Effects of 2.625% NaOCl - 20% Citric Acid and 2.625% NaOCl - 17% EDTA on Cleanliness of Smear Layer on Apical One Third

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Abstract. Smear layer, as a result of instrumentaion, could influence the outcome of treatment. The aim of this study was to analyze the effectiveness of two combinations of irrigants in removing the smear layer in the root canal. Thirty permanent premolars were divided in 3 groups equally. Group I, II, and III used NaOCl 2.625%-citric acid 20%, NaOCl 2.625%-EDTA 17%, and saline solution (control) respectively. The remnants of smear layer were evaluated by SEM and were scored. The score was 0, 1, 2, or 3, if the remnants of the smear layer was less than 25%, between 25 to 50%, 50 to 75%, and more than 75% of the surface respectively, and were analyzed by Kolmogorov Smirnov. There was no significant different in removing the smear layer between the Group I and the Group II (p = 0.447), while comparing to the control group, the experimental groups were significantly better (p = 0.000). No difference in ability to remove the smear layer between combinations of irrigant of NaOCl 2.625%-citric acid 20% and NaOCl 2.625%-EDTA 17%.

1. Introduction
The prognosis or success of root canal treatment is determined by the level of access, root canal preparation (cleaning and shaping of the root canal), and good obturation. The cleaning and shaping of the root canal is a chemomechanical procedure performed mechanically using an instrument and chemically using medication including irrigants [1,2].

Root canal preparation (root canal instrumentation) generates a smear layer, which is a debris layer with a thickness of 0.5–5 µm. This layer contains organic and inorganic components and seals the connection between the dentin tubules, the lateral canal, and the isthmus, which are anatomically found in the apical one third [3,4]. The smear layer can interfere with the penetration of medicament to the dentin tubules and the lateral canal, thus, interrupting the disinfection process in the root canal since debris, microorganisms, and their products are left in the root canal [5]. The smear layer can also interrupt the adaptation of obturation material with the root canal wall, leading to micro leakage and creating a pathway for bacterial contamination [6-8].

Cleaning the smear layer in the apical one third is very difficult, necessitating the use of irrigants, which in addition to having antimicrobial effects and biocompatibility, have also the ability to remove
organic and inorganic material in the smear layer [9]. One commonly used irrigant is NaOCl (Sodium Hypochlorite). Besides having antimicrobial effects, NaOCl has the ability to eliminate organic tissue from the smear layer. Though 2.625% NaOCl has antimicrobial effects and is fairly effective at dissolving organic debris, it is ineffective against inorganic debris [10].

Since NaOCl dissolves organic debris only, researchers have attempted to combine NaOCl irrigants with other materials with the ability to remove inorganic debris. One such material is ethylenediaminetetra acetic acid (EDTA), a chelator substance reported to be effective in removing smear layers when used alongside NaOCl. Research has shown that 17% EDTA can remove inorganic components from the smear layer because it reacts with calcium ions from the dentin, forming a calcium-chelate solution. However, according to in vitro studies by Malheiro et al (2005), 17% EDTA has higher cytotoxicity than 10%, 15%, and 25% citric acid [11].

Due to the high toxicity of 17% EDTA, alternative biocompatible irrigants that can be combined with NaOCl to remove inorganic components from the smear layer are actively being researched. Citric acid is an organic acid capable of removing the smear layer when combined with NaOCl. It can also demineralize the intertubular dentin, thus, opening the dentin tubules [12]. Citric acid is available at concentrations ranging from 1% to 40%. In an in vitro cytotoxicity study, 10%–25% citric acid was demonstrated to be more biocompatible than 17% EDTA [1,11].

To date, there has been no irrigant, sole or combined, that meets the ideal requirements for smear layer removal. Therefore, several attempts have been made to combine different materials to obtain the desired results such as in the studies conducted by Lenarda et al. (2000) [13]. The authors stated that the combination of 1 mol L⁻¹ (19%) citric acid with 5% NaOCl was more effective in removing the smear layer compared to a combination of 15% EDTA with 5% NaOCl. Later, Khedmat et al. (2008) [14] found that the effectiveness of three irrigants (Smear Clear, 17% EDTA, and 10% citric acid) when combined with 5.25% NaOCl showed no statistically significant differences in removing the smear layer in the corona, middle, and apical third.

