

Comparative Analysis of Endodontic Smear Layer Removal Efficacy of 0.1% Polyhexanide, Combination of 5% Sodium Hypochlorite, 0.1% Polyhexanide and 0.02% Hypochlorous Acid and 17% Ethylenediaminetetra Acetic Acid Using Scanning Electron Microscope: An Invitro Study

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Abstract: Aim: The aim of the study is to evaluate the efficiency of different endodontic irrigants; 0.1% Polyhexanide, combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid and 17% Ethylenediaminetetraacetic acid in removing smear layer using scanning electron microscope through in vitro method. Materials and Methods: The present in vitro study was carried out on 45 single-rooted extracted human mandibular premolar teeth with single canal and complete root formation. Teeth were randomly assigned to three groups with 15 teeth in each group. Group I samples were irrigated with 17% ethylenediaminetetraacetic (EDTA) irrigation, Group II with combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid, and Group III with 0.1% polyhexanide irrigation. Scanning electron microscope evaluation was done for the assessment of smear layer removal in the coronal, middle, and apical thirds. Comparison of the smear layer removal between the three different groups was done by Kruskal–Wallis test, followed by Mann–Whitney U test for comparing individual groups. A “P” value less than 0.05 was considered to be statistically significant. Results: Statistically significant difference was seen between the two test groups (17% EDTA vs. 0.1% Polyhexanide) in smear layer removal at coronal, middle, and apical thirds of the root canal. The most efficient smear layer removal was seen in Group I with 17% EDTA irrigation followed by combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid and the least by 0.1% Polyhexanide in apical third. Conclusion: The present study showed that all the three experimental irrigants removed the smear layer from different tooth levels (coronal, middle, and apical). Final irrigation with 17% EDTA and combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid is more efficient than 0.1% Polyhexanide in the removal of smear layer from the apical third of root canal.

Keywords: Endodontic Irrigants, Smear Layer Removal, Polyhexanide, EDTA, Root Canal

1. Introduction

Bacteria and their metabolites are the primary etiological factors for pulp and periapical disorders, by invading the root canal system. Because of this, root canal therapy aims to thoroughly eradicate germs and stop re-infection in the affected root canal systems and periapical tissues. In addition to mechanical cleaning, a variety of irrigation solutions must be employed to remove bacteria and their products, tissue remnants and smear layer removal from root canals and dentinal tubules.⁽¹⁾

The smear layer consists of both organic and inorganic substances such as fragments of odontoblastic processes, microorganisms, and necrotic material covering the root canal walls and openings of the dentinal tubules. The smear layer itself may be infected and may protect the bacteria within the dentinal tubules. It can hinder the penetration of intracanal medicaments and sealers into the dentinal tubules. The smear layer can also act as a barrier between obturating materials and the canal wall, and thus, interfere with the formation of an appropriate seal.⁽¹⁾

Hence, thorough debridement of the root canal system for removing smear layer is crucial for long term success of root canal treatment.

Ethylenediamine tetra acetic acid (EDTA) is the most widely used irrigant for smear layer removal. In addition to the cleansing function, it acts on inorganic material by reacting with calcium ions in dentine, resulting in calcium chelation, promoting decalcification of dentine within 5 minutes.⁽²⁾ Because of its harmful effects on periapical tissues, the search for more biocompatible solutions other than EDTA continues.

The biocide, Polyhexamethylene biguanide or Polyhexanide (PHMB) 0.1%, which belongs to the bisbiguanide family, has a wide range of applications as a disinfectant for surfaces, objects and instruments. It is effective against Gram positive (*S. epidermidis*, *S. aureus*, and *E. faecalis*) and Gram negative (*E. coli*) bacteria. The mechanism of polyhexanide's antimicrobial action as a cationic antiseptic is based on its connection with negatively charged phospholipids of the bacterial cell membrane by forming a poly-hexanide-phospholipid complex.⁽³⁾

Among the various irrigating solutions, sodium hypochlorite (NaOCl) is the most widely used because of its strong antimicrobial activity and its ability to dissolve organic tissue remnants. In clinical practice, NaOCl is used in concentrations ranging from 0.5% to 5.25%, with 2.5% being commonly employed. Although higher concentrations provide improved antimicrobial efficacy, they are also associated with increased cytotoxicity and may cause irritation to the periapical tissues if extruded beyond the root apex.⁽³⁾

