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Abstract

Aims: The aim of the study was to evaluate the ability of a mixture of (castor detergent and papain enzyme) MCP to remove the smear layer by using scanning electron microscope.

Materials and Methods: Samples of 45 human extracted was divided in to 3 groups (A, B, and C) n = 15 and prepared endodontically using pro taper system up to size F 3, each group was irrigated with corresponding solution 3 ml in between each file size and 5 minutes as a final irrigant as following: Group A irrigated with distilled water (control negative). Group B irrigated with 2.5% (Sodium hypo chloride) NaOCL and 17% (Ethylene di amine tetra acetic acid) EDTA (control positive). Group C irrigated with 20% castor detergent and 4% papain enzyme as a mixture (MCP). Each sample was irrigated with 15 ml of distilled water and dried with paper points. The samples were sent for SEM photograph. Each sample was evaluated at three levels (apical, middle, and cervical part of the canal)

Results: MCP solution showed partial removal of both organic and inorganic parts (dual action) of smear layer from the 3 levels of root canals, but the apical one was significantly less clean than the other two, also it has a more gentle effect of erosion than EDTA with NaOCl. EDTA with NaOCl has the ability to remove the smear layer completely from the canal, but still the apical third was significantly less clean than that of other two. It is impossible to remove the smear layer completely by NaOCl and EDTA without erosion of the inner surface of irrigated canal when EDTA was used as a final irrigant for 5 minutes.

Conclusion: solution had the ability to remove the smear layer partially at the three levels of a root canal without dentin erosion. While EDTA had the ability to remove the smear layer completely at the three levels of canal with obvious dentinal erosion. Still the apical area has mechanical and anatomical limitation in root canal irrigation.

Keywords

new endodontic solution, smear layer removal, SEM

Disciplines

Biomaterials | Dentistry | Endodontics and Endodontology | Materials Science and Engineering

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A Newly Prepared Solution for the Removal of the Smear Layer

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Abstract Aims: The aim of the study was to evaluate the ability of a mixture of (castor detergent and papain enzyme) MCP to remove the smear layer by using scanning electron microscope. **Materials and Methods:** Samples of 45 human extracted was divided in to 3 groups (A, B, and C) n = 15 and prepared endodontically using pro taper system up to size F 3, each group was irrigated with corresponding solution 3 ml in between each file size and 5 minutes as a final irrigant as following: **Group A** irrigated with distilled water (control negative). **Group B** irrigated with 2.5% (Sodium hypo chloride) NaOCL and 17% (Ethylene di amine tetra acetic acid) EDTA (control positive). **Group C** irrigated with 20% castor detergent and 4% papain enzyme as a mixture (MCP). Each sample was irrigated with 15 ml of distilled water and dried with paper points. The samples were sent for SEM photograph. Each sample was evaluated at three levels (apical, middle, and cervical part of the canal) **Results:** MCP solution showed partial removal of both organic and inorganic parts (dual action) of smear layer from the 3 levels of root canals, but the apical one was significantly less clean than the other two, also it has a more gentle effect of erosion than EDTA with NaOCl. EDTA with NaOCl has the ability to remove the smear layer completely from the canal, but still the apical third was significantly less clean than that of other two. It is impossible to remove the smear layer completely by NaOCl and EDTA without erosion of the inner surface of irrigated canal when EDTA was used as a final irrigant for 5 minutes. **Conclusion:** solution had the ability to remove the smear layer partially at the three levels of a root canal without dentin erosion. While EDTA had the ability to remove the smear layer completely at the three levels of canal with obvious dentinal erosion. Still the apical area has mechanical and anatomical limitation in root canal irrigation.

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1. Introduction

Contemporary methods of root canal instrumentation produce a layer of organic and inorganic material called the smear layer that may also contain bacteria and their by-products. It is known that the smear layer may harbor bacteria, preventing the canal from being disinfected [1]. This smear layer may prevent the penetration of intracanal medicaments into the dentinal tubules and interfere with the close adaptation of root filling materials including sealers to canal walls, the removal of the smear layer for more thorough disinfection of the root canal system by improves the efficacy of intracanal medications and irrigants and reduces the time needed for canal disinfection [2].

