Original Article

Efficiency of Canal Brush and Ultrasonic Activated Irrigation in Smear Layer Removal Using Two Different Chelating Agents (In Vitro Study)

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Abstract

Objective: This study aims to evaluate the efficiency of two different activation methods after using two different chelating agents in the removal of the smear layer.

Methods: In this study**,** seventy single-rooted mandibular premolar teeth were included, and divided into six study groups and one control group according to the chelating agents and activation methods used. In groups 1,3, and 5, EDTA was used as the chelating agent, where it was activated by Canal Brush in G3 and by Ultra Smart in G5. In groups 2,4, and 6, 7% Maleic acid was used instead and activated by Canal Brush in G4 and Ultra Smart in G6. While in G1 and G2, no activation systems were used. The 7th group was a negative control and purposed to verify the internal microstructure, so only distal water was irrigated (no activation and no chelating agent was used). Samples were sectioned longitudinally and prepared for Scanning Electron Microscope (SEM) analysis in the coronal, middle, and apical parts. Statistical analysis was performed using Kruskal-Wallis and Mann-Whitney U tests. The level of significance was set to 0.05 ($P < 0.05$).

Results: Although the G5 (Ultra Smart +EDTA) median score showed the best smear layer removal score, it was not statistically significant in comparison with the other 5 study groups. In all groups (whether Maleic acid or EDTA were used), smear layer removal was effective in the coronal and middle thirds while less effective in the apical third, with no statistically significant difference between the chelating agents.

Conclusions: Under the limitations of this in vitro study, no activation system was able to remove the smear layer from the root canal wall completely. However, NaOCl and EDTA's irrigation sequence combined with Ultra Smart (ultrasonic activation) obtained better results than the other techniques.

Keywords: *Canal Brush, EDTA, Maleic acid, Smear layer, Ultra Smart.*

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Received: March 14, 2023, Accepted: July 17, 2023, Published: April 1, 2024.

Cite this article as: Fakhruldeen ZE, Faraj BM. Efficiency of Canal Brush and Ultrasonic Activated Irrigation in Smear Layer Removal Using Two Different Chelating Agents (In Vitro Study). Sulaimani Dent J. 2024;11(1):48-60.

DOI: https://doi.org/10.17656/sdj.10186

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Introduction

The success of root canal treatment depends on effective chemo-mechanical cleaning and disinfection to eliminate the etiological factors that cause an endodontic infection, mainly the pathogenic microorganisms and the remnants of dentin debris[1]. In response, the instrumentation phase of the endodontic treatment must be accomplished in combination with deep irrigation to remove the remains of the pulp tissue, dentin, and pathogenic microorganisms. Without irrigation, instruments would be ineffective due to accumulating the remaining debris[2].

The "smear layer" consists of organic and inorganic debris formed during mechanical root canal preparation. Necrotic tissue and bacteria cover the canal walls in this layer, which hinders the capacity of disinfecting agents and intracanal medications to penetrate the dentinal tubules and inhibits the filling materials' ability to attach to the canal wall[3].

Conflicting studies exist concerning the benefit of maintaining or eliminating the smear layer. But generally, most data in the literature demonstrate the importance of removing the smear layer to enhance the adaptation of filling materials and proper disinfection of the root canal system. Any accumulated debris or bacterial colonies left in the root canal have a potential side effect on the sealing ability of the root canal filling materials. In addition, their presence may impede the proper disinfection in cases with apical periodontitis[4].

Different methods have been used to remove the smear layer, including chemical, mechanical, and laser, which were applied to enhance the canal wall's cleaning effect. So far, no single method has been universally accepted to clean root canals' entire length. However, the approach most commonly used is the sequential application of Sodium hypochlorite (NaOCl) solutions and Ethylenediaminetetraacetic (EDTA) [5].

Ethylenediaminetetraacetic acid, or EDTA, is a chelator used after NaOCl as the final irrigant. EDTA solution is neutral or slightly alkaline; EDTA precipitates when the pH is acidic. It is typically used as a 17% or 15% solution. EDTA affects the inorganic portion of the smear layer's dentin, so NaOCl must be used prior to removing the organic debris. EDTA has little or no antimicrobial activity, although some studies have indicated antifungal activity for EDTA[3].

Another irrigation solution used in this study is Maleic acid, a mild organic acid used in adhesive dentistry as an acid conditioner. It has been discovered that this agent can remove the smear layer when used as an acid etchant in restorative dentistry. It is usually used as 7% Maleic acid and has a pH of 1.3. Ballal et al. have suggested that 7% Maleic acid achieves the high-quality removal of the smear layer from root canal dentin and is more efficient than EDTA at the apical third of the root canal[6,7].

The Canal Brush is an irrigation activation file that was introduced a few years ago, while Blue Flex Canal Brush is a new product. It is a unique grinding file for the irrigant activation and final processing of the root canal. Rotating at high speed from 900 to 4000 rpm, the brush untwists inside the canal into separate wires, which are perfectly adjusted for the individual anatomy of the root canal and inaccessible to the usual rotary instruments. Blue Flex Canal Brush does not enlarge the canal diameter[8].

Two types of ultrasonic irrigation have been described in the literature: the first type combines simultaneous ultrasonic instrumentation and irrigation (UI), and the second type, often referred to as passive ultrasonic irrigation (PUI), is performed without simultaneous instrumentation. In this study, we used the second type, in which the file only activates the irrigation solution[9].

