An *in vitro* scanning electron microscopic study: smear layer removal by chelat activation methods

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Abstract

Background: The process of mechanical root canal preparation creates smear layer. The smear layer can prevent the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling materials to canal walls. The purpose of this study is to compare the smear layer removal ability of root canal irrigation methods with chelat solution. **Materials and Methods:** 45 extracted human premolar roots were selected and randomly divided into 3 groups (n=15) based on root canal irrigation methods with 17% EDTA solution: (1) Conventional needle irrigation, (2) Ultrasonic activation, (3) Sonic activation. The roots were prepared with Reciproc Blue 25 file and was removed smear layer by 3 different methods of irrigation. The study sample was then sectioned longitudinally with a diamond cutting disc, randomly selecting half of the root. After undergoing sample processing, the half roots were observed and evaluated for the presence of smear layer under a scanning electron microscope with a magnification of 1000 times according to Torabinejad (2003). **Results:** In cervical, the average smear layer score of the 3 study groups was not statistically significant (p>0.05). In the middle, apical and over the root canal, the average smear layer score of the sonic activation method was lower than that of the other 2 groups. **Conclusion:** Sonic irrigation is more effective in removing smear layer than conventional needle irrigation and ultrasonic irrigation activation.

Key word: *smear layer, ultrasonic, sonic.*

1. INTRODUCTION

In endodontic treatment, the mechanical preparation produces a smear layer, this is a 1- to 2-mm-thick amorphous structure containing both inorganic dentin debris and organic substances, including fragments of the odontoblastic process, microorganisms, and necrotic pulp tissue. Smear layer can prevent the penetration of intracanal medicaments into dentinal tubules and influence the adaptation of filling materials to canal walls [1]. Therefore, to achieve good endodontic treatment results, it is necessary to remove the smear layer.

NaOCl is the most commonly irrigant currently. However, NaOCl at different concentrations is not able to completely remove smear layer because it can only dissolve organic substances. Using NaOCl in combination with chelators has been shown to be effective in removing smear layer [1], [2].

One of the most popular chelat solutions for smear layer removal is the use of EDTA. However, the combination of EDTA and conventional irrigating needles does not seem to be able to effectively remove the smear layer [3]. Therefore, different activation methods to enhance the effects of EDTA have been proposed and investigated. One of the methods of chelat activation is using an ultrasonic, sonic, laser, or XP-Endo Finisher file [3], [4], [5].

Currently, there have been many studies comparative the effective of removing smear layer when irrigated by different methods. According to the study of Qiang Li (2020) and Mancini M. (2021), the results show that the chelat activation methods have a higher efficiency in removing smear layer than the conventional irrigation method [4], [5]. However, study by Machado R. (2021) showed that in the apical third, conventional needle irrigation have similar smear layer removal efficiency compared with the activated methods while in the middle and cervical thirds, conventional needle irrigation remove more smear layer [3].

Therefore, in order to clarify the effectiveness of removing smear layer between methods, we conducted a study:*"***An *in vitro* scanning electron microscopic study: smear layer removal by chelat activation methods***"* with the goal of comparing the ability to remove smear layer by chelat activation methods observed by scanning electron microscope.

2. MATERIALS AND METHODS

2.1. Research subjects: 45 roots of premolar mandible teeth taken from patients with indications for extraction in orthodontic treatment.

2.2. Study design: in vitro experimental study, carried out at the Preclinical Department of Odonto-

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2.3. Research methods

- Step 1: Biomechanical preparation

45 roots of premolars mandibular are standardized with a length of 15mm. The working length (WL) was determined by inserting a K-type #15 instrument (Dentsply/Maillefer) until it could be visualized at the apical foramen, and subtracting 1 mm from this measurement. Simulation of the apical periodontal membrane using OpalDam Green gum protector (Ultradent Products, South Jordan, UT, USA). A #15 K-file was inserted before the layer was applied, to prevent the gingival barrier from entering the canal [4]. Preparing root canals with a Reciproc Blue R25 file (VDW, Munich, Germany) attached to the X Smart Plus endodontic machine (Dentsply Sirona, USA). The R25 file will be gradually moved down to the apex until WL is reached. During this procedure, the instrument was used in a reciprocating motion, with slight apical pressure and a slow in-and-out pecking motion, at an approximate amplitude of 3 mm. Each file was used for 5 canals. Root canal irrigation: insert a Elsodent 30G single sideport needle (France) into the canal with a length shorter than WL 1mm. Irrigate with a total volume of 10 ml of 3% NaOCl solution for each canal during preparation [6]. Finally, the canals were further irrigated with 2 ml of distilled water to restrict the interaction between irrigant solutions [7].