The studies above revealed that the efficacy of a combination of NaOCl, EDTA, and citric acid in removing the smear layer remains controversial. The researchers used 5% NaOCl, which is known to be more toxic than lower concentrations. Furthermore, citric acid is more biocompatible than EDTA. In this study, the effectiveness of a combination of 2.625% NaOCl with 20% citric acid and 2.625% NaOCl with 17% EDTA in removing the smear layer in the apical third is investigated. This study is focused on the apical third area as it is reported to be the most difficult area to clean.

2. Methods

Thirty human mandibular premolar teeth were used in this study according to the inclusion criteria: 20 ± 2 mm mean length of teeth, one rooted tooth, straight root canal, perfectly closed apex, and no tooth defects. The samples were randomly divided into three groups: Group I (2.625% NaOCl–20% citric acid), Group II (2.625% NaOCl–17% EDTA), and Group III (saline). All the samples were prepared with the crown down technique using the ProTaper instrument. Group I (n = 10) was irrigated using a combination of 2.5 ml of 2.625% NaOCl and 5 ml of 20% citric acid. The root canal was irrigated with 2.5 ml of 2.625% NaOCl after access preparation as well as after the use of every instrument using a 30 G irrigation gauge for 1 min, followed by 5 ml of 20% citric acid for 1 min, and a final irrigation with 2.5 ml of 2.625% NaOCl. Group II (n = 10) was irrigated using a combination of 2.5 ml of 2.625% NaOCl and 5 ml of 17% EDTA. The root canal was also irrigated with 2.5 ml of 2.625% NaOCl after access preparation as well as after the use of every instrument using a 30 G irrigation gauge for 1 min, followed by 5 ml of 17% EDTA for 1 min, and a final irrigation with 2.5 ml of 2.625% NaOCl. Group III (n = 10) was irrigated with 2.5 ml saline (0.9% NaCl) after the use of every instrument using a 30 G irrigation gauge for 1 min and a final irrigation with 5 ml and 2.5 ml saline for 1 min. Afterward, all the root canals were dried using a paper point and the orifice was closed using a temporary filling. The teeth were then vertically dissected into two parts using a stainless steel chisel along a groove made earlier. One part of the tooth was randomly selected for examination using scanning electron microscopy (SEM) with two points for measurement and identical magnification at
1000 times. The images were stored digitally and were rated by a trained operator blinded to the procedures. Hundred imaginary columns were created from the sample images and the samples were rated as follows.

0: if >75% of the wall in the apical one third was free of a smear layer and the dentin tubules were open
1: if <75% and >50% of the wall in the apical one third was free of a smear layer and some parts of the dentin tubules were open
2: if <50% and >25% of the wall of the apical one third was free of a smear layer and the dentin tubules were visible but limited
3: if >75% of the wall of the apical one third was covered by a smear layer

Statistical analysis using the Kolmogorov–Smirnov test was performed to determine the cleanliness of the dentin wall from the smear layer on the apical third. A p value < 0.05 was considered significant.

3. Results
The cleanliness of the apical third root canal wall in Groups I, II, and III is shown in Table 1. All 30 samples in Group 3 had a score of 3, whereas none of the samples in Group II did. Root canal walls with a score of 3 were found in Group I but the proportion with a score of 0 in Group I was less than that in Group II. The difference between Groups I and II was not statistically significant. However, both Groups I and II showed statistically significant differences compared to Group III (control). Figure 1 shows representative SEM images of the apical third root canal wall for smear layers with each possible score.