Shahravanetal (2007) concluded that removal of smear layer reduces apical leakage [3]. An ideal smear layer, removing agent should eliminate both organic and

inorganic phases from all canal surfaces without harmful erosive effects on dentin[4,5]. Different solutions have been used to remove the smear layer. Sodium hypochlorite (NaOCL) in a 1% to 5.25% concentration is an irrigant solution widely used in root canal treatment because of its bactericidal properties and ability to dissolve organic tissues [6]. Also Sodium Hypochlorite has several undesirable characteristics such as tissue toxicity, risk of emphysema when overfilling, allergic potential, disagreeable smell and taste and inability to remove the smear layer completely [7].

Unfortunately, no irrigating solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer [8]. The most commonly used chelating agents are based on different concentrations of ethylenediamine tetra-acetic acid (EDTA) [9,10]. The use of EDTA alone or prior to NaOCL resulted in the maximum decrease in dentin micro hardness [11]. Silva (2013) determined that 15% EDTA effectively removed

the smear layer from the middle and apical thirds of the root canal, 15% EDTA was associated with the greatest effect on root dentine demineralization [12]. Morgental (2013) founded that EDTA had no measurable antibacterial effect when used as a root canal irrigant [13].

Papain acts as a debris-removing agent. It acts only on the affected tissues, which lack the α 1-antitrypsine plasmatic antiprotease that inhibits proteolysis in healthy tissues [14]. Bhardwaj (2002) find that papain enzyme has the comparable antibacterial effect of calcium hydroxide when used in gel form as an intracanal medicament against *Efaecalis* [15]. Castor oil is phytotherapeutic polymer which is obtained from the seeds of the Ricinus communis plant. Ricinus communis plant polymer at 10% has been suggested not only because of its excellent biological properties, but also because of its antimicrobial activities, as demonstrated by Ferreira *et al* [16].

Aguiar *et al* (2010) concluded that castor detergent presented better results at the middle third, while 0.5% NaOCL presented better results at the cervical third, and both irrigants were similar at the apical third. ENDOQUIL a 3.3% Ricinus common is detergent [17]. This substance was also reported to increase root dentin permeability similarly to a 0.5% solution of NaOCL and a 0.4% papaine gel [18]. The Ricinus communis detergent acts by breaking sugar leakage of the cellular wall of pathogenic microorganisms, consequently the loss of cytoplasmic material leads to cell destruction [16,19]. However, further studies are necessary to indicate Endoquil during the root canal biomechanical preparation in the endodontic treatment [17]. The debridement ability of the Ricinus communis gel was comparable to that of the 1% sodium hypochlorite solution, but none of these agents were able to completely remove the smear layer [20].

The aims of this in vitro study were to evaluate the efficacy of newly prepared endodontic irrigant solution containing 20% castor oil detergent and 4% papain as a mixture (MCP) to remove the smear layer after chemo-mechanical preparation of the root canal using scanning electron microscopy.

2. Materials and Methods

2.1. Sample Preparation and Distribution

Samples of 45 humans extracted single straight rooted teeth of age between 25-35 year old were examined under a stereo microscope to exclude root resorption, crack or fracture and incomplete apex. Straight root only used in this study. The teeth were stored in distilled water until the start of the study. The teeth were de-coroneted at the cement-enamel junction (CEJ), using a diamond disk with the straight hand piece. The potency of each root canal was confirmed by inserting size 15 K file. The working length of each canal was determined by inserting size 15 K type file inside the canal until the tip of the file was just becoming visible at the apical foramen under stereomicroscope. The file was reduced 1 mm. from the measured working length; each canal length was adjusted to 14 mm. working length by cutting from the cervical part of the root. The samples were divided randomly into three groups, 15 teeth for each group.

Group A in which distilled water only was used as a root canal irrigant during instrumentation 3 ml between

each file and 5 ml as a final irrigant and wait for 5 minutes (remain inside in the canal) as a negative control.

Group B in which 2.5% NaOCL (Clorox KSA) was used during instrumentation 3 ml between each file size and 5 ml 17% EDTA (Master-Dent, Dentonics, Inc. USA) as a final irrigant for 5 minutes (remain in the canal) then washed with 15 ml of distilled water as a control positive.

Group C in which the (MCP) is an acronym of a mixture of castor detergent and papain enzyme) experimental solution was used as a root canal irrigant during instrumentation 3 ml between each file and 5 ml of MCP solution and wait for 5 minutes as a final irrigant (remain in the canal) then washed with 15 ml of distilled water.

