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Comparative Analysis of Endodontic Smear Layer Removal Efficacy of 0.1% Polyhexanide, Twin Kleen, and 17% Ethylenediaminetetraacetic Acid Using Scanning Electron Microscope: An *In Vitro* Study

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Abstract
Aim: The aim of the study is to evaluate the efficiency of different endodontic irrigants; Irritrol, 0.1%Polyhexanide, 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 singles-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 0.1% polyhexanide irrigation, and Group III with twin kleen 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. twin kleen) 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 0.1% polyhexanide and the least by twin kleen 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 0.1% Polyhexanide is more efficient than Twin kleen in the removal of smear layer from the apical third of root canal.

Keywords: Endodontic irrigants, smear layer removal, polyhexanide, twin kleen, ethylenediaminetetraacetic acid, scanning electron microscope, root canal irrigation, *in vitro* study

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 [3]. 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 [2].

Twin Kleen is an average chelating agent (9% HEBP) that is short-term compatible with sodium hypochlorite. The combo decreases AHTD (Accumulated Hard Tissue Debris) and inhibits smear layer development following rotary instrumentation. Twin Kleen serves as an antimicrobial remedy, moderate chelating agent, proteolytic agent, lubricant, and smear layer preventer that might be added to endodontic irrigating solutions throughout root canal instrumentation in addition to a last rinse solution. The feature of this combo is that NaOCl retains its natural, antimicrobial, and tissue-dissolving characteristics, while etidronic acid also reduces and eliminates inorganic ions [3].

However, the comparative studies on the effect of smear layer removal of 0.1% Polyhexanide is not discussed in Literatures. So, this study is to evaluate the efficiency of 0.1% Polyhexanide as an irrigant in removing smear layer.
Hence, the present study was undertaken to evaluate the efficiency of 17% EDTA, 0.1% Polyhexanide, and Twin kleen endodontic irrigants in smear layer removal through scanning electron microscopic image analysis.

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 is estimated using a number 10 K file until it is 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 is 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 are divided into three groups.
The samples were divided into Groups 1, 2, and 3 containing 15 samples each:

- **Group 1:** 15 samples with 17% EDTA irrigation.
- **Group 2:** 15 samples with 0.1% Polyhexanide irrigation.
- **Group 3:** 15 samples with Twin kleen 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 is 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 humidor

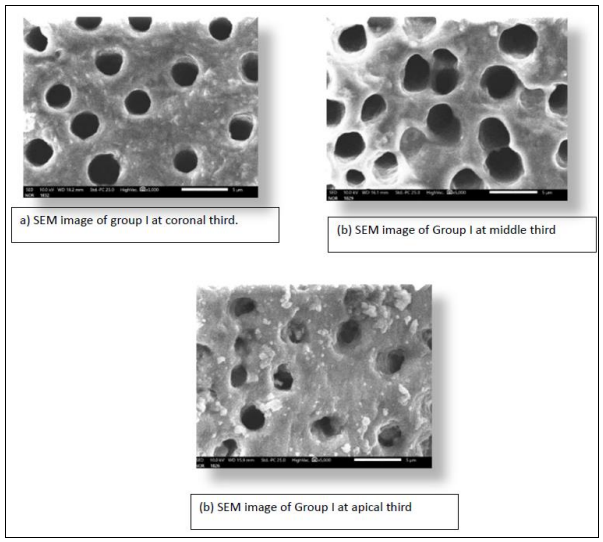
Sem Analysis

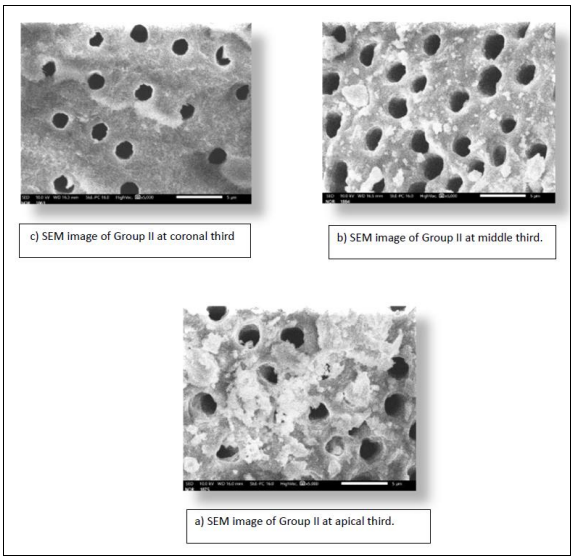
Longitudinal grooves were prepared on buccal and lingual surfaces of each root using a diamond disc at a slow speed without penetrating the canal. The roots were then split into two halves using a chisel, and then the samples were observed under scanning electron microscope at apical (2mm to apex), middle (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 vaccum 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* [4]. which measure the presence, quantity, and distribution of the smear layer.