Table 1. Score distribution of apical third root canal wall cleanliness

| Group     | Cleanliness Scale | Total |
|-----------|-------------------|-------|
|           | 0     | 1     | 2     | 3     |       |
|           | n     | %     | n     | %     | n     | %     |
| Group I   | 2     | 20.00 | 5     | 50.00 | 2     | 20.00 | 1     | 10.00 | 10   |
| Group II  | 4     | 40.00 | 3     | 30.00 | 3     | 30.00 | 0     | 0.00  | 10   |
| Group III | 0     | 0.00  | 0     | 0.00  | 0     | 0.00  | 10    | 100.00| 10   |
| Total     | 6     | 20.00 | 8     | 26.70 | 5     | 16.70 | 11    | 36.70 | 30   |

Group I: 2.625% NaOCl–20% citric acid, Group II: 2.625% NaOCl–17% EDTA, Group III: Saline

According to the Kolmogorov–Smirnov test results, there was no statistically significant difference in the apical third root wall cleanliness between Groups I and II (p = 0.988). There were statistically significant differences between Group I and the control as well as and between Group II and the control. These results are shown in Table 2.
Table 2. p value of apical third of root canal wall cleanliness

| Group                  | p-value |
|------------------------|---------|
| Group I vs Group II    | 0.447   |
| Group I vs Group III   | 0.000   |
| Group II vs Group III  | 0.000   |

Group I: 2.625% NaOCl–20% citric acid, Group II: 2.625% NaOCl–17% EDTA, Group III: Saline. *p<0.05, **p>0.05

4. Discussion

This research used mandibular first premolar with single root and one root canal to simplify the examination [15]. Thirty teeth were used in this study, determined using the Federer formula. The teeth were kept in a saline solution to maintain the humidity of the tooth and to ensure a similar condition as the biological condition in the oral cavity. The root canal was prepared while preserving the crown to duplicate the clinical treatment condition. In addition, the criteria used were teeth with a length of 18–22 mm and a working length of 0.5 mm from the apical foramen.

Access and preparation of the root canal were achieved using the crown down technique to facilitate maximum penetration of irrigants in removing the dentin debris produced by instrumentation, facilitating a cleaner root canal. The ProTaper was utilized in this study to minimize the possibility of bias by reducing the smear layer. This is in accordance with the report by Ferlinasari (2011) stating the ProTaper descriptively produces fewer smear layers compared to the Revo-S for root canal instrumentation [16]. Ruddle (2005) and Low (2010) stated it takes at least one to two ProTaper finishing files to prepare the apical third followed by confirmation using the K-file [17,18]. The ProTaper F3 instrument file with an apex diameter of #30 and 9% taper were used for standardization of the final preparation results based on a pre-determined file size (K-file # 15).

The irrigants used in this study were 2.625% NaOCl, 17% EDTA, 20% citric acid, and saline. The 2.625% NaOCl was used due to its better biocompatibility and lower concentration compared to 5.25% NaOCl while retaining the antibacterial effect and the ability to dissolve organic pulp tissue. This is in accordance with the study by Baumgartner et al. (1992) using SEM, which demonstrated that 5.25% NaOCl, 2.5% NaOCl, and 1% NaOCl could completely remove organic tissue on freshly extracted teeth. However, the same results were not obtained with 0.5% NaOCl. In his report, So (2011) stated also 2.5% NaOCl could remove completely pulp tissue from cows, unlike 1% NaOCl and 0.5% NaOCl [10]. NaOCl was used after access preparation and after the use of every root canal preparation instrument in this study. In addition to removing the debris from the root canal instrumentation, NaOCl served also as a lube, and extended the duration of the antimicrobial effect. However, as stated before, NaOCl could not remove the smear layer completely, necessitating the combination of the chelator materials EDTA and citric acid.

The 17% EDTA was chosen because it has the ability to eliminate inorganic smear layer tissue. The pH of 17% commercial EDTA is 7.3 and it has the effect of calcium chelation. The binding of these calcium ions depends on the solubility of the dentin hydroxyapatite, and the chelation process continues until the EDTA is exhausted. On the other hand, the EDTA with a high pH, such as pH 11.3 at 25% and 50%, and EDTA with a pH less than 6, such as at 15%, is less effective in the dentin demineralization due to the constant solubility of the dentin and the reduction of calcium ions in the chelation process [19,20].