However, NaOCl has limited action on the inorganic component of the smear layer. For this reason, complete smear layer removal usually requires the combined use of sodium hypochlorite with chelating agents, which can dissolve the inorganic portion of dentin debris.⁽⁴⁾

Therefore, the combined use of NaOCl and chelating agents during root canal irrigation is considered an effective approach for the removal of the smear layer and for improving canal cleanliness, thereby enhancing the overall success of endodontic treatment

Hypochlorous acid (HOCl) has been shown to contribute to smear layer removal and improve the cleanliness of root canal walls, and therefore has been proposed as a potential alternative to sodium hypochlorite (NaOCl) as an irrigating solution. HOCl is a biocompatible antimicrobial compound that is naturally generated by the human immune system as part of the defense mechanism against microbial infection. Compared with NaOCl, HOCl exhibits considerably lower cytotoxicity while maintaining comparable antibacterial activity against *Enterococcus faecalis*. Because of these properties, the combined use of HOCl with NaOCl has been suggested to potentially produce a synergistic antimicrobial effect.⁽⁵⁾

Although the antimicrobial activity of polyhexanide (PHMB) has been widely reported, its effectiveness in smear layer removal as a root canal irrigant has not been sufficiently explored in the literature.

Therefore, this study intends to investigate and compare the smear layer removal ability of polyhexanide when used alone and in combination with 0.02% hypochlorous acid and 5% sodium hypochlorite as irrigating solutions.

2. Materials and Methods

Selection of Samples

Forty-five freshly extracted single rooted human mandibular premolars were collected with fully formed apices and complete root formation. Teeth with fractures, calcification and open apices were eliminated after radiographic examination.

Teeth Preparation for the Study

Buccal and proximal radiographs were taken to ensure that the teeth had only single canal. The teeth were cleaned of debris and soft tissue remnants and were stored in saline solution.

The teeth were decoronated to obtain uniform working length

of 17 mm for all samples using a diamond disk (Flexiflex). The root canals were accessed and working length was estimated using a number 10 K file until it was visible at the apical foramen of each root canal and subsequently by subtracting 1 mm from this point. The apical foramen of each root was sealed using sticky wax (Pyrex) in order to restrict the flow of irrigants through it and also to simulate the clinical situation. Chemo mechanical preparation of root canals were performed in a step back technique using K files. The canals were enlarged apically up to ISO size number 40. A Gates Glidden (Dentsply Sirona) drill number 2–4 was used to enlarge the coronal third of the root canal. Irrigation was done using 1 ml of 3% sodium hypochlorite solution for 10 seconds between each instrument change, and subsequently the specimens were divided into three groups.

The samples were divided into Groups I, II, and III containing 15 samples each:

- Group I: 15 samples with 17% EDTA irrigation.
- Group II: 15 samples with combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid irrigation.
- Group III: 15 samples with 0.1% Polyhexanide irrigation.

All the irrigating solutions were introduced into the canal using stainless steel 27-gauge bevelled needle. The needle was placed within 1–2 mm of working length within each canal and irrigation was performed.

After irrigation, all the root canals were dried with absorbent paper points, and a sterile cotton pellet was placed in the access cavity. The teeth were stored in a plastic bag placed in a humidior

SEM Analysis

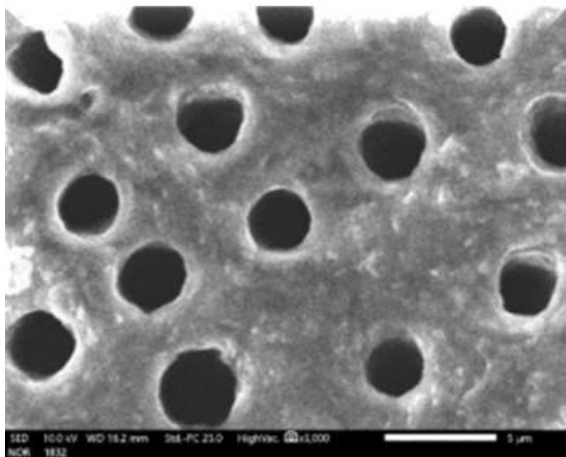
Longitudinal grooves were prepared on buccal and lingual surfaces of each root using a diamond disc at a slow speed without penetrating the canal. The roots were then split into two halves using a chisel, and then the samples were observed under scanning electron microscope at apical (2mm to apex), middle (6mm to apex), and coronal (10mm to apex) levels. The samples were dried and mounted on a single stub, sputter-coated with gold in high vacuum evaporator and analysed using JSM-6510 LV Scanning electron microscope to evaluate the smear layer removal efficacy. For each sample, photomicrographs were taken at the coronal, middle and apical sites of the root canal x 5000x.