Preparation of irrigant solution:

The experimental solution was prepared by converting the castor oil (HEMANI Pakistan) to sodium castorate powder by adding NaOH to castor oil. 20 gm of sodium castorate powder was dissolved in 100 ml deionized water to produce 20% of castor detergent, then 4 gm of papain enzyme powder (HIMEDIA Company, molecular weight = 23000, protolytic activity \geq 4.5 ml of 0.1 M NaOH) was added to the same 100 ml solution to give 4% papain enzyme.

2.2. Root Canal Preparation

The root samples were closed from the apical area with sticky wax and all root canal samples were prepared by using pro taper NiTi rotary system (Dentsply maillefer Switzerland) starting from S X, S 1, S 2, F 1, F 2 & F 3 respectively at 3000 rpm speed and torque 2.5 N/cm rotary endodontic handpiece (NSK Japan). Each file was inserted four times in the canal. The time was standardized to 10 minutes for each canal [21].

The irrigation was performed by endodontic syringe with flexible silicon tip (Diaflex DiaDent Korea) inserted up to 2 mm. from the apex. The speed of irrigation during canal preparation in between each file was 1 ml/5 seconds [5]. Finally, each canal was irrigated with 15 ml of distilled water and dried with sterile endodontic paper point size F 3.

2.3. SEM Sample Preparation

A sterile paper point was left inside the canal to protect the prepared canal from being contaminated by dentinal chips or debris during the splitting process, a sterile paper point was left inside each canal [22]. The canal orifices were closed by piece adhesive tape to prevent the insertion of dentin particles during the root notching by diamond disc. Each root was notched carefully longitudinally buccally and lingually without penetrating the canal using a diamond disc with straight handpiece. The roots were split, gently into two halves with the aid of a mixing spatula and the optimum half of each root was used for the SEM examination for apical, middle, and cervical third [Figure 1](#). The other half of each specimen was discarded. The specimens were dehydrated with ascending concentrations of ethyl alcohol (30%–100%) for 5 min for each concentration except for 100% was 30 minutes and placed in desiccators for 24 hours [21].

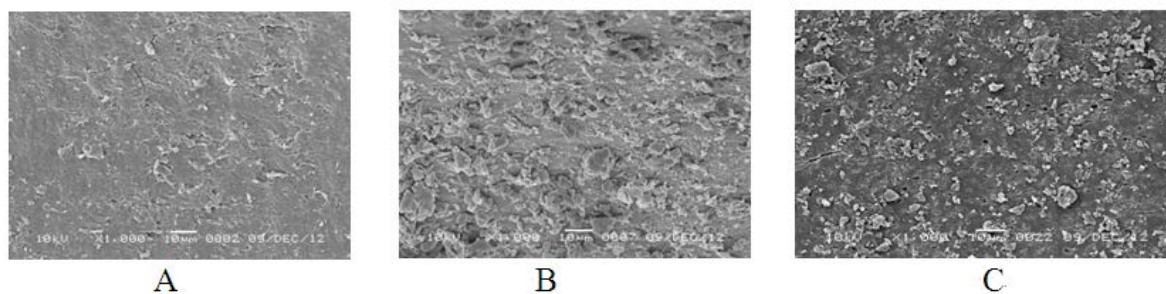


Figure 1. Representative SEM image of control negative (distilled water) reveals heavy smear layer covering all the dentin surface A apical third, B Middle third, and C cervical third all X 1000