The 20% citric acid was used in this study because in addition to its biocompatibility, it has the ability to eliminate inorganic smear layer tissue. This is consistent with Schafer’s (2007) assertion that 1–40% citric acid can be used in root canal treatment because it is effective in removing smear layers, in dissolving the dentin powder, and in demineralizing the intertubular dentin to open the dentinal tubules [12]. Additionally, 20% citric acid has been reported to have good biocompatibility. This is in accordance with research by Malheiros (2005) showing that 10–25% citric acid had better biocompatibility than 17% EDTA [11].
In this study, irrigation was performed for 1 min in accordance with Schafer’s (2007) and Serper’s (2002) procedure [21,22]. Serper (2002) stated that the demineralization effect increased over a 1-min timeframe [22]. In addition, Gesteira (2009) stated in his report 10% EDTA and 17% EDTA had a demineralization effect by eliminating the smear layer and this result was not significantly different after 1 min and 3 min [24]. The volume of 2.625% NaOCl used was 2.5 mL after access preparation, after every instrument use, and for the final irrigation [12]. This procedure is supported by the results obtained by Sluis et al. (2006), showing that 2 mL of 2% NaOCl per minute was as effective as 2 mL of 2% NaOCl every 0.5 min for 3 min with vibration activation [24].

The last irrigant used after the completion of root canal preparation was 2.625% NaCl. The dentin tubules were opened due to irrigation with 17% EDTA, as well as 20% citric acid, allowing 2.5% NaOCl to enter the opened dentin tubule and enabling the bactericidal effect to reach the dentin tubule. In addition, NaOCl could dissolve the remaining organic components, and neutralize the EDTA effects, which can weaken the dentin through continuous demineralization [10].

Root canal irrigation can be performed through conventional means or with the help of both sonic and ultrasonic activation techniques. However, based on the results of several studies, including Kuah (2009) [15], Torrez (2009), and Nair (2010), the use of activation during the irrigation of root canals is not significantly different from the conventional method [24,25]. In addition, since there are still many clinicians using conventional methods, we used conventional irrigation techniques in this study. Torez (2009) and Kuah (2009) stated that EDTA eliminated the smear layer with and without the use of sonic or ultrasonic activation [15,26].

Important aspects of irrigation techniques to be considered are the kinds of irrigants, concentrations, timing, and amount of irrigants applied. In addition, the diameter of the irrigation needle must be considered according to the size of the apex preparation. In vitro studies conducted by Sedgley et al. (2005) showed that the use of irrigation needles with a working length of 1 mm resulted in fewer bacteria in the root canal compared to needles with a working length of 5 mm [27]. The use of irrigation needles with the shortest working length possible can optimize the efficiency of irrigants [27]. In this study, the preparation was completed using the ProTaper F3 with D0 (0.30), followed by a size 30 irrigation needle with an outer diameter of 0.32 mm, per the medical standardization of stainless steel needles (ISO 9626: 1991/Amd 1: 2001). Therefore, the irrigants could be efficiently delivered 1–2 mm from the working length to the prepared root canal with no resistance, increasing the effectiveness in dissolving the smear layer [28]. In addition, the use of needles with holes on the side can prevent the extrusion of irrigants and debris to the periapical tissues [15].

The preparation of the specimens in this study was based on the techniques adopted in the previous studies, specifically making longitudinal cuts with sharp tools (stainless steel chisel) [28]. After part of the tooth was randomly selected, it was visualized using the SEM at two different locations, and the mean value was used. The SEM was used because the technique is capable of producing images with high resolution and maximum magnification. A magnification of 1000 times was used because it provided a wider view with a detailed surface and could detect the smear layer and orifices of the tubule dentin root canal clearly. In contrast, a lower magnification of 50–150 times could detect large pulp fragments only and debris derived from dentin flakes, and it was difficult to detect both the smear layers and the orifices. After the SEM images were obtained, each image was rated by two examiners in a blind manner, i.e., the examiner did not know the type of materials or the sample preparation procedure used. The samples were assessed by creating 100 imaginary columns from the sample image, then, adjusted according to the scoring method. The creation of imaginary columns was used for ease and equality of perception in determining the score, thereby reducing bias between the two observers in the objective assessment. The Kappa test results from the two observers showed 93% agreement. The scoring method employed in this study was based on the study by Foschi [29].