The images were scored according to the criteria given by Hulsmann *et al* ⁽⁶⁾ which measure the presence, quantity, and distribution of the smear layer.

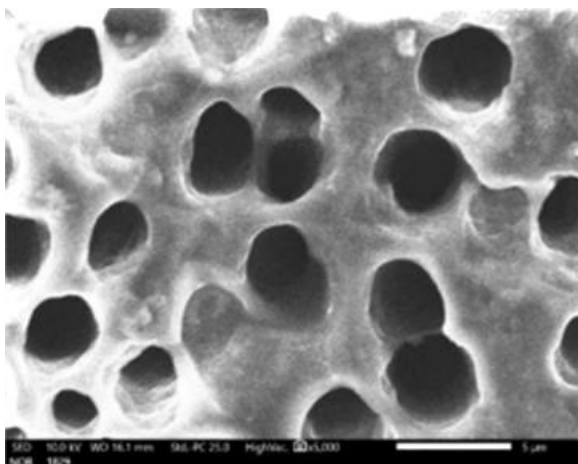
Table 2: Hulsmann et al scoring criteria

Score	Interpretation
1	No Smear layer, dentinal tubuli open
2	Small amount of smear layer, some dentinal tubuli open
3	Homogeneous smear layer covering the root canal wall, only few dentinal tubuli open
4	Complete root canal wall covered by a homogeneous smear layer, no open dentinal tubuli
5	Heavy, non- homogenous smear layer covering the complete root canal wall

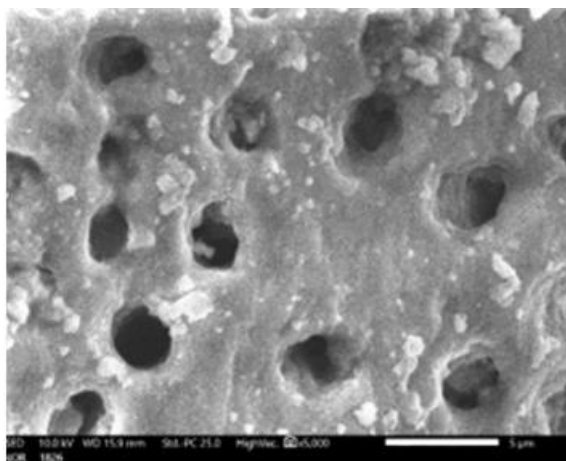
Photomicrographs ($\times 5000x$) of these areas on each of the coronal, middle and apical thirds were taken [Figures 1-3].



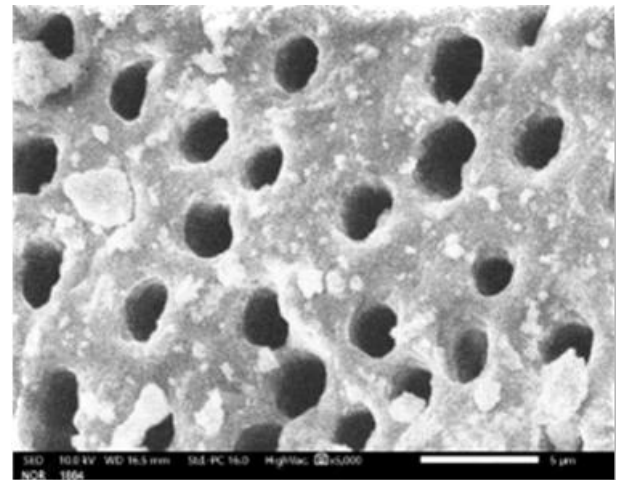
(a) SEM image of group I at coronal third.