Passive Ultrasonic-activated irrigation technique includes utilizing an ultrasonic file that oscillates at a frequency of 30 kHz. Such frequency achieves cavitation and miniature acoustic streaming, thus producing shear stresses to disturb and separate the bacterial biofilm and debris throughout the canal's dentinal wall[10]. Ultra Smart by COXO Co.Ltd was the ultrasonic device used in this study, by applying an oscillating file with a working tip of 25#02, 38kHz frequency, and a working time of 20 seconds, in accordance with the manufacturer's instructions[11].

When reading and comparing the literature, many studies were found that showed the effectiveness of the activation techniques using the most widely used irrigation solutions: EDTA and NaOCl[3]. However, little research has been done on maleic acid activation, specifically in combination with a canal brush activation. This study compares this combination of chelating agent/activation technique with EDTA, the most common chelating agent, accompanied by ultrasonic activation.

The null hypothesis to be tested is that there is no difference in the effect of Canal Brush and ultrasonic activation methods on smear layer removal, with or without the use of EDTA or maleic acid chelating agents.

Methods

Teeth Preparation for the Study

The present study obtained approval from the ethics committee of the College of Dentistry – University of

Sulaimani (no. 21/87 on 9/11/2021). The study design is considered an "In vitro comparative study", and the sample size was determined by power calculation using G power 3.1.9.4 software to select the minimum total sample size of 70 teeth, divided equally into seven groups, ten samples per group.

In this study, seventy single-rooted mandibular premolar teeth that were extracted for orthodontic reasons (age 14-30 years), of similar length and dimensions, were inspected through a stereomicroscope Microscope (KOPPACE Technology, Shenzhen, China) with 20× magnification. In addition, buccal and proximal radiographs were taken for the purpose of excluding teeth that matched the exclusion criteria: open apices (not fully formed), root resorption, prior root canal treatment, root caries, calcified canals, and cracks, and to select teeth that matched the inclusion criteria: teeth with only a single canal, intact roots, fully formed apex, teeth without any root fractures or cracks and with non-calcified canals.

The teeth were cleaned of debris and soft tissue remnants using a soft toothbrush and saline-wetted cotton and stored in a saline solution. For specimen standardization, the crowns were removed with a high-speed long tapered chamfer[12] diamond bur to establish a 16mm length for all samples. In addition, root canal degree of curvature was standardized, including only straight to averaged curved canals, i.e. <20º according to Schneider's Classification[13].

A stainless-steel size 10 K-File (ROGIN Dental Medical CO.LTD, Shenzhen, China) was introduced in the root canals until it was visible at the level of the apical foramen under stereomicroscope magnification. The working length was calculated by reducing this length by 1 mm. Consequently, the apical two-thirds (including the apex) of the specimens were sealed with pink modelling wax (POLWAX, bilkim, Izmir, Turkey) to simulate a closed system and prevent the leakage of the irrigant through the apical foramen during canal preparation. Samples were then included in a condensation silicon impression material matrix (alphasil PERFECT, Muller-Omicron GmbH & Co.KG, Lindlar, Germany) to make a customized model for each sample.

Following the preparation of a manual glide path working with size 15 and 20 K-Files (ROGIN Dental Medical CO.LTD, Shenzhen, China), the root canals were instrumented from Sx to F3 files using ProTaper Universal system (Dentsply, Maillefer, Switzerland) with 1ml of 5.23% NaOCl (AQUA, Istanbul, Turkey) irrigation between each file.

Teeth grouping according to the final irrigation regime

Teeth were divided into seven groups (as shown in Figure 1) according to the final irrigation regime:

Group 1(EDTA): 10 teeth were used, in which, after instrumentation, 2 ml of 5.23% NaOCl was irrigated with a 30-gauge side-vented endodontic needle (HunterLine, Germany) 2 mm short of the working length for 30 seconds, followed by irrigation with 1 ml of 17% EDTA (Prevest DenPro LTD. Co, India) for 40 seconds without use of any activation method.

Group 2 (Maleic acid): 10 teeth were used after instrumentation and final irrigation with 2 ml of 5.23% NaOCl for 30 seconds, and 1 ml of freshly prepared 7% Maleic acid irrigated for 40 seconds with no activation method. The 7% Maleic acid was prepared by dissolving 7 gm of Maleic acid powder (Sigma-Adrich Co., Germany) into 100 ml of pure distal water.

Group 3 (Blue Flex Canal Brush -EDTA) : (n=10) irrigation with 1 ml of 5.23% NaOCl for 30 seconds and activation with Blue Flex Canal Brush (Blue Flex, made in Russia, packed in India) was performed by placing in a slow-speed handpiece (900-4000 rpm) and advancing to 2 mm short of the working length for 10 seconds. Then the Brush was taken out, re irrigation with 1mL of 5.23% NaOCl was done and activation by Blue Flex Canal Brush was repeated once for 10 seconds.

After that, irrigation with 0.5 mL of 17% EDTA for 10 seconds and activation of the solution with Blue Flex Brush for 10 seconds was performed, followed by re-irrigation with 0.5 ml of EDTA for 10 seconds and activation again for 10 seconds (a total of 40 seconds during which a total 1 ml of EDTA was inside the canal).

A final flush of 5 ml of Normal Saline was irrigated into the canal.

