

COMPARATIVE EVALUATION OF Q-MIX, CHITOSAN NANOPARTICLES, EDTA ON SMEAR LAYER REMOVAL USING SCANNING ELECTRON MICROSCOPY: AN IN VITRO STUDY.

Dental Science

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ABSTRACT

Aim: The aim is to evaluate the smear layer removal efficacy of qmix and compare it with different endodontic irrigating solutions chitosan and ethylenediaminetetraacetic acid [EDTA] under scanning electron microscope (SEM).

Materials and Methods: Forty single-rooted teeth were decoronated. All the root canals were shaped with rotary protaper gold instruments (#30, .06) and irrigation was done with 2ml of 5.25% sodium hypochlorite (NaOCl) solution between each file change. The samples were divided randomly into the 4 groups (n = 10) depending on the final irrigant used as follows: (Group 1) Saline, (Group 2) Qmix, (Group 3) Chitosan, and (Group 4) 17% EDTA. The final irrigation was done using 5 ml of the respective irrigating solutions, each for 60 s. Specimens were sectioned longitudinally. For each root, the half containing most visible part of apex was selected for the SEM analysis. The presence/absence of smear layer was evaluated at the coronal, middle, and apical third of the root canal using three score criteria. Data were analyzed using Mann-Whitney U-test and Kruskal-Wallis test.

Results: QMix showed the least smear layer scores followed by chitosan, EDTA, NaOCl.

Conclusion: Within the limitation of this study, it can be concluded that the test irrigant qmix removed the smear layer throughout the entire length of the root canal.

KEYWORDS

Ethylenediaminetetraacetic acid, chitosan, QMix, scanning electron microscope, smear layer removal.

INTRODUCTION

The success of endodontic treatment depends on the eradication of microbes from the root-canal system and prevention of reinfection. Biomechanical preparation is done with hand and rotary instruments under constant irrigation to remove the inflamed and necrotic tissue, microbes/biofilms, and other debris from the root-canal space. Several studies using advanced techniques such as microcomputed tomography (CT) scanning have demonstrated that proportionally large areas of the main root-canal wall remain untouched by the instruments, emphasizing the importance of chemical means of cleaning and disinfecting all areas of the root canal.¹ There is no single irrigating solution that alone sufficiently covers all of the functions required from an irrigant. Optimal irrigation is based on the combined use of 2 or several irrigating solutions, in a specific sequence, to predictably obtain the goals of safe and effective irrigation.²

During biomechanical preparation, the use of hand or rotary files for instrumentation will result in the production of considerable amount of mineralized debris what is called smear layer.³ Eick et al. were the first who identified the smear layer using scanning electron microscope (SEM) and found that smear layer is made from different size of particles ranging from of smear layer on instrumented root canals was first reported by McComb and Smith in 1975.⁴ They showed that this layer is made of remnants of dentin, odontoblastic processes, necrotic or viable pulp tissues, and bacteria. This content can act as a reservoir for further microbial irritants⁵ and may serve as a substrate for microorganisms⁶ to survive, multiply,⁷ and then proliferate deeply inside the dentinal tubules.^{8,9} Removal of smear layer after root canal instrumentation and before canal obturation improves the adaptation of root filling materials to the canal walls, resulting in a superior seal.¹⁰ So far the most commonly used method of smear layer removal has been the chemical method using chelating agents, with

ethylenediaminetetraacetic acid (EDTA) being the most common agent used.¹¹ The most advocated combination of 17% EDTA plus 5.25% NaOCl removes the smear layer completely in the coronal and middle thirds but is less effective in the apical third.¹¹

However, EDTA results in excessive peritubular and intertubular dentinal erosion that results in decreased microhardness of root dentin.¹² Due to the deleterious effects of EDTA on dentin, need for more biocompatible material continues. Reviewing all these facts, this leads in search for an alternative agent which is more biodegradable to replace EDTA continues.