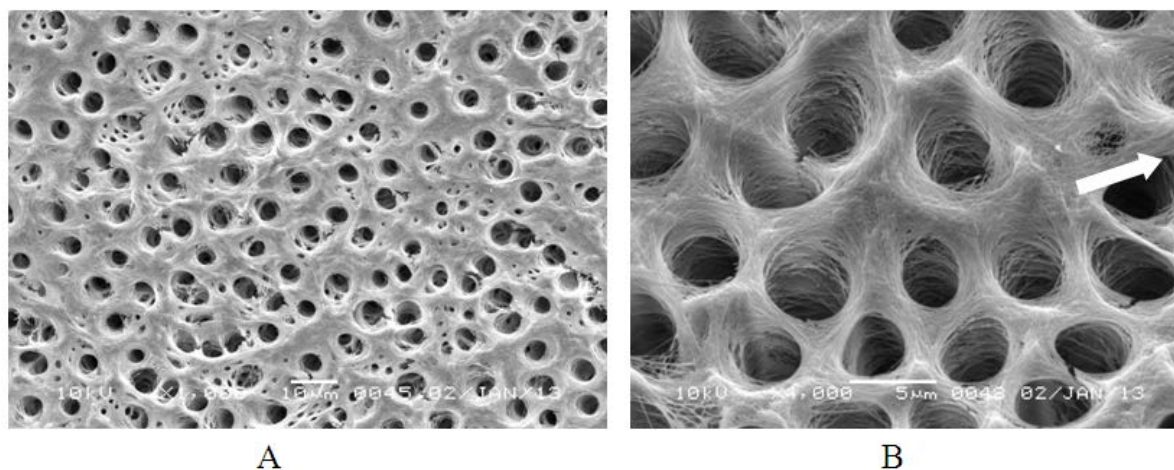


Figure 2. Representative SEM image of the control positive 17% EDTA & 2.5% NaOCL apical third showed complete removal of smear layer with patent dentinal tubules with intertubular dentin crack and destruction (arrows) A X 1000 and B X 4000

The specimens were left to dry overnight, mounted on copper stubs, coated with gold, and examined and photographed using a scanning electron microscope (Jeol, JSM, T330A, Electron Optical Laboratory, Tokyo, Japan) at an accelerating voltage of 10 KV at low vacuum. The examination was performed at the center of each third [Figure 2](#). Smear layer removal was evaluated using the three-point scoring system reported by Torabinejad *et al* (2003) as follows: [5]

1. No to minimal smear layer: No or minimal 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 the tubules contained debris.

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

Degree of erosion of dentinal tubules was scored according to Peeters *et al* (2011) as follows: [22]

1. No to minimal erosion: All tubules looked normal, or some tubules had minimal erosion in appearance and size.

2. Moderate erosion: The peritubular dentin was eroded.

3. Severe erosion: The intertubular dentin was destroyed, and tubules were connected with each other.

The (Mann-Whitney and Wilcoxon Signed Rank,) tests were used to analyze the data and the significance was tested at 0.05 level.

3. Results

The results of the study showed that only EDTA and MCP detergent had the ability to remove the smear layer in compared with distilled water which has no any efficacy in smear layer removal. For group A control negative, in which (distilled water) was used as irrigant there is a heavy smear layer throughout the entire length of the root canals and there is no any appearance of patent dentinal tubules [Figure 1](#) (A & B) since distilled water has no any ability to remove whether organic and inorganic part of smear layer, there are no significant difference between three levels of root canal ($p > 0.05$). [Table 1](#).

Table 1. Comparison for Smear Layer Removal Using Wilcoxon Signed Ranks Test for Three Groups

Group A	Middle-Apical	Cervical-Apical	Cervical-Middle
Z	0.000	0.000	0.000
p-value	1.000	1.000	1.000
Group B			
Z	-2.000	-2.646	-1.732
p-value	0.046	0.008	0.083
Group C			
Z	-2.236	-2.070	-1.732
p-value	0.025	0.038	0.083

Examination of group B scores control positive (EDTA & NaOCL) at the apical third there was complete with partial removal of smear layer in most of samples [Figure 2](#)

(A & B), while in the middle and cervical third there was complete removal of smear layer in which the dentinal tubules were clearly opened and even inside the tubule

there are clear removal of plug [Figure 3](#) (A & B) and [Figure 4](#) (A & B). Statistical analysis of data of three levels showed that there was a significant difference between apical and middle ($p < 0.05$) [Table 1](#) the same for

comparison of apical and cervical there was also a significant difference ($p < 0.05$). [Table 1](#) while between middle and cervical third there are no significant difference ($p > 0.05$).