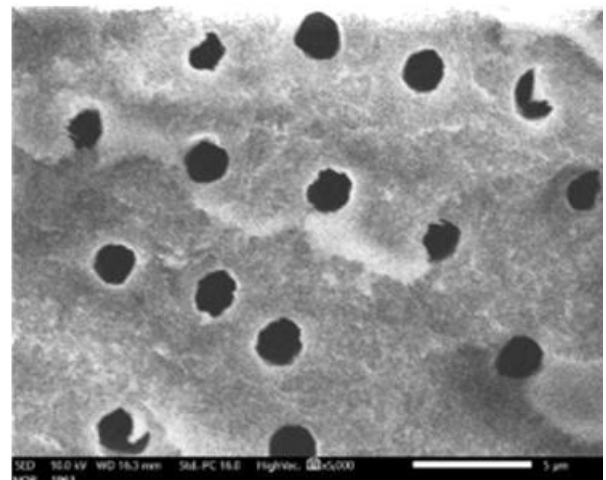
(b) SEM image of Group I at middle third



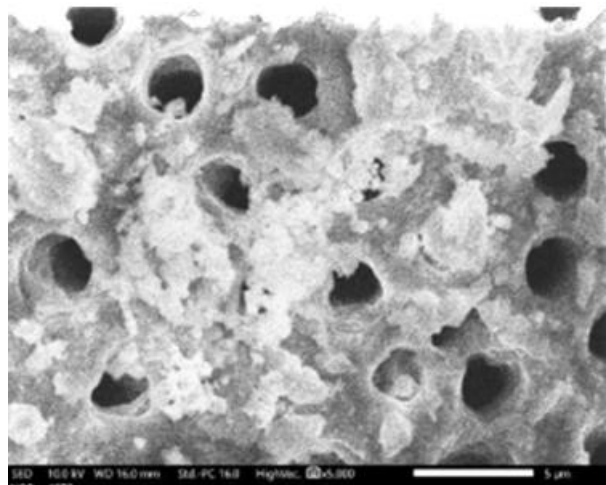
(b) SEM image of Group I at apical third



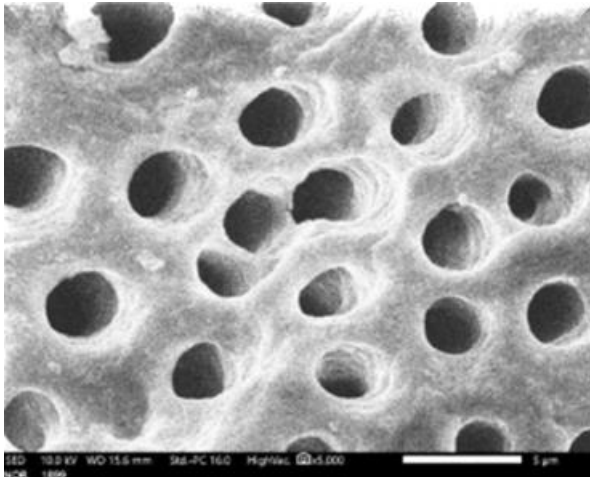
c) SEM image of Group II at coronal third



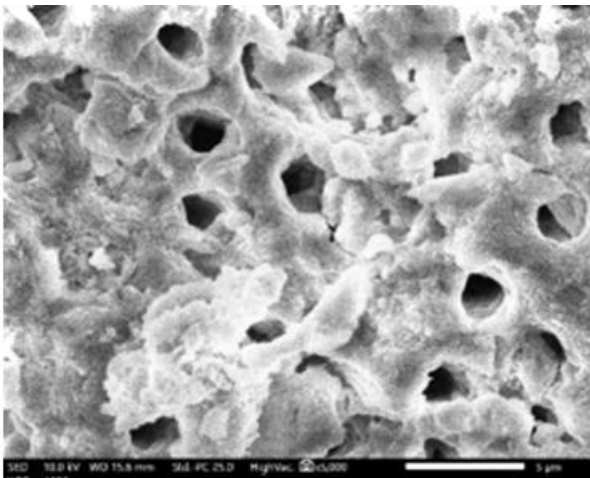
b) SEM image of Group II at middle third