Group 4 (Blue Flex Canal Brush- Maleic acid): (n=10), The chemo-mechanical preparation was identical to that in the Blue Flex Brush-EDTA group, but 7% Maleic acid irrigation was used instead of 17% EDTA for the final irrigation protocol.

Group 5 (Ultrasonic activation -EDTA): (n=10), After performing the chemo-mechanical preparation, ultrasonic activation was done in the final irrigation regime by using an ultrasonic file with a tip size of 25#2 mounted on an ultrasonic device (Ultra Smart, Foshan COXO Medical Instrument Co., Ltd, China) with an activation time of 20 seconds, and a frequency of 38KHz, in accordance with the manufacturer's instructions. Final Irrigation regime: 2 ml of 5.23% NaOCl was applied for 30 seconds, then activated for 20

seconds, followed by 1 ml of 17%EDTA for 20 seconds and further activation for 20 seconds.

Group 6 (Ultrasonic activation - Maleic acid): (n=10) The chemomechanical preparation and the final irrigation regimen were the same as in the Ultra Smart-EDTA acid group, but 7% Maleic acid was used instead of 17% EDTA.

Group 7 (Negative Control): 10 teeth were used as a negative control group without activation, and no sodium hypochlorite or chelating agents were used to verify the roots' microstructure, with only instrumentation and distal water used for irrigating the canals.

The canals were dried with absorbent paper points (ProEndo, HunterLine Dental Inc. Germany), and then F3 Gutta-Percha (ProEndo, HunterLine Dental Inc. Germany) was inserted as an indicator to avoid cutting into the root canal during root sectioning.

Teeth Preparation for SEM Analysis

Longitudinal grooves were created on the roots using a double-sided diamond disc (SUNSHINE DIAMONDS, DR. HOPF FmbH &Co.KG, Germany). A diamond disc of 0.1 mm thickness was mounted on a laboratory handpiece. Sectioning was performed without reaching the root canal. Subsequently, the roots were separated into two parts using a disposable scalpel blade nº 15 (Kehr Surgical Private Limited, Kanpur, India). Each half sample was sputter coated with gold and then analyzed by a scanning electron microscope (SEM) (Bruker Co., USA) in the canal's apical, middle, and coronal thirds, respectively, by using 1000X and 4000X magnifications and this last magnification was used for scoring since it was more detailed regarding the visualization and counting of the dentinal tubules.

Each root third was evaluated blindly for smear layer removal by two experienced endodontists. If any score didn't match, an agreement was made on selecting the most appropriate score. The inter-rater reliability or the agreement between the two examiners' scores was determined statistically using Cohen's Kappa coefficient. Scoring was based on the following scale by Hulsmann[18]:

Score 1: No smear layer, dentinal tubules open.

Score 2: Small amount of smear layer and some dentinal tubules open.

Score 3: A Homogenous smear layer covers the root canal wall, with only a few dentinal tubules open.

Score 4: Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.

Score 5: Heavy, nonhomogeneous smear layer covering the complete root canal wall.

Statistical analysis

For statistical analysis, the data were analyzed by the SPSS 25.0 program, and the following non-parametric tests were used as the data did not display normal distribution:

1. The Kruskal-Wallis test was used for inter-group comparisons.

2. The Mann-Whitney U test was used for intra-group comparisons.

Additionally, Cohen's Kappa coefficient was used to check the inter-examiner agreement.

All tests were performed at a significance level of $p < 0.05$.

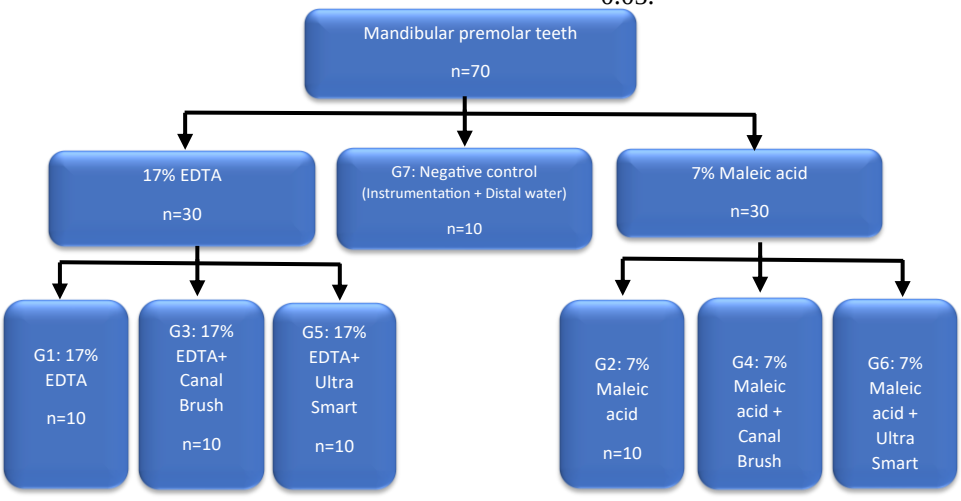

Figure 1: A diagram illustrating the study groups distribution according to the final irrigation regime.