- Step 2: Smear layer removal: The teeth were randomly divided into 3 groups (n = 15) according to the protocol for smear layer removal that was used.

+ Group 1 (n=15) (Conventional needle irrigation) (CNI): The root canals were filled with 2.5 mL of 17% EDTA using a Elsodent 30G single sideport needle (France) calibrated to reach 1 mm short of the WL.

+ Group 2 (n=15) (Ultrasonic activation) (PUI): The root canals were filled with 2.5 mL of 17% EDTA using a Elsodent 30G single sideport needle (France) calibrated to reach 1 mm short of the WL. PUI was performed with Irrisonic E1 (Helse, Santa Rosa de Viterbo, Brazil) attach to the P5 Booster (Satelec, France) according to the manufacturer's instructions (energy level 4, insert into the canal with a length shorter than WL 1mm, avoiding the instrument touching the canal wall for 20 seconds).

+ Group 3 (n=15) (Sonic activation): The root canals were filled with 2.5 mL of 17% EDTA using a Elsodent 30G single sideport needle (France) calibrated to reach 1 mm short of the WL. Sonic activation method is performed by inserting the Endo Activator tip into the canal with a length shorter than WL 1mm attach to Waterpik power floss, activated in 20 seconds.

In each group, the solution used was renewed and/or activated for 3 cycles of 20 seconds each, totaling an irrigation/activation time of 1 minutes. The canals were then irrigated with 2 mL of distilled water, and dried with 3 absorbent paper points (R25, Reciproc, VDW).

- Step 3: Analysis by SEM

The R25 obturator cone insert into canal with full WL (to prevent debris from falling onto the root canal wall during cutting). Cut along the root in the mesial and distal direction with a diamond disc. Replacing the disc after each cut. Then, using an enamel chisel between the two halves of the tooth root and rotate it slightly, separating the tooth root into two halves, randomly selecting one half of the root. Dehydrate the samples before SEM reading according to the following procedure: soak the half roots in 30% ethanol for 10 min, 50% for 20 min, 90% for 30 min, 100% for 30 min. Samples after dehydration were fixed on round metal plates with Carbon glue and coated on the surface with a 30 nm thick gold layer. On each half root, the smear layer was observed under SEM with 1000x magnification at 3 positions: apical third, middle third and cervical third. The technician takes the observed images.

Three observers were trained on how to assess participation. Each observer observes and evaluates over 135 images. Evaluation of the presence of smear layer according to the Torabinejad M. (2003) scale [8]:

1: No smear layer. No smear layer on the surface of the root canals; all tubules were clean and open.

2: Moderate smear layer. No smear layer on the surface of root canal, but tubules contained debris.

3: Heavy smear layer. Smear layer covered the root canal surface and the tubules.

2.4. Data analysis

Data was statistically analyzed using SPSS software ver 20.0. Calculate the mean and standard deviation of the measured values.

+ Compare 2 groups that are related by Wilcoxon test, the test is used with 95% confidence.

+ Compare 3 independent groups by Kruskall - Wallis test, the test is used with 95% confidence.

+ Compare 2 independent groups by Mann - Whitney's U test, the test is used with 95% confidence.

3. RESULTS

Kappa values of 0.90 and above were obtained, demonstrating excellent agreement among examiners for the scores given.