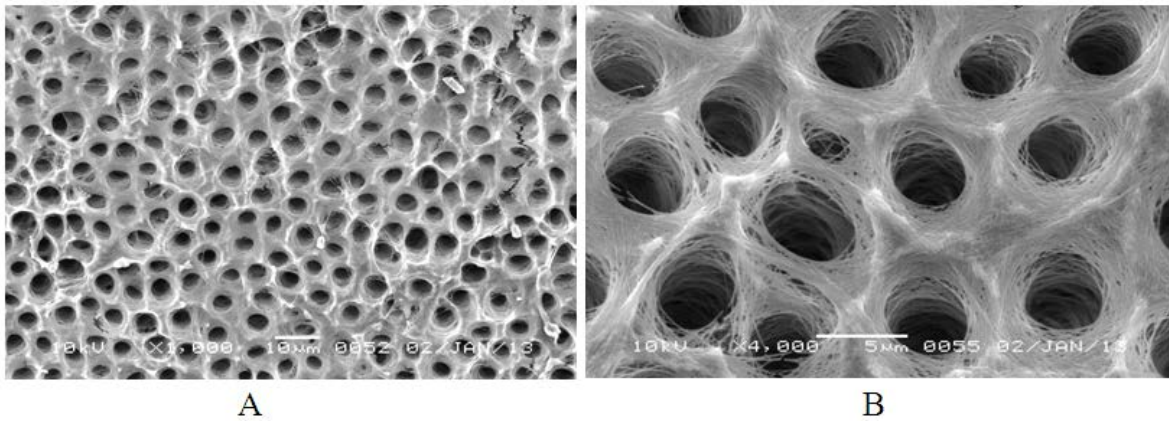


Figure 3. Representative SEM image of the control positive 17% EDTA & 2.5% NaOCL middle third showed complete removal of smear layer with intertubular dentin crack (arrows) A X 1000, B X 2000 and B X 4000

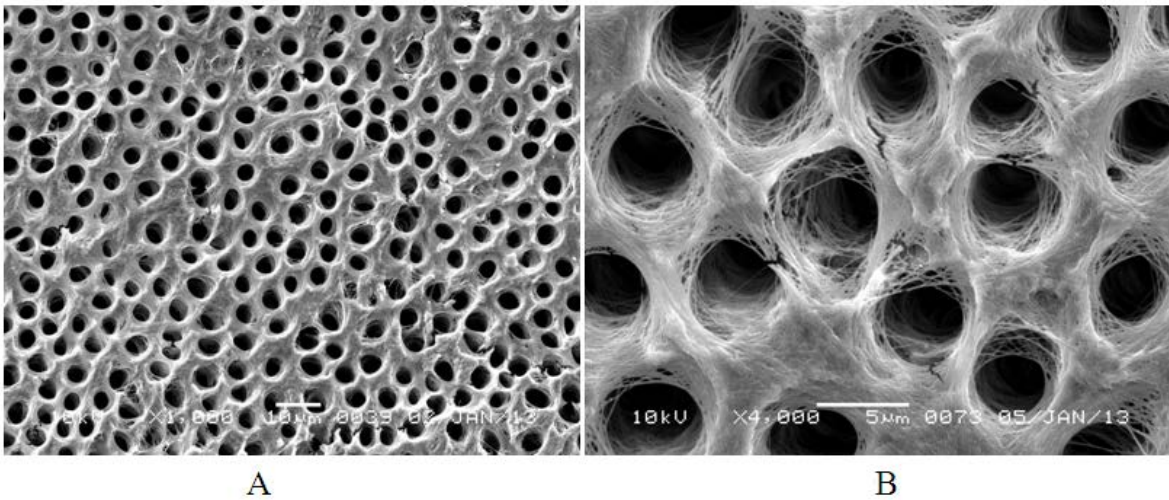


Figure 4. Representative SEM image of the control positive 17% EDTA & 2.5% NaOCL cervical third showed complete removal of smear layer with intertubular dentin crack (arrows) A X 1000 and B X 4000