Results

Cohen's Kappa Coefficient = 0.836, indicated excellent inter examiner agreement[14], as shown in Table 1. Following are the results for inter and intra-group comparisons:

Kruskal Wallis test results for inter-group comparisons

Maleic acid and EDTA removed the smear layer almost equally, and statistically, there was no significant difference between these two chelating agents. There was no statistically significant difference between Blue Flex Canal Brush and Ultra Smart (Ultrasonic) activated irrigation in smear layer removal using EDTA or Maleic acid chelating agent in any of the three-thirds of the teeth: at the coronal (p=0.418), middle (p=0.641) and apical (p=0.917), as shown in Table 2. In addition, Figures 2 and 3 show some SEM photographs of these examined groups.

The distal water group was used only for verifying the microstructure of the root canal wall without following the irrigation protocol (only instrumentation and distal water irrigation were done), so it was excluded from the statistical analysis. The median of the scores were 4,4, and 5 for the coronal middle and apical parts, respectively; a sample image is shown in Figure 3 (G7).

EDTA groups comparisons with and without activation (groups 1, 3, and 5)

EDTA groups comparison: Group 1(EDTA), group 3(EDTA+ Canal Brush), and Group 5(EDTA+ Ultra Smart) comparisons showed that Ultra Smart improved the performance of EDTA in smear layer removal at Coronal and apical thirds as group 5 had lower median scores than group 1 in those parts. On the other hand, Canal Brush slightly improved the action of EDTA in the apical third, as indicated by the lower median score in the apical third. Nevertheless, it did not improve the action of EDTA in the coronal and middle thirds, as illustrated by Table 3.

However, these differences were statistically non-significant, as shown by Table 2 (Kruskal-Wallis test). SEM images from groups 1, 3, and 5 are shown in Figure 2.

Maleic acid groups comparison with and without activation (Groups 2, 4, and 6)

Maleic acid group comparison: Group 2 (Maleic acid), group 4 (Maleic acid + Canal Brush), and group 6 (Maleic acid + Ultra Smart) showed that the Blue Flex Canal Brush improved the action of Maleic acid in the middle third only, as group 4 had a lower median score than Group 2 in that part. Ultra Smart slightly improved the action of Maleic acid in the middle third, as group 6 had a lower median score than group 2, though these differences were statistically insignificant. None of the activation methods improved the action of Maleic acid in the apical or coronal thirds, as shown in Tables 2 and 3. SEM images from groups 2,4, and 6 are shown in Figure 3.

Intra-group comparisons

When comparing the smear layer removal scores at the coronal, middle, and apical parts of each group, it was found that in all groups, there was a significant difference between the coronal and the apical part ($p < 0.05$), wherein the coronal third smear layer removal was generally better than the apical third. There was no significant difference between the coronal and the middle parts ($p > 0.05$) in any of the groups. Regarding the comparison between the middle and the apical thirds, there was a significant difference ($p < 0.05$) in G1 (EDTA), G3 (EDTA+ Canal Brush) and G6 (Maleic acid + Ultra Smart), while in G2 (Maleic acid), G4 (Maleic acid + Canal Brush) and G5 (EDTA +Ultra Smart) there was no significant difference ($p > 0.05$), as shown in Table 4.

Table 1: Overall assessment analysis on the level of agreement between examiners.

	Value	Asymptotic Standard Error	Monte Carlo Significance		
			Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
The measure of Agreement; Kappa	0.835	0.030	.000[c]	0.000	0.014
N of Valid Cases	210				

Table 2: Comparison of smear layer removal scores between coronary, middle, and apical thirds of all groups.

Region	Group	Mean Rank	Kruskal-Wallis Test Result (Significance)
Coronal Third	EDTA	31.55	NS*
	Maleic acid	29.8	
	EDTA+Canal Brush	30.35	
	Maleic acid+Canal Brush	34	
	EDTA+Ultra Smart	21.15	
	Maleic acid+Ultra Smart	36.15	
Middle Third	EDTA	27.2	NS*
	Maleic acid	35.95	
	EDTA+Canal Brush	31.5	
	Maleic acid+Canal Brush	30.2	
	EDTA+Ultra Smart	24.2	
	Maleic acid+Ultra Smart	33.95	
Apical Third	EDTA	31.9	NS*
	Maleic acid	29.9	
	EDTA+Canal Brush	31.2	
	Maleic acid+Canal Brush	30.7	
	EDTA+Ultra Smart	25.6	
	Maleic acid+Ultra Smart	33.7	

*NS: Non-Significant

Table 3: Differences in mean, median and percentiles between each group.

Groups	Region	Mean	Median	25 percentile	75 percentile
1. EDTA	Coronal	1.9	2	1	3
	Middle	2.2	2	1	3
	Apical	3.3	4	2.75	4
2. Maleic acid	Coronal	1.8	2	1	2.25
	Middle	2.7	3	2	3.25
	Apical	3.3	3.5	2.75	4
3. EDTA+Canal Brush	Coronal	1.8	2	1	2
	Middle	2.4	3	1.75	3
	Apical	3.4	3.5	3	4
4. Maleic acid+ Canal Brush	Coronal	2.1	2	1	3
	Middle	2.4	2	1.75	3.25
	Apical	3.3	3.5	3	4
5. EDTA+ Ultra Smart	Coronal	1.4	1	1	2
	Middle	2	2	1	3
	Apical	3	3	2	4
6. Maleic acid+ Ultra Smart	Coronal	2.1	2	1.75	3
	Middle	2.6	2.5	2	3
	Apical	3.5	4	3	4

Table 4: Intra-group comparisons (comparing the coronal, middle and apical thirds of the same group) using Kruskal Wallis and Mann-Whitney U tests.