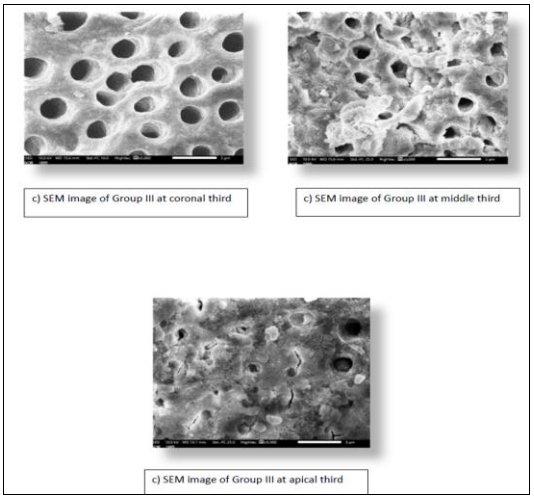
Table 2: Hulsmann et al scring Criteria

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

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







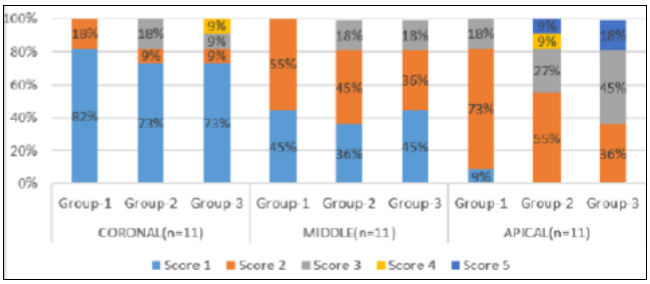
Statistical methods

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.

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 0.1% Polyhexanide (Group II) showed comparatively better results than Twin Kleen (Group III) at the apical third [Table 1]. There was no significant difference between Polyhexanide 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 twin kleen group.



Intergroup comparison of all groups at apical, middle and coronal third

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	Coronal	1.1818	.40452	9.500	.001*
	Middle	1.5455	.52223		
	Apical	2.0909	.53936		
GROUP 2	Coronal	1.7273	1.34840	2.968	.067
	Middle	1.8182	.75076		
	Apical	2.7273	1.00905		
GROUP 3	Coronal	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

Intragroup comparison of the groups at apical, middle and coronal third

S- Statistically Significant

NS- Not Statistically Significant

INTRAGROUP COMPARISON	Coronal vs middle	Apical vs coronal	Apical vs middle
GROUP 1	NS	S	S
GROUP 2	NS	NS	NS
GROUP 3	NS	S	S

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	6.808	.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

INTER-GROUP COMPARISON	S- Statistically Significant NS- Not Statistical Significant		
	Coronal	Middle	Apical
GROUP 1 VS GROUP 2	NS	NS	NS
GROUP 1 VS GROUP 3	NS	NS	S
GROUP 2 VS GROUP 3	NS	NS	NS

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 [5].

Straight single-rooted and single canal mandibular premolar were selected, which was confirmed using radiograph as suggested by Wu *et al* [6]. 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 [7]. 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 [8].

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 has to be 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* (9) where the smear layer removal efficacy of 17% EDTA in coronal, middle and apical thirds was evaluated

According to Ahamed *et al* [10] the chelating effect of EDTA was almost negligible in the apical third of root canals. The decreased content of noncollagenous proteins and the smaller 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 this study, Twin Kleen removed the smear layer more in coronal third followed by middle third because it has a chelating agent, specifically containing 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) which targets and removes the inorganic components of the smear layer. It also prevents the formation of the smear layer when combined with sodium hypochlorite, allowing the hypochlorite to better clean and disinfect the canal according to Mohammed *et al* [11].

And in this study Twin kleen showed least smear layer removal in apical third as HEBP is a weak chelating agent and due to the challenges of reaching the apical third. According to Subrahmanya *et al* [12] etidronic acid has a lower chelating activity than EDTA, and its diminished effectiveness on sclerosed dentin may also potentially play a role.

PHMB had removed smear layer more in apical third like EDTA primarily through dissolving the inorganic components. It has superior antimicrobial action without the chemical incompatibility issues seen with other irrigants.

This is in accordance with the study done by Kalpana *et al* [13], where a combination of PHMB and macrogol, together known as Lavasept solution is used for smear layer removal.

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*) [14]

Compare to EDTA and Naocl, PHMB preserves modulus of elasticity of dentin, thereby showing reduced risk to dentin integrity (Ackay *et al*) [15]

PHMB has shown low cytotoxicity and good tissue tolerance compared to other irrigants (Rita *et al*) [16]

Conclusion

The present study shows that 17% EDTA efficiently removes the smear layer from root canal walls.

17% EDTA proved to be superior to TWIN KLEEN solution in removing the smear layer from apical third of root canals. No significant difference was found between 17% EDTA and 0.1% polyhexanide in the removal of the smear layer from the coronal, middle and apical thirds of the root canal. 0.1% polyhexanide solution 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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