Table 1. Mean of smear layer score in 3 positions of each groups

Positions \ Value		Mean ± std	Median	p
Group 1	Apical (1)	2.27 ± 0.46	2	$p_{(1-2)}$ = 0.011
	Middle (2)	1.73 ± 0.59	2	$p_{(1-3)}$ = 0.002
	Cervical (3)	1.4 ± 0.51	1	$p_{(2-3)}$ = 0.059
Group 2	Apical (1)	2,2 ± 0.56	2	$p_{(1-2)}$ = 0.059
	Middle (2)	1.87 ± 0.64	2	$p_{(1-3)}$ = 0.034
	Cervical (3)	1.8 ± 0.68	2	$p_{(2-3)}$ = 0.655
Group 3	Apical (1)	1.67 ± 0.82	1	$p_{(1-2)}$ = 0.107
	Middle (2)	1.27 ± 0.46	1	$p_{(1-3)}$ = 0.141
	Cervical (3)	1.27 ± 0.46	1	$p_{(2-3)}$ = 1

- In group 1, mean of smear layer score in apical is higher than middle and cervical ($p<0.05$).
- In group 2, mean of smear layer score in apical is higher than cervical ($p<0.05$).
- In group 3, mean of smear layer score in all positions was not statistically significant.

Table 2. Mean of smear layer score in 3 groups

Positions / Group	Apical (Mean ± std)	Middle (Mean ± std)	Cervical (Mean ± std)	Overall (Mean ± std)
Group 1 (n=15)	2.27 ± 0.46	1.73 ± 0.59	1.4 ± 0.51	1.8 ± 0.37
Group 2 (n=15)	2.20 ± 0.56	1.87 ± 0.64	1.8 ± 0.68	1.96 ± 0.52
Group 3 (n=15)	1.67 ± 0.82	1.27 ± 0.46	1.27 ± 0.46	1.4 ± 0.36
p	0.033	0.018	0.051	0.005

- In cervical third, mean of smear layer score of 3 groups was not statistically significant.
- In apical third, middle third and overall, mean of smear layer score of the 3 groups were statistically significant.

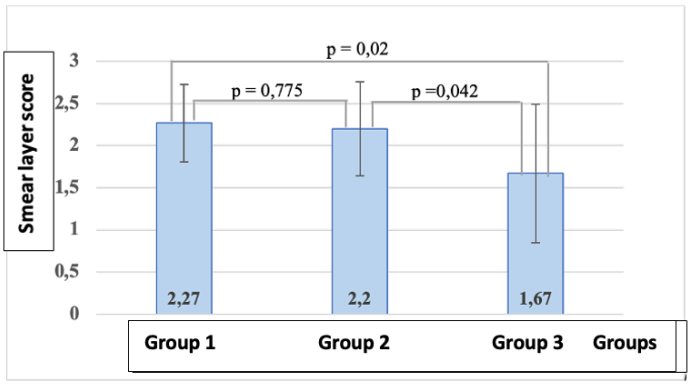

Chart 1. Mean of smear layer score in apical third of 3 groups

- Mean of smear layer score in apical third of group 1 and group 2 was not statistically significant.
- Mean of smear layer score in apical third of group 1 and group 2 were higher than group 3 ($p<0.05$).

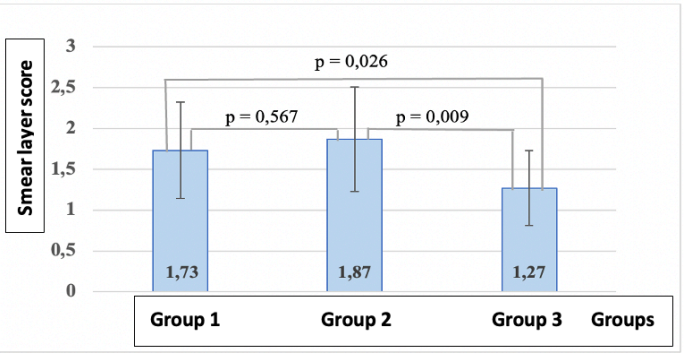

Chart 2. Mean of smear layer score in middle third of 3 groups

- Mean of smear layer score in middle third of group 1 and group 2 was not statistically significant.
- Mean of smear layer score in middle third of group 1 and group 2 were higher than group 3 ($p<0.05$).