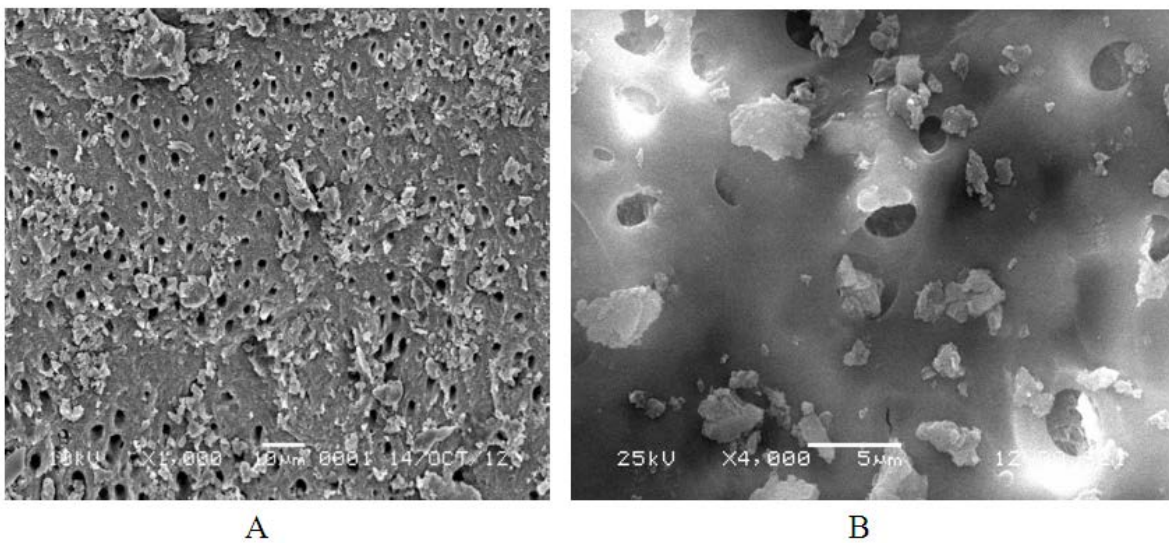


Figure 5. Representative SEM image of the MCP solution (20% castor detergent + 4% papain enzyme) apical third showing partial removal of smear layer without any sign of erosion and different patent dentinal tubules with remnants of smear layer, apical third A X 1000 and B X 4000

The results of group C (MCP) solution showed that there is partial removal of smear layer in three levels of

the prepared canals [Figure 5](#) (A & B), [Figure 6](#) (A & B), and [Figure 7](#) (A & B). The cervical and middle third was

cleaner than that of apical third. The results also showed that the smear plugs (extending part into dentinal tubules)

are completely removed in the clean area of dentin.

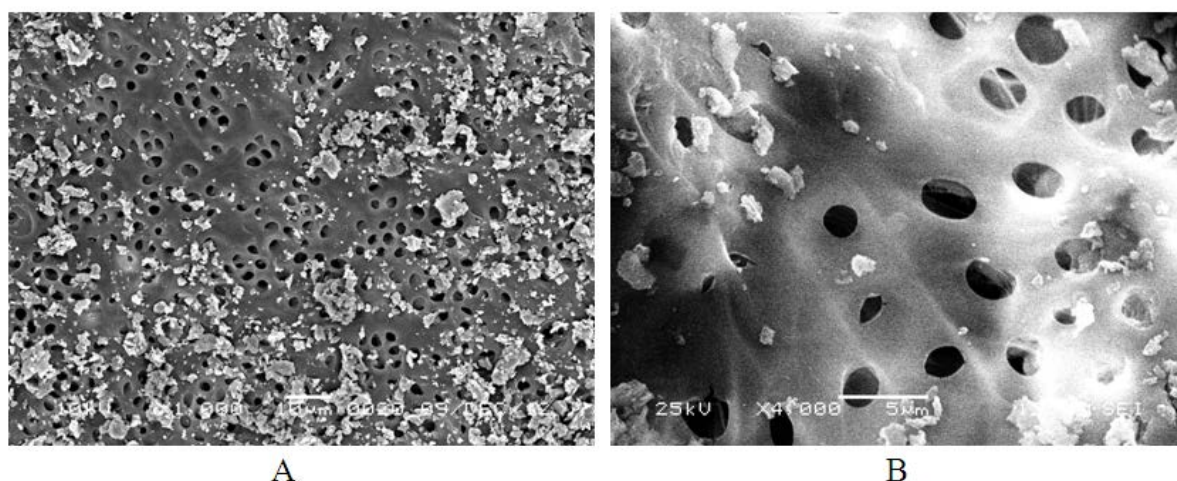


Figure 6. Representative SEM image of MCP solution (20% castor detergent + 4% papain enzyme) middle third showing partial removal of smear layer without any type of erosion and different patent dentinal tubules with remnants of smear layer, A X 1000 and B X 4000