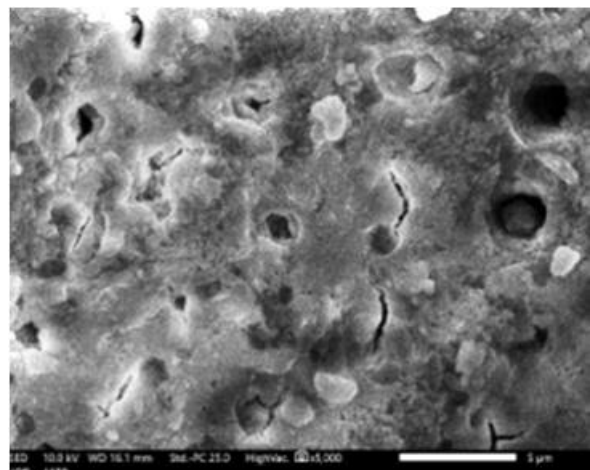
a) SEM image of Group II at apical third.



c) SEM image of Group III at coronal third



c) SEM image of Group III at middle third



c) SEM image of Group III at apical third

Statistical methods

Statistical Package for Social Sciences (SPSS) version 23 was used for analysis:

- 1) Kruskal-Wallis analysis of variance (ANOVA) was used for intragroup comparisons.
- 2) Mann-Whitney U test was used for intergroup comparisons.

3. Results

All irrigants tested, removed smear layer effectively form

coronal and middle third. At the apical third, all irrigants showed poor smear layer removing property, but 17% EDTA (Group I) and combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid (Group II) showed comparatively better results than 0.1% polyhexanide (Group III) at the apical third [Table 1]. There was no significant difference between combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid and EDTA. Intragroup comparison showed highly significant difference in coronal against apical region in EDTA group, significant difference in coronal vs apical and middle vs apical in 0.1% polyhexanide group.

Table 1: Comparison of Smear Layer Mean Scores at Coronal, Middle and Apical Third Using Kruskal Wallis Test

Groups	Position	Mean	Std. Deviation	X ² Value	P Value
Group 1	Control	1.1818	.40452	9.500	.001
	Middle	1.5455	.52223		
	Apical	2.0909	.539369		
Group 2	Control	1.7273	1.34840	2.968	.067
	Middle	1.8182	.75076		
	Apical	2.7273	1.00905		
Group 3	Control	1.4545	.68755	14.652	.000*
	Middle	2.1818	.98165		
	Apical	3.3636	.80904		

Table 2: Pair Wise Comparison of Mean Scores of Smear Layer Removal Using Mann Whitney U Test

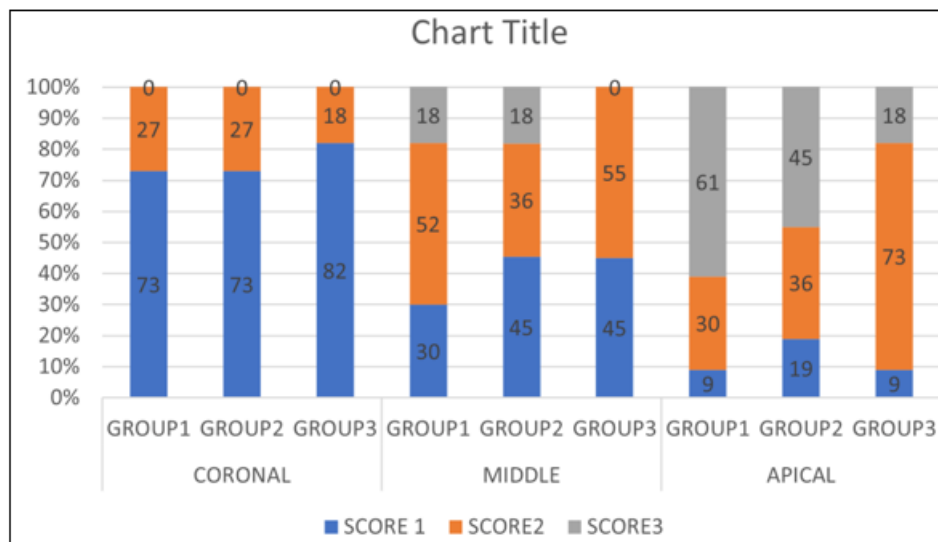
Pair-wise comparison in groups		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
GROUP 1						
Coronal	Middle	-.36364	.20995	.210	-.8812	.1539
Coronal	Apical	-.90909*	.20995	.000*	-1.4267	-.3915
Middle	Apical	-.54545*	.20995	.037*	-1.0630	-.0279
GROUP 2						
Coronal	Middle	-.09091	.45394	.978	-1.2100	1.0282
Coronal	Apical	-1.00000	.45394	.087	-2.1191	.1191
Middle	Apical	-.90909	.45394	.129	-2.0282	.2100
GROUP 3						
Coronal	Middle	-.72727	.35598	.119	-1.6049	.1503
Coronal	Apical	-1.90909*	.35598	.000*	-2.7867	-1.0315
Middle	Apical	-1.18182*	.35598	.007*	-2.0594	-.3042