After the score of each sample was determined, the results were analyzed and are presented in Table 1. The results of the test between two groups using Kolmogorov–Smirnov showed that there was no statistically significant difference between Groups I and II, with a p value of 0.988 (p > 0.005). This might be because both of the groups were treated using a combination of NaOCl as an antimicrobial.
agent and an organic tissue solvent with EDTA or citric acid as a chelating agent, which equally had the ability to eliminate the inorganic components of the smear layer [1,10]. Although not significantly different, the percentage of root canal walls with a score of 0 in Table 2 shows that the root canal wall was cleaner in Group I (40%) than in Group II (20%), as the smear layer could be seen in the sample. This was probably because 17% EDTA has a normal pH, which is effective in the chelation process. The binding of calcium ions from soluble hydroxyapatite was also more effective when the process of chelation reached equilibrium and stopped until the material was exhausted. When using citric acid with a more acidic pH, the process of chelation will reach the equilibrium point faster, limiting the number of calcium ions needed in the process of chelation [10].

Smear layers with a score of 2 were visible in both Groups I (2 samples) and II (3 samples). One sample had a score of 3 in Group I, indicating that it was difficult to remove the smear layer in the apical third area. This is consistent with the conclusion by Foschi (2004) that it was difficult to completely eliminate the smear layer in the root canal especially in the apical third area using irrigation solutions or other methods. The author’s solution was to minimize the number of smear layers [29]. Khedmat (2008) reported that 1 mL of Smearclear, 1 mL of 17% EDTA, and 1 mL of 10% citric acid combined with 5 mL of 5.25% NaOCl showed no differences in removing the smear layer. This was because the duration of the application and the volume of Smearclear, EDTA, and citric acid were less effective in eliminating the smear layer, thus, requiring the use of irrigation techniques [14]. Kuah’s study (2009), which compared the use of 1% NaOCl with 17% EDTA with different application times ranging from 1 min to 3 min with and without ultrasonic irrigation techniques, showed that 1 min was efficient in removing the smear layer both with and without irrigation techniques. The important factor in increasing the effectiveness is ensuring the irrigants are in direct contact with the surface of the root canal wall, in this case, the apical third [15].

Single saline irrigation materials (0.9% NaCl) were used as controls because saline is a physiological solution that contains sodium chloride salt dissolved in sterile water, making it biocompatible. However, saline alone is not capable of eliminating smear layers containing organic and inorganic components [30]. This was demonstrated in this study; almost all the samples in the saline group had smear layers on the root canal wall with a score of 3, whereas Groups I and II had significantly cleaner results (p = 0.000). This is in line with the research conducted by Ring (2008) showing that saline is not recommended as a root canal irrigant since it has no antimicrobial or tissue solvent properties. Saline can be used as a control only, as a benchmark in research [31].

This research demonstrated that use of a combination of 2.625% NaOCl, 17% EDTA, and 20% citric acid is effective at removing the smear layer from the root canal wall, resulting in good adaptation. Supporting Balaji’s statement (2010), combining NaOCl irrigants with chelator materials to remove organic and inorganic smear layer components is more effective than using the irrigants alone [7]. This study reinforces the benefits of combining NaOCl irrigants with EDTA or citric acid in cleaning the smear layer.

5. Conclusion
It can be concluded from this study that both the combination of 2.625% NaOCl with 20% citric acid and 2.625% NaOCl with 17% EDTA have similar efficacy in cleaning the smear layer in the apical third area.

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