Groups	Kruskal Wallis Test (H value, D. F, p Value) ^	Regional Difference	Mann-Whitney U value	Mean Rank	p-value
1. EDTA	H=8.41, D.f= 2, p value= 0.015	Coronal-Middle	58.000	9.70 - 11.30	0.580
		Middle-Apical	78.000	7.70 - 13.30	0.035*
		Coronal-Apical	84.500	7.05 - 13.95	0.007**
2. Maleic acid	H=10.46, D. F= 2, p value= 0.005	Coronal-Middle	76.000	7.90 - 13.10	0.520
		Middle-Apical	68.000	8.70 - 12.30	0.190
		Coronal-Apical	89.000	6.60 - 14.40	0.002**
3. EDTA + Canal Brush	H=14.18, D. F= 2, p=0.001	Coronal-Middle	72.000	8.30 - 12.70	0.105
		Middle-Apical	81.000	7.40 - 13.60	0.019*
		Coronal-Apical	94.000	6.10 - 14.90	0.000**
4. Maleic acid + Canal Brush	H= 6.25, D. F =2, p=0.44	Coronal-Middle	58.000	9.70 - 11.30	0.579
		Middle-Apical	68.000	8.70 - 12.30	0.190
		Coronal-Apical	89.000	6.60 - 14.40	0.002**
5. EDTA + Ultra Smart	H=10.37, D. F= 2 p=0.006	Coronal-Middle	68.000	8.70 - 12.30	0.190
		Middle-Apical	76.000	7.90 -13.10	0.052
		Coronal-Apical	82.000	6.30 - 14.11	0.001**
6. Maleic acid + Ultra Smart	H=10.08, D. F=2, p=0.006	Coronal-Middle	66.500	8.85 - 12.15	0.218
		Middle-Apical	80.500	7.45 - 13.55	0.019*
		Coronal-Apical	90.000	6.50 - 14.50	0.002**

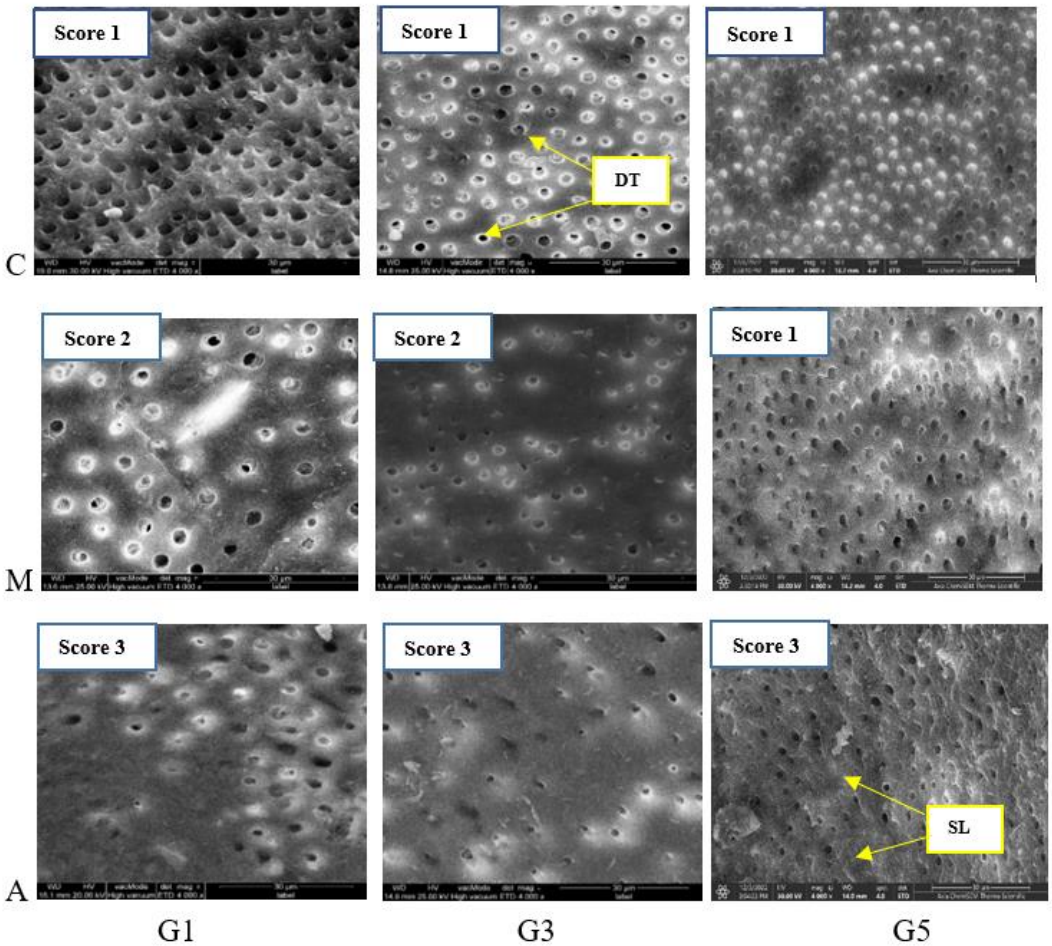

Figure 2: SEM photographs and relative scores, taken at 4000X for EDTA groups: G1(EDTA), G3 (EDTA +Canal Brush) and G5 (EDTA+ Ultra Smart). C =Coronal, M=Middle, A=Apical, DT= Dentinal tubules, SL= Smear Layer.