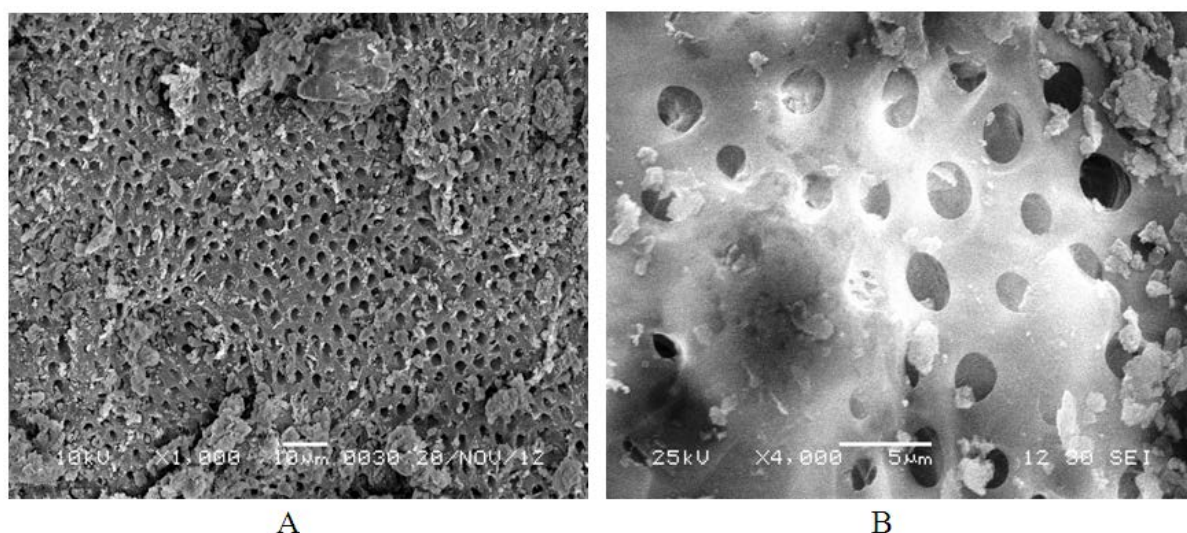


Figure 7. Representative SEM image of the MCP solution (20% castor detergent + 4% papain enzyme) cervical third showing partial removal of smear layer with patent dentinal tubules and remnants of the smear sign of erosion appear, A X 1000 and B X 4000

Statistical analysis showed that there were significant differences between apical and middle third ($p > 0.05$), and between apical and cervical there are also significant

differences ($p > 0.05$), in addition, comparison between middle and cervical showed that there was no significant difference ($p < 0.05$) [Table 1](#).

Table 2. Comparison Among Three Groups for Smear Layer Removal Using Mann-Whitney Test

Group A vs. B	level	Apical	Middle	Cervical
	Mann-Whitney U	0.000	0.000	0.000
	Wilcoxon W	120.000	120.000	120.000
	Z	-5.078	-5.182	-5.385
	p-value	0.000	0.000	0.000
Group A vs. C	level	Apical	Middle	Cervical
	Mann-Whitney U	37.500	0.000	0.000
	Wilcoxon W	157.500	120.000	120.000
	Z	-3.808	-5.385	-5.182
	p-value	0.000	0.000	0.000
Group B vs. C	level	Apical	Middle	Cervical
	Mann-Whitney U	35.000	0.000	0.000
	Wilcoxon W	155.000	142.500	142.500
	Z	-3.605	-4.397	-4.397
	p-value	0.000	0.000	0.000

Comparison between experimental groups C with group A & B at the three levels showed that there was a significant difference between the three groups of each level ($p > 0.05$) at level apical, middle, and cervical, [Table](#)

2. Examination of erosion of erosion scores revealed that group B (EDTA & NaOCL) solution produce different forms of erosion (there was clear dentin surface erosion with exposed collagen fibers, erosion of peritubular dentin,

and cracking of the inter tubular dentin the intertubular dentin was destroyed, and tubules were connected with each other) this can be seen clearly from the SEM photographs at X 4000 magnification [Figure 2 \(B\)](#), [Figure 3 \(B\)](#), and [Figure 4 \(B\)](#). While the results of the group C (MCP) revealed that the solution has more gentle effect on the dentin than EDTA, this can be seen clearly from the SEM photographs at X 4000 magnification the dentin surface looked normal, smooth and free from peritubular dentin erosion, also there is no intertubular dentin destruction or crack [Figure 5 \(B\)](#), [Figure 6 \(B\)](#), and [Figure 7 \(B\)](#).

Statistical analysis of the erosion score showed that there are significant difference between group B (EDTA & NaOCL) and group C (MCP) at the three levels (cervical, middle, and apical). ($p < 0.05$) [Table 3](#). This means that the EDTA produce cleaner dentin surface than MCP solution. Comparison of three levels of group B (EDTA & NaOCL) showed that there are significant differences between both (apical and middle) and (apical and cervical), ($p < 0.05$) while between the (cervical and middle) there was no significant differences ($p > 0.05$), [Table 4](#).

Table 3. Erosion Scores at Three levels for group (B) and (C) Using Mann-Whitney Test

Level	Apical	Middle	Cervical
Mann-Whitney U Value	52.500	0.000	0.000
Wilcoxon W Value	172.500	120.000	120.000
Z	-3.247	-5.182	-5.385
p-value	0.001	0.000	0.000

Table 4. Erosion Scores for Group (B) and (C) Using Wilcoxon Signed Ranks Test

Group B	Value	Middle-Apical	Cervical-Apical	Cervical-Middle
	Z	-3.578	-3.508	-1.732
	p-value	0.000	0.000	0.083
Group C	Z	0.000	0.000	0.000
	p-value	1.000	1.000	1.000

Comparison of three levels of group C (MCP) showed that there are no significant differences between the three groups ($p > 0.05$) [Table 4](#), this mean that at the three levels there are similar efficacy in smear layer removal.