One such material which is used in this study was Chitosan is a naturally occurring polysaccharide, which has been utilized in different fields of dental exploration due to its alluring properties like biocompatibility, biodegradability, bio adhesion.¹³ Chitosan is acquired by the deacetylation of chitin, which is found in shells of Crustaceans. It has a high chelating ability for various metal ions in acidic conditions and has been applied widely for the removal or recovery of metal ions in different industrial areas. Applications for chitosan are being seen more in the fields of medicine and pharmaceuticals.^{13,14}

QMix 2 in 1 (DENTSPLY Tulsa dental, OK, USA), is a novel root canal irrigating solution, and it contains a mixture of a bisbiguanide an antimicrobial agent chlorhexidine, a polyaminocarboxylic acid, EDTA a calcium-chelating agent, saline, and a surfactant.¹⁵

MATERIALS AND METHODS

Forty single-rooted human teeth extracted for periodontal reasons were selected for this study. They were stored in normal saline at room temperature immediately after extraction until use. Inclusion criteria were morphological similarity; single-canal roots; straight roots;

absence of root decay; absence of previous endodontic treatment, cracks. Teeth were sterilized by autoclaving for 15min at 15 psi pressure and 121°C temperature immediately before use. The presence of a single root canal in each tooth was confirmed on radiographs. All the teeth were decoronated to get the flat reference point and to standardize the root canal length of 14 mm. Patency of the root canal was established by passing a stainless steel number 15 K-file (Mani, Japan) just beyond the apex of all canals. When the tip of the file was visible at the apex, 1 mm short of the file length was considered to be the working length. Canals were prepared using ProTaper Gold rotary system (Dentsply Maillefer, Ballaigues, Switzerland). Each canal was prepared up to an apical preparation of #F3. About 2 ml solution of 3% NaOCl (Vishal dentocare Pvt., Ltd., India) was used as an irrigant between each subsequent file size in all experimental groups using 5 ml syringe (BD, India) and 32-gauge side-vented needle. For effective irrigation in the apical third of root, the needle was inserted till 1 mm short of the apex.

The roots were randomly assigned in 4 groups of 10 specimens each depending on the type of the final irrigant used, as follows: Group 1 Saline (n = 10): Each root canal was flooded with 5 ml of saline irrigating solution for 60 s as a final rinse. Group 2 Qmix (n = 10): Each root canal was flooded with 5 ml of Qmix (Dentsply Tulsa, OK, USA) irrigating solution for 60 s as a final rinse. Group 3 Chitosan (n = 10): Each root canal was flooded with 0.2% chitosan (Nanobiotech Pvt.Ltd, Chennai, India) irrigating solution for 60 s as a final rinse. Group 4 EDTA (n = 10): Each root canal was flooded with 17% EDTA (Prevest Dentpro, India), irrigating solution for 60 s as a final rinse. Irrigation in each sample was done using NaviTip 32 G so as to reach within the apical third of the canal. The root canals were irrigated using 5 ml of the respective irrigating solution, each for 60 s. Each canal was finally flushed with 5 ml of distilled water and dried with absorbent paper points. The teeth were split longitudinally, with the aid of chisel and a surgical mallet, through the longitudinally placed grooves. For each root, the half containing the most visible part of apex was selected for SEM evaluation, and the remaining half was discarded.

Scanning electron microscope evaluation

Coded samples were mounted on metallic stubs, gold sputtered and viewed under SEM (ZEISS EVO 40). Photographs at magnifications of $\times 2500$ were taken for each specimen.

All the samples were evaluated at different levels of root canal that is coronal, middle, and apical to evaluate the presence and absence of smear layer. The amount of smear layer remaining on the surface of the root canal and dentinal tubules was scored according to a three-score system developed by Torabinejad et al.