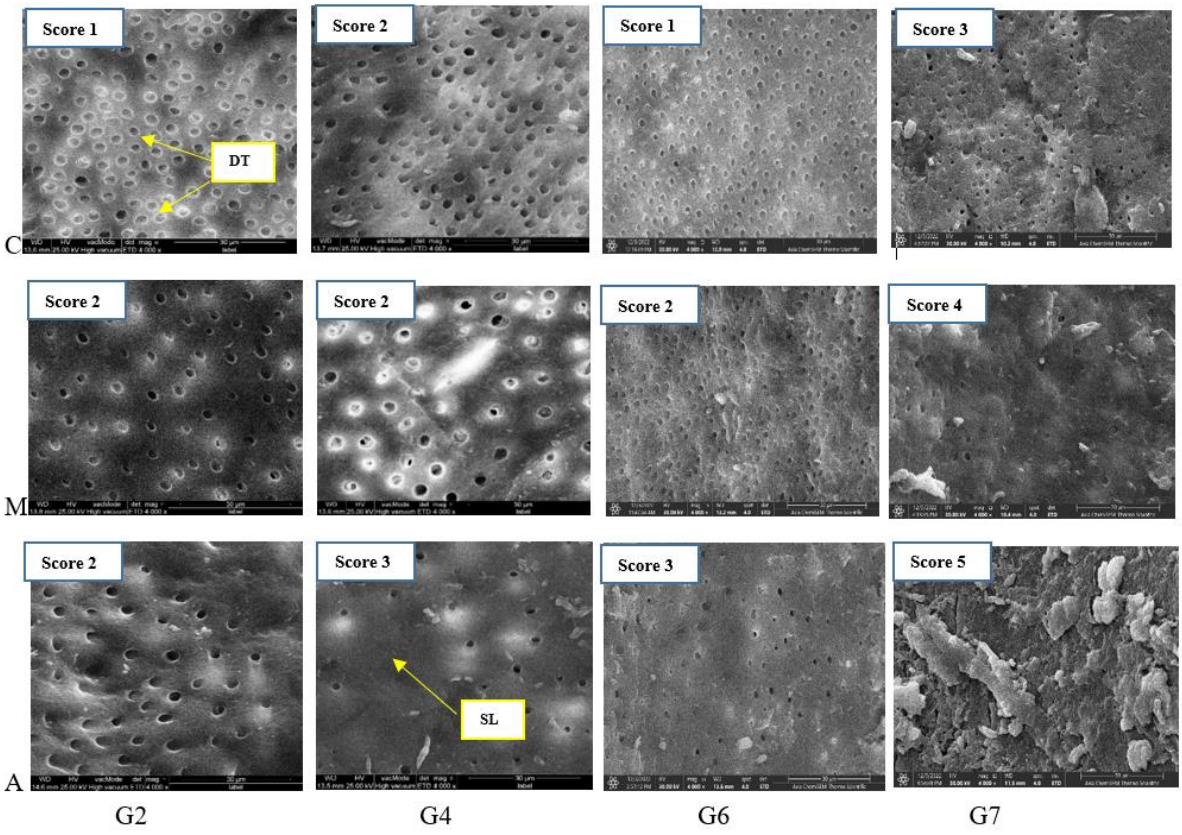

Figure 3: SEM photographs and relative scores, taken at 4000X for Maleic acid groups: G2 Maleic acid, G4 (Maleic acid +Canal Brush), G6 (Maleic acid Ultra Smart), and negative control group G7 (Distal water). C =Coronal, M=Middle, A=Apical. DT= Dentinal tubules, SL= Smear Layer

Discussion

One of the main goals of endodontic treatment is to eliminate microorganisms and any factor that aids in their recruitment, such as organic and inorganic debris, from the root canal system. Irrigation, as an essential component of root canal treatment, can almost achieve such a goal by reaching areas not touched by instrumentation and aiding in removing the smear layer[15].

Using hand or rotary files for instrumentation during mechanical preparation will produce abundant mineralized debris as part of the smear layer. The first researchers to use a scanning electron microscope (SEM) to identify the smear layer were Eick et al.[16], who discovered that the layer is composed of particles ranging in size from 0.5 to 15 µm.

Several studies stressed the importance of smear layer removal[3,4]. Many activation techniques, such as hydrodynamic irrigation, sonic, ultrasonic devices, and Endovac (Negative pressure irrigation), were introduced, tested, or studied in the literature for their effectiveness. In the present study, we aimed to use a different approach to the standard protocol of smear layer removal (An irrigation sequence of NaOCl followed by EDTA)[5]. The research was done using two different types of chelating agents activated by two techniques.

In this study, we used Maleic acid and compared it to EDTA because research has demonstrated that 7% Maleic acid is as effective as 17%EDTA or even more so in the apical third[6]. We selected Blue Flex Canal Brush because few studies have evaluated this product[8], and there was a need for more research to prove its effectiveness further. Also, it was chosen as a different activation method from ultrasonic activation, a widely used approach that has been reported by many previous studies to be effective in smear layer removal[17,18].

The use of (Blue Flex Canal Brush) to activate the irrigation solutions and the combination of Canal Brush and Maleic acid chelating agent is considered a modification made to previous research that focused on

the effect of Maleic acid alone[7,9,20]. This last-mentioned combination has been compared in the present study with the method most reported and tested[18] in the literature for its effectiveness in irrigation activation and removing inorganic particles, namely the use of EDTA and ultrasonic activation technique.