Comparison of Mean Scores of Smear Layer Removal in Different Groups Using Kruskal Wallis Test Using 5000x

Groups	Position	Mean	Std. Deviation	X ² value	P value
CORONAL	GROUP 1	1.1818	.40452	1.000	.380
	GROUP 2	1.7273	1.34840		
	GROUP 3	1.4545	.68755		
MIDDLE	GROUP 1	1.5455	.52223	1.869	.172
	GROUP 2	1.8182	.75076		
	GROUP 3	2.1818	.98165		
APICAL	GROUP 1	2.0909	.53936	6.806	.004*
	GROUP 2	2.7273	1.00905		
	GROUP 3	3.3636	.80904		

Pair Wise Comparison Of Mean Scores Of Smear Layer Removal Using Mann Whitney U Test

Pair-Wise Comparison between groups		Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval	
					Lower Bound	Upper Bound
Coronal						
Group 1	Group 2	-.54545	.38569	.347	-1.4963	.4054
Group 1	Group 3	-.27273	.38569	.761	-1.2236	.6781
Group 2	Group 3	.27273	.38569	.761	-.6781	1.2236
MIDDLE						
Group 1	Group 2	-.27273	.33029	.690	-1.0870	.5415
Group 1	Group 3	-.63636	.33029	.149	-1.4506	.1779
Group 2	Group 3	-.36364	.33029	.521	-1.1779	.4506
APICAL						
Group 1	Group 2	-.63636	.34498	.173	-1.4868	.2141
Group 1	Group 3	-1.27273	.34498	.002*	-2.1232	-.4223
Group 2	Group 3	-.63636	.34498	.173	-1.4868	.2141



Intergroup Comparison of All Groups at Apical, Middle and Coronal Third

4. Discussion

Necrotic tissue remnants in the root canals serve as a nutrient source for any remaining microorganisms. The successful outcome of an endodontic treatment depends on the complete eradication of the microorganisms from the root canal system prior to obturation. Irrigation is a crucial step during and after instrumentation for effective removal of smear layer as well as for lubrication of root canal system. ⁽⁷⁾

Straight single-rooted and single canal mandibular premolar were selected, which was confirmed using radiograph as suggested by Wu *et al.* ⁽⁸⁾ Standardized crown down technique was followed for preparation of root canal using K-files up to size 40 reaching full working length which facilitates penetration of irrigants to the apical third and produces a greater reduction in remaining bacteria and dentin debris as compared with smaller preparation. ⁽⁹⁾ This study used a 27-gauge needle tips, which can penetrate more deeply into the apical one-third because of the small bore size.

In the present study, 2.5% NaOCl was used for the chemomechanical preparation between each instrument, which could also remove the organic contents of smear layer. Scanning electron microscope is one of the most commonly used technique for evaluating smear layer removal, and hence, was used in the present study. ⁽¹⁰⁾

Before using root canal irrigants on human beings, laboratory studies have to be conducted to determine the benefits and consequences. Hence, the efficiency of 0.1% Polyhexanide and its combination is evaluated.

In this study, Smear layer was removed more at coronal and middle third region by 17% EDTA because it acts as a chelating agent, binding to and removing the inorganic, calcium-rich components of the smear layer and demineralizing the dentin. This is in accordance with the study conducted by Abraham *et al* ⁽¹¹⁾ where the smear layer removal efficacy of 17% EDTA in coronal, middle and apical thirds was evaluated

According to Ahamed *et al* ⁽¹²⁾ the chelating effect of EDTA was almost negligible in the apical third of root canals. The decreased content of noncollagenous proteins and the less number of dentinal tubules in the apical region of root canal have been correlated to this reduction in chelating effect of EDTA and the resulting prominent removal of smear layer from the coronal third. So, in apical third it shows less smear layer removal compared to coronal and middle thirds.

