved root canals [9]. But the results are in contrast to a study by Rödig et al. wherein they had reported that PUI was as ineffective as conventional irrigation in smear layer removal in the apical thirds [12]. The possible difference may be because Rödig et al. used tooth samples with a mean canal curvature of 38.5 degrees.

In comparison with other groups, EndoVac has the highest smear layer removal efficacy in the middle third. This could be because of the placement of macrocannula up to the middle third of the canal. In addition, there was no significant difference between MDA and PUI at the middle third. This is in accordance with the earlier study by Saber et al. in straight root canals [15]. NI was least effective in smear layer removal at all the three levels of the root canal amongst all the groups in the present study. Tay et al. suggested that the presence of an apical vapor lock would adversely affect debridement efficacy for needle irrigation [20]. Further studies are needed to comparatively evaluate the efficacy of newer irrigant delivery systems in canals with severe curvature.

**CONCLUSION**

Within the limitations of this study, it may be concluded that final irrigant activation with apical negative pressure (EndoVac system) results in better smear layer removal in curved root canals when compared with manual dynamic agitation, passive ultrasonic irrigation and needle irrigation.

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