Score 1: No smear layer – no smear layer was detected on the surface of the root canal, and all tubules were open

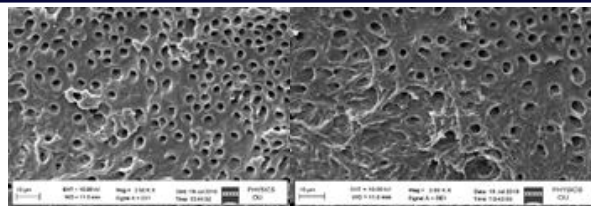
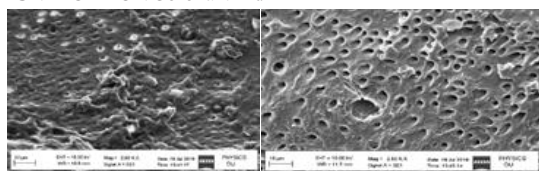
Score 2: Moderate smear layer – no smear layer was detected on the surface of the root canal walls but tubules contained debris

Score 3: Heavy smear layer– smear layer covered the root canal wall surface and the tubules.

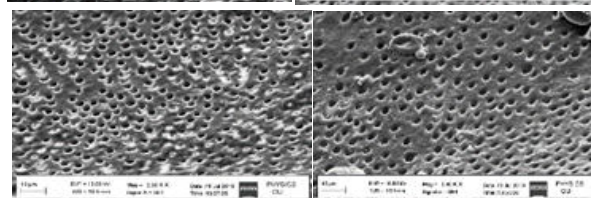
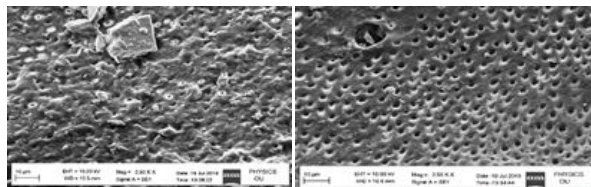
RESULTS AND OBSERVATION

The results of the smear layer scores for each group with their mean and standard deviation values were obtained. Intragroup comparison of mean smear layer scores of coronal, middle, and apical third in each group was done using Friedman test along with post hoc pair-wise comparison using Wilcoxon test, which showed irrigating solutions (QMix, chitosan, and EDTA) were more effective in coronal third area followed by middle third and apical third area, and their intergroup comparison was done using Kruskal–Wallis test in the coronal, middle, and apical third area. The results showed that QMix had the least smear layer scores when compared to others. This was followed by chitosan, EDTA, Saline group.

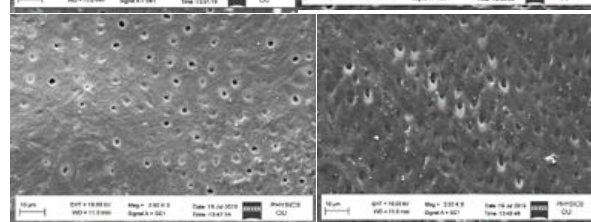
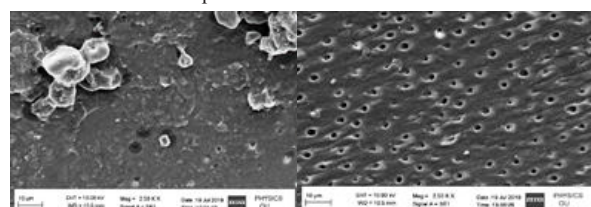
SCANNING ELECTRON MICROSCOPE IMAGES -AT 2500 X MAGNIFICATION Coronal third



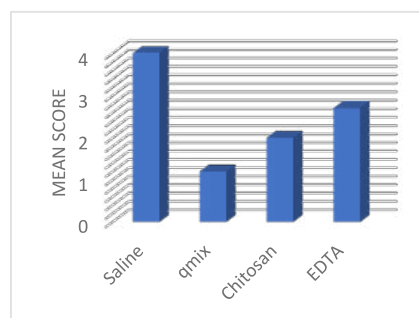
SCANNING ELECTRON MICROSCOPE IMAGES -AT 2500 X MAGNIFICATION Middle third