In the present study, 0.1% polyhexanide (PHMB) demonstrated the least efficacy in smear layer removal across the apical, middle, and coronal root canal thirds compared to the other experimental groups. This suggests that PHMB interacts less aggressively with the dentin surface, a finding potentially attributable to its limited capacity for calcium

chelation and collagen dissolution, as previously proposed by Khabadze et al. ⁽¹⁴⁾. The efficacy of polyhexanide as a standalone endodontic irrigant for smear layer removal has not been previously evaluated, and our results indicate that it may not be sufficient for comprehensive debris management when used in isolation

According to Lapinska et al. ⁽¹³⁾, 0.1% PHMB preserves dentin's Vickers microhardness and Young's modulus more effectively than conventional NaOCl and EDTA regimens. This can be attributed to PHMB's minimal affinity for the organic and inorganic components of dentin, which allows for effective disinfection without inducing structural alteration. This distinction is critical when developing endodontic irrigation protocols that aim to balance long-term tooth preservation with microbial control. Furthermore, PHMB exhibits potent antimicrobial activity while avoiding the chemical incompatibilities often encountered with traditional irrigant combinations.

Studies evaluating polyhexanide in combination regimens, particularly with hypochlorous acid, have reported an absence of adverse chemical reactions such as precipitate formation.⁽¹⁵⁾ This compatibility suggests that polyhexanide is suitable for sequential irrigation protocols designed to balance effective debris removal with the preservation of dentin structural integrity. Consequently, this study incorporated these combined irrigation strategies to evaluate their collective clinical efficacy.

In intergroup comparisons, the combined irrigation protocol of 5% sodium hypochlorite (NaOCl), 0.1% polyhexanide (PHMB), and 0.02% hypochlorous acid (HOCl) effectively removed the smear layer across the coronal, middle, and apical thirds of the root canal system. Hypochlorous acid has been previously advocated as an alternative to conventional irrigants due to its efficacy in smear layer removal and its ability to cleanse dentinal walls ⁽¹⁶⁾. Furthermore, HOCl is inherently biocompatible and has been shown to exhibit significantly lower cytotoxicity than NaOCl while maintaining comparable antimicrobial activity against *Enterococcus faecalis*. Notably, the use of HOCl as an irrigant in this study did not induce dentinal discoloration. These findings, which align with those reported by Kalpana et al. ⁽¹⁷⁾, suggest that integrating HOCl with NaOCl may offer a synergistic clinical benefits providing comprehensive cleaning efficacy without the aesthetic drawbacks or precipitate formation often associated with other chemical combinations.

Sodium hypochlorite remains a cornerstone in endodontics for its unparalleled antimicrobial efficacy and tissue-dissolving capabilities. However, its interaction with the collagen matrix of dentin has been shown to lead to deproteinization, thus reducing elasticity and hardness. Therefore, combining NaOCl with PHMB increases the young's modulus of elasticity of dentin.

PHMB differs from other irrigants with regards to:

- It has broad spectrum of antibacterial activity and had shown more permanent antibiofilm effect than CHX (Khabadze et al)⁽¹⁸⁾
- Compare to EDTA and Naocl, PHMB preserves modulus

of elasticity of dentin, thereby showing reduced risk to dentin integrity (Ackay et al)⁽¹⁹⁾

- PHMB has shown low cytotoxicity and good tissue tolerance compared to other irrigants (Rita et al) ⁽²⁰⁾

5. Conclusion

This study provides valuable evidence that 17% EDTA efficiently removes the smear layer from root canal walls and proved to be superior to 0.1% PHMB solution in removing the smear layer from apical third of root canals. No significant difference was found between 17% EDTA and combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid in the removal of the smear layer from the coronal, middle and apical thirds of the root canal. Combination of 5% sodium hypochlorite, 0.1% polyhexanide and 0.02% hypochlorous acid could be used as an alternative to 17% EDTA as a irrigation solution.

The results obtained from the present *in vitro* study do not necessarily allow any definite actions of the tested substances *in situ*. Blood, tissue remnants, and various other variables may affect the actions of irrigating agents in the root canal system. Curved canals are more challenging and make effective cleaning of the root canal system more difficult. As deeper penetration of the needle takes place in the single-rooted premolar tooth because of wider canals, the results may vary in teeth with narrow canals. Further studies are necessary to confirm the results, preferably of longer duration, to validate their effectiveness and contribution in the quality of treatment.

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