We compared the root in the coronal, middle, and apical thirds separately due to the morphological differences, as a previous study has shown that by moving downward from coronal to the apical zone, the tubular lumen area progressively decreases from 15.47 ± 7.06 μm2 of the coronal zone to 12.77 ± 10.23 μm2 of the middle zone, reaching a minimum of 3.033 ± 2.43 μm2 in the apical zone. In addition, the surface occupied by the intertubular dentin was calculated to progressively increase, moving from coronal to apical, while the ratio between the tubular lumen area and dentinal surface progressively decreases[21]. Furthermore, irregular structures were found more in the middle and apical thirds than in the coronal third of the root canal[22].

A study has reported that a neutral to slightly alkaline EDTA solution reduces dentin's mineral and non-collagenous protein (NCP) components. Thus, EDTA removes calcium ions and calcium bonded to NCPs. Because the content of NCPs decreases in the apical third of the root canal system, the degree of decalcification of EDTA in this part is low[23].

The slightly better performance of 7% Maleic acid in the apical third might also be attributed to the increased surface tension of 17% EDTA (0.0783 N/m) compared to that of 7% Maleic acid (0.06345 N/m), indicating that the surface tension of 17% EDTA is higher than that of 7% Maleic acid and that Maleic acid can penetrate the tubules more easily[7].

In this study, we used 17% EDTA applied for 40 seconds (less than 1 minute) as this has shown to be effective in smear layer removal and to prevent the erosion of internal dentin[15]. Regarding Maleic acid, research has indicated that 1 min of 7% Maleic acid application is enough to remove smear layer effectively. Furthermore, another research showed that 45 seconds of 7% Maleic acid is the most effective duration to remove smear layer without decreasing the dentin's microhardness[24]. So, in this study, 7% Maleic acid was used for 40 seconds (less than 1 minute) to be comparable with EDTA. The time of the application was near to the time suggested above.

The present study showed that 17% EDTA and 7% Maleic acid were equally efficient in smear layer removal in the coronal and the middle parts of the root canal, which was similar to other research results (Ballal, Jain, and Tay, 2016[19] and Kuruvilla et al., 2015[6]). Nevertheless, in the apical part, the studies mentioned above stated that 7% Maleic acid showed better results in smear layer removal, while in our study, although 7% Maleic acid performed slightly better, the results were not statistically significant when comparing to EDTA.

Another study also showed no statistically significant difference between 7% Maleic acid and 17% EDTA, which suggests that Maleic acid can be used instead of EDTA[25].

On the other hand, a study by Attur et al.[20] showed that EDTA had better smear layer removal in all three-thirds compared to 7% Maleic acid.

It is known that the efficiency of a chelating agent depends on several factors, including application time, concentration, amount of the solution, and pH value[23].

The differences in the results between the current and previous studies could be attributed to the irrigation time and amount of the irrigation solutions, including the chelating agents. For example, studies by Ballal et al.[19], Attur et al.[20] and Kuruvilla et al.[6] used 5ml of chelating agents that were applied for 1 min. In this study, we used only 1ml, which was applied for 40 seconds. We selected 1 ml in this study because EDTA irrigation volume greater than 1 ml did not improve debris removal, and the smear layer was removed efficiently with a final rinse of 1 ml of 17% EDTA for 1 minute[26,27]. We used 1 ml of 7% Maleic acid to be equal and comparable with the volume of EDTA used. Increasing the volume of the EDTA used, and the activation cycles could result in better smear layer removal, as indicated by the other studies mentioned above. Still, on the other hand, this might enhance dentinal erosion[15].

Although the pH value of EDTA is 8.5[28] (slightly alkaline) and Maleic acid is 1.3 (strong acid)[24], both remove the smear layer effectively due to the different mechanisms of action that allow each solution to chelate appropriately, even though they have different pH values, as discussed in the following paragraphs:

The pH of EDTA solutions affects their efficacy and calcium ion availability in several ways. As the pH increases, the availability of calcium ions from hydroxyapatite (HAp) for chelation decreases. At the same time, a more remarkable dissociation of the EDTA produces an increased attraction for calcium ions. Conversely, calcium ions become more available for chelation at lower pHs. However, the efficacy of EDTA decreases, so the optimal pH for EDTA solutions seems to be from 6–10 and is more effective at neutral pH to slightly alkaline pH[29]. EDTA functions appropriately at a slightly alkaline pH[28].

Effective decalcification action of EDTA at a slightly alkaline to neutral pH may be attributed to the mechanism of capturing metallic ions like calcium, which binds to the chelating agent, meaning that the calcium ions from the external layer of the apatite crystals will be removed[30].

The 7% Maleic acid is highly acidic, with a pH of (1.3). This acidic pH might have caused demineralization of the root canal dentin and subsequent reduction in the microhardness[31]. The mechanism of acidic interaction with HAp was found to involve two phases. In the first phase, carboxylic acids bond to the calcium of HAp. Depending on the diffusion rate of the calcium–acid complexes into the solution, the acid will in the second phase either remain attached to the HAp surface with only limited decalcification involved, or the calcium–acid complex will debond, resulting in a substantial decalcification effect[31].