4. Discussion

The key role of root canal irrigant is to clean the root canal during the Chemo-mechanical preparation. Consequently, one or more irrigants must be used for the complete elimination of two components of smear layer (organic and inorganic) from the root canal system [23]. Any solution that expected to remove the smear layer must have both chelating actions for removal of inorganic part of smear layer and proteolysis action for removal of organic part of the smear layer. Papain is a well-known proteolytic enzyme, and castor detergent was selected due to its chelating action. The 4 hydroxyl groups that present in EDTA plays an imported role and are responsible for severe erosion of dentin as in group B control positive, while in castor oil detergent there are 3 hydroxyl groups and Its role are similar to 4 hydroxyl groups in EDTA so its attack both the smear layer and dentin but it has no erosive effect like EDTA as in group C in which there is no any signs of erosion. On those hydroxyl groups the Ca^{++} attached to produce the chelating process.

According to above explanation the EDTA takes 2 Ca^{++} ions while castor detergent takes one Ca^{++} ion and this possibly explains the results of group C (MCP) solution that produce no erosion when contact to dentin for 5 minutes, in comparison with EDTA. For that reason all EDTA samples showed an erosive effect on dentin, which is the main disadvantage of 17% EDTA. These results coincide with the results of Sampaio *et al* (2005) [24].

who found that castor-oil, detergents showed partial removal of the smear layer in compare with EDTA detergent.

Also the above results coincide with the results of Bolhari *et al* who concluded that EDTA (17%) more effective chelating agent in smear layer removal from the root canals than an herbal solution (citrus aurantifolia extracts) which is not able to remove the smear layer completely [25].

Proteolytic activity of papain enzyme are more activated in this solution by the presence of chelating agent as Arnon *et al* (2002) founded that papain activity is enhanced and activated when heavy metal chelating agent are present [26]. Also the use of fine silicone endodontic needle which inserted up to 2 mm from the apex ensures the optimum arrival of endodontic irrigant to the apical area which explain there are no significant differences between the apical third in compare with middle and cervical third of group C (MCP).

On the other hand, the rotary systems usually produce a heavier smear layer than that of manual [27]. For this cause the complete removal of smear layer in prepared root canal with rotary system still not an easy goal. Also, any agitation technique weather it is manual, sonic and ultrasonic which improve the cleaning action of any irrigant was not used in this study to avoid any factor that may affect the results, this mean that any removal of smear layer was due to the chemical action of solution weather EDTA or MCP in group B and C respectively.

The removal of smear layer in both group B and group C is better in the cervical and middle third than apical, this is possibly due to the larger diameter of the canal in the middle and cervical than that of apical third also because of the dentinal tubules in the apical third is less in number and diameter than that of middle and cervical [5]. So the

effectiveness of irrigation will be higher due to larger volume and velocity of fluid. There for the removal of smear layer was better in middle and cervical third than the apical and also the degree of erosion more severe in middle and cervical third, For that reason the results of statistical analysis showed that for both group B & C there are no significant differences between the cervical and middle third because the conditions of these two levels are similar (large in diameter, dentinal tubules larger in diameter, and receive larger volume of fluid than apical one). These results coincide with the results of David Uroz-Torres (2010) [28] and Saito *et al* (2008) [29] who found that the removal of smear layer was more complete in cervical and middle thirds then in the apical third.

5. Conclusions

Under the limitation of this study it has been concluded that the MCP solution (20% Sodium castorate and 4% papain enzyme) is a single solution that has dual action (chelation and proteolytic action) for removal of organic and inorganic debris. MCP solution has the ability to remove partially both organic and inorganic debris at three levels apical, middle and cervical third, but the apical third had significantly less effect than at the middle and cervical third. EDTA and NaOCL has the ability to remove the smear layer completely at the cervical, middle, and apical third with a less effect on the apical third. EDTA and NaOCL cannot remove the smear layer completely without dentin erosion, while MCP solution had the ability to remove partially the smear layer without dentin erosion. Still the apical area has mechanical and anatomical limitation in root canal irrigation.

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