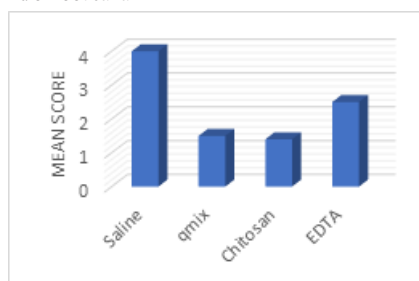
SCANNING ELECTRON MICROSCOPE IMAGES -AT 2500 X MAGNIFICATION Apical third

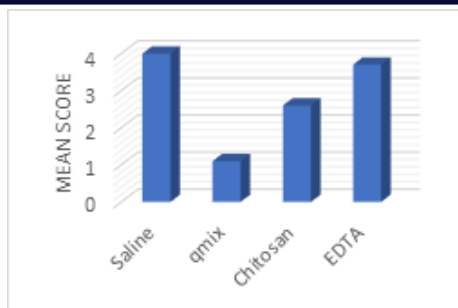


The mean score for each treatment type for all samples (n=10) for coronal third of root canal



The mean score for each treatment type for all samples (n=10) for middle third of root canal





The mean score for each treatment type for all samples (n=10) for apical third of root canal

DISCUSSION

Mechanically prepared dentin surfaces are always covered with a so-called smear layer, a loosely bonded amorphous layer of organic and inorganic debris.¹⁶ According to Shahravan et al. in a systematic review and meta-analysis, the smear layer removal improved fluid-tight seal of root canal system of permanent teeth.¹⁷ In this respect, several endodontic irrigating solutions have been used to remove such smear layer with varying degrees of success. They have ranged from acids and chelates, to those intended to dissolve organic debris.

Irrigation is an important step during and after instrumentation for effective removal of smear layer and also for lubrication of root canal system.¹ NaOCl is the traditional irrigant which is used most commonly, however due to its inability to remove inorganic part of smear layer other irrigants were introduced.¹⁸ Chelating agents have been suggested to improve the chemomechanical debridement in root canal treatment by removing the smear layer from the root canal as well as the demineralizing and softening dentin.¹⁹ The chelating agents used in this study are chitosan, Qmix, EDTA.

Smear layer removal from canal walls was evaluated with SEM as it is one of the effective qualitative tools to study the surface morphology. The dentinal tubules are very well appreciated at $\times 2500$. The smear layer removal evaluation was done using criteria given by Torabinejad et al. as it is the most commonly used criteria to evaluate smear layer in other studies.²⁰

There was a statistically significant difference between group I when compared with other groups II, III, IV.

In Group 2, QMix 2 in 1 solution showed promising results in smear layer removal from the coronal, middle, and apical thirds of the root canal. The same results were also shown by Dai et al.,²¹ Stojicic et al.,²² Eliot et al.,²⁴ and Vemuri²³ et al. The possible reason for effective smear layer removal could be attributed to combined effect of its constituent chelating agent, that is, EDTA, that dissolves the inorganic content of the root canal by reacting with calcium ions which forms soluble chelates and a surface active agent, that is, cetrinide, which enables better penetration of an irrigant in the root canal. The introduction of the surfactant seems to reduce the contact angle of the EDTA solution when placed on the dentin surface and enhances cleaning efficacy. This was also supported by studies done by Ballal et al.,²⁵ who showed that wetting of root canal dentin was better with QMix 2 in 1 due to its low surface tension.

A study done by Dai et al.²¹ reported that QMix was as effective as 17% EDTA in removal of smear layer from the root canal system. Eliot et al. demonstrated that QMix was superior to 17% EDTA in removal of canal wall smear layer.²³ These findings support the result obtained in the present study. Venghat²⁹ et al. did a study to evaluate smear layer removal efficacy and reported that EDTA performed better than QMix, though the results were not significantly different. This difference in the results obtained from that of our study could be attributed to the different scoring system used in their study.

Stojicic et al. evaluated efficacy of a novel root canal irrigant, QMix, against *Enterococcus faecalis* and its ability to remove smear layer was examined, using SEM.²² It was concluded that QMix and NaOCl were superior to CHX and MTAD under laboratory conditions in killing *E. faecalis* and ability to remove smear layer by QMix was comparable to EDTA.²² These studies correlate with the present study QMix 2 in 1 showing better smear layer removal compared with other final irrigating solutions and there is a significant difference among all the groups.