In the present study, Ultra Smart (Ultrasonic) activated irrigation improved smear layer removal of 17% EDTA in the coronal and the apical thirds. Although this improvement was not statistically significant, it was similar to other studies[32,33]. However, a systematic review showed that ultrasonically activated irrigation did not improve EDTA action[34].

Furthermore, studies by Iandolo[35] and Spirito[36] proved that Passive Ultrasonic Irrigation (PUI) improved smear layer removal; however, in the first study, Ultra Smart was used for ultrasonic activation of an intracanal-heated NaOCl. In addition, the irrigant volume of 5 ml NaOCl during activation was used. The number of activation cycles was five, while in the second study, there was a total of eight activation cycles using 8 ml of NaOCl and 3 ml of EDTA versus 2 ml of non-heated NaOCl and 1 ml of EDTA, each activated for 20 seconds only, in the current study (1 activation cycle for each irrigant).

Using Ultra Smart (ultrasonic activation) improved the action of Maleic acid in the middle third but statistically was not significant; similar non-significant results were reported by Ramachandran et al.[37], but a study by Billal and Rao[38] reported that ultrasonic activation improved 7% Maleic acid in the apical third only. However, it is worth mentioning that in that study, Maleic acid application and activation time was 1 minute, unlike in our study, where the entire application and activation time was just 40 seconds.

The only thirds that Blue Flex Canal Brush slightly improved using 7% Maleic acid and 17% EDTA were the middle third and the apical thirds, respectively, but the results were not statistically significant, and this could be attributed to the action of the wire untwisting, that is, on the mid to end part of the Brush[39]. Furthermore, the coronal third has a larger diameter, allowing for more irrigation volume to contact the dentinal wall, and the smear layer was almost completely removed with no help of an activation mean[40]. Only one study was found in the literature to evaluate Blue Flex Canal Brush, but their result is not comparable to our's, as they did not use this product to activate EDTA[8].

PUI (passive ultrasonic irrigation) relies on transmitting acoustic energy from an oscillating file or smooth wire to an irrigant in the root canal. The energy is transmitted by means of ultrasonic waves and can induce acoustic streaming and cavitation of the irrigant. The acoustic stream can be defined as a rapid movement of the fluid in a circular or vortex shape around the vibrating file. Cavitation is the creation of steam bubbles or the expansion, contraction, and distortion of preexisting bubbles in a liquid[10]. This mechanism could be why Ultra Smart performed slightly better than syringe irrigation and Blue Flex Canal Brush.

PUI should be introduced in the canal once the root canal system has a final apical size and taper. A fresh irrigant solution should be introduced, and a small file or smooth wire (e.g., size #15- 25) should be ultrasonically activated. Since the root canal has already been shaped, the file or wire can move more freely, and the irrigant can penetrate the apical part of the root canal system, with the cleaning effect being more significant[10,15].

Blue Flex Canal Brush rotates at high speed from 900 to 4000 rpm, and the brush untwists inside the canal into separate wires, which are perfectly adjusted for the individual anatomy of the root canal and inaccessible for the usual rotary instruments. Blue Flex Canal Brush does not enlarge the canal diameter.[8] The non-significant effect of this Brush could be attributed to its short time of application (20 seconds) for each irrigant. Future research is required further to investigate its effectiveness at a longer activation time.

Limitations of this study include the in vitro use of the samples, since factors present among in-vivo samples, such as the presence of blood in the canal and more necrotic tissues, could affect the result. In addition, including only straight to averaged curved canals < 20º limits the results to this type of curvature.

Other limitations include using NaOCl at room temperature, as some research shows a better action

when it is pre-heated[36], and it is well-known that NaOCl has a vital role in removing the organic part of the smear layer even before EDTA or Maleic acid removes the inorganic part[35].

The results from the current study assure that using NaOCl followed by EDTA remains the gold standard in smear layer removal, offering satisfying outcomes during clinical work. However, using Ultra Smart or ultrasonic activation could show promising results in further enhancing smear layer removal, as it showed slightly better results than Blue Flex Canal Brush. As discussed earlier, its effectiveness has been demonstrated in other studies, mainly when used for more extended periods.

Further studies are needed to evaluate Blue Flex Canal Brush and Ultra Smart (ultrasonic activation) but by using more irrigant volumes, which are mainly NaOCl, EDTA, and Maleic acid, and utilizing more activation cycles, thus extending their activation time (more than 20 seconds; one activation cycle for Ultra Smart and two activation cycles for Blue Flex Canal Brush) to further assess Blue Flex Canal Brush performance at higher irrigant volume and provide more proof for the significance of Ultra Smart using such volumes and activation times of the irrigation solutions.

Conclusion

Within the limitations of the present study, it is concluded that:

1. Using Blue Flex Canal Brush for 20 seconds (2 activation cycles) for 17% EDTA and 7% Maleic acid did not enhance the action of either chelating agent. Similar results were obtained for smear layer removal.

2. Applying 5.23% NaOCl followed by 1 ml of 17% EDTA combined with Ultra Smart activation for 20 seconds obtained better results than activation using Blue Flex Canal Brush.

3. Ultra Smart activation did not enhance 7% Maleic acid action.

4. The 7% Maleic acid and 17% EDTA application (with no activation) showed similar effective results for removing the smear layer.

5. All groups generally showed better results in the coronal third compared to the middle and apical thirds, where smear layer removal was less effective.

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