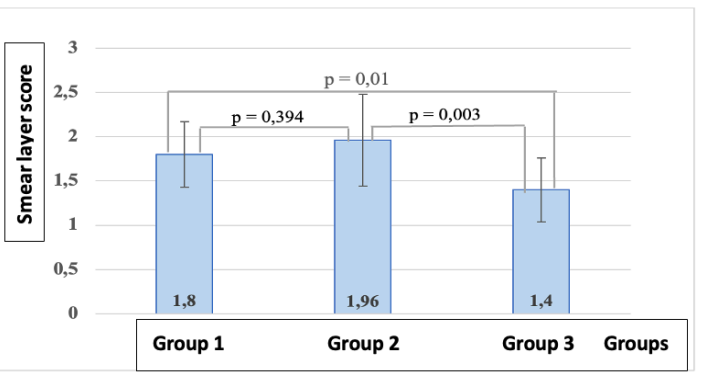

Chart 3. Mean of smear layer score in overall of 3 groups

- Mean of smear layer score in overall of group 1 and group 2 was not statistically significant.
- Mean of smear layer score in overall of group 1 and group 2 were higher than group 3 ($p<0.05$).

4. DISCUSSION

The results from Table 1 show that in group 1, the score of smear layer in the apical third was higher than that in the middle and the cervical thirds. This can be explained by the "vapor lock" effect (formed by a closed end at the end of the apical third and the further apical approach, the narrower the root canal diameter becomes, this prevent the circulation of irrigant solutions) [9]. Gulabivala (2010) also explained that it is not possible to clean the apical because of the lack of penetration of the needle tip and the formation of a "stagnation plane" below the needle tip [10].

In group 2, the score of smear layer at the apical third was higher than that of the cervical third. Our results are quite consistent with the study of Qiang Li (2020) [5].

In group 3, mean of score smear layer in apical, middle and cervical thirds was not statistically significant.

The results from Table 2 show that in the cervical third, mean of score smear layer of the 3 groups was not statistically significant. Our results are quite consistent with the results of Qiang Li (2020): At the cervical third, the ultrasonic and sonic activation methods have no difference in the smear layer score [5].

According to our research, at the apical third, the smear layer score of group 3 was lower than that of group 1 and group 2 with $p < 0.05$ (Chart 1). This means that at the apical third , the smear layer removal ability of the sonic method is better than that of the other two methods. Our results are consistent with the results of Qiang Li (2020) [5]. This result can be explained through the difference in frequency and amplitude of sonic and ultrasonic irrigation. The maximum amplitude of oscillation occurs at the tip of the trigger device, equivalent to the apical position of the canal during irrigation. Compared with ultrasonic activation, the sonic

acttivation have lower frequency and higher amplitude, resulting in greater irrigation energy [11]. In the narrow range of the apical region, the high-energy irrigators easily contact the root canal wall, thus increasing the cleaning capacity of the smear layer [12], [13]. However, according to the study of Machado (2021), it was shown that in the apical third, the amount of smear layer residue in the canal of the 3 methods was not statistically significant [3]. The author also explained that the reason for this was that the constriction of the canal in the apical third prevented the circulation of irrigant and chelating solution, leading to a decrease in the removal efficiency of dentin. For ultrasonic and sonic irrigation methods, it must be ensured that the instrument does not touch the root canal wall during irrigation [3].

The results from Chart 1 also show that at the apical third, the smear layer removal efficiency of CNI and PUI is the same. Our results are not consistent with the conclusions of Matos (2020). According to this author's study, at the apical third, the canal irrigation with 17% EDTA solution combined with PUI removed smear layer better than CNI [14]. The author interpreted this result as the existence of a "vapor lock" effect in the CNI method. Meanwhile, ultrasonic activation is able to eliminate this effect, improving the efficiency of the irrigant [15].

The results from Chart 2 show that at the middle third, the smear layer score of the group activated by sonic is lower than that of the PUI group and the CNI group ($p < 0.05$). According to the study of Mancini (2013), at the position 8mm from the foramen (corresponding to the middle third), the group activated by sonic had better smear layer removal efficiency than the CNI group. Our results are also consistent with the results of Qiang Li (2020): in the middle third, the smear layer score of the CNI group was higher than that of the sonic activated group [5]. However, Machado (2021) suggests that in the middle third, CNI has better cleaning capacity of smear layer than the PUI and sonic activation method. The author also explained that the reason for this difference may be due to the contact between the activator tip and the root canal wall, thereby creating a new smear layer [3].

The mean of smear layer score in overall was lower in the sonic activated group than in the PUI group and the CNI group (Chart 3). Our results are quite consistent with the results of Qiang Li (2020): Over the entire canal, CNI showed the highest mean of smear layer score compared with activated methods [5].

5. CONCLUSIONS

Sonic irrigation is more effective in removing smear layer than CNI and PUI.

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