In Group 3, 0.2% Chitosan solution showed better results in smear layer removal from the coronal, middle, of the root canal. Chitosan acetate to remove the smear layer at the apical third could be attributed to its viscosity which may interfere with its flow ability to reach the apical part especially in curved and relatively narrow canals used in the current study.²⁷ The efficiency of chitosan for smear layer removal could be attributed to the hydrophilic nature of chitosan which favors its intimate contact with root canal dentin and its adsorption to root canal wall. Additionally, chitosan was dissolved in 1% acetic acid to form the solution. In an acid medium, the amino groups present in the chitosan are protonated ($-NH_3^+$), resulting in attraction to other molecules for adsorption to root dentin to occur.³⁰ However, the incomplete ability of 0.2% Chitosan acetate to remove the smear layer at the apical third could be attributed to its viscosity which may interfere with its flow ability to reach the apical part especially in curved and relatively narrow canals.²⁸

The use of chitosan in endodontics is of interest due to its biocompatibility, antimicrobial and chelating properties. Researchers have hypothesized two theories on the mechanism involved in chelating with chitosan. The first theory is based on the bridge model, where the same metal ion is bound by two or more amino groups on the chitosan chain. The second theory claims that only one of the amino groups on the chitosan chain is anchored to the metal ion.³⁰

Kamble et al. reported that 0.2% chitosan removed the smear layer with to greater extent than 17% EDTA.³¹ Silva et al. reported that that 15% EDTA, 0.2% chitosan, and 10% citric acid removed the smear layer effectively from apical thirds of the root canal.³² Mittal et al. reported that 0.2% chitosan was very effective in removing smear and smear plug with less peritubular dentin erosion compared to apple cider vinegar and 15% EDTA.^{28,33}

In the present study, 0.2% chitosan acetate showed better results in removing the smear layer compared to 17% EDTA at all root canal levels apically. The coronal and middle levels within group II, (Qmix), group IV (17% EDTA) as well as group III (0.2% chitosan acetate) showed a lower mean scores (more smear layer removal) compared to the group I (saline group).

In Group 4, 17% EDTA was found to be effective in removal of smear layer from coronal and middle third area and was less effective in the apical third of root canal, which is in accordance with a study done by Torabinejad et al. and Mancini et al.^{34,35} where 17% EDTA may not have pronounced action as described by Vasilidis et al. and Paqué et al. EDTA efficiently removed the smear layer due to its chelating property.^{36,37} This might be attributed to formation of stable complex with the calcium ions in dentin. However, it didn't show a pronounced effect in the apical third as compared to middle or coronal thirds of the root canal as the solution not only removes calcium ions but also calcium bonded to noncollagenous proteins of dentin (NCPs). As the content of NCPs decreased at the apical third of the root canal system, accordingly the decalcifying effect of EDTA at the apical part is low.³⁸

CONCLUSION

Within the limitations of this study, it was observed that among the final irrigating solutions used QMix 2 in 1 displayed effective smear layer removal when compared to Chitosan, EDTA, and Saline.

Table 1: Intragroup and intergroup comparison of smear layer scores

	Coronal third	middle third	Apical third	p value	inter group comparision
Group I(Saline)					
Mean	3.70	4.00	4.00	0.135	Coronal <apical
SD	0.42	0.00	0.00		
Group II(Qmix)					
Mean	2.3	1.6	1.8	0.05	Coronal <apical
SD	0.52	0.79	0.79		
Group III(Chitosan)					
Mean	2.5	2.00	2.4	0.002	Coronal <apical
SD	0.52	0.52	0.52		
Group IV(EDTA)					
Mean	2.7	2.30	3.0	0.32	Coronal <apical
SD	0.00	0.32	0.32		
Intergroup comparison					
p ^c	0.0001	0.0001	0.0001		
Post hoc pair wise comparision	2,3,4<1	2,3,4<1	2,3,4<1		

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