Original Article

Effectiveness of Three Different Irrigants - 17% Ethylenediaminetetraacetic Acid, Q-MIX, and Phytic Acid in Smear Layer Removal: A Comparative Scanning Electron Microscope Study

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Abstract

Background: Removal of smear layer from the root canal walls is important for long-standing endodontic success. **Aim:** The aim of this study is to evaluate and compare smear layer removing ability among 17% ethylenediaminetetraacetic acid (EDTA), Q-MIX, and phytic acid by scanning electron microscopy (SEM). **Materials and Methods:** This *in-vitro* experimental study assessed smear layer removal using three different irrigants. Thirty single-rooted freshly extracted human permanent premolars were collected, disinfected, and decoronated to a standardized root length of 13 mm. Root canals were cleaned and shaped till F2 universal rotary protaper at working length 1 mm short of the apex. They were randomly divided into three groups, and final irrigation was done accordingly. Group 1 (n = 10): with 1 ml of 17% EDTA, Group 2 (n = 10): with 1 ml of Q-MIX, Group 3 (n = 10): with 1 ml of phytic acid. Samples were then longitudinally sectioned and evaluated under SEM at coronal, middle, and apical levels. Statistical Analysis: Two-way analysis of variance and Tukey's *post hoc* test were performed. The level of significance was set at 0.05. **Results:** Smear layer removing ability among irrigants and sections in descending order: 17 EDTA > Q-MIX > phytic acid; coronal > middle > apical. **Conclusion:** 17% EDTA showed better and promising results followed by Q-MIX and then phytic acid.

Keywords: *17% ethylene diaminetetraacetic acid, phytic acid, Q-MIX, scanning electron microscope, smear layer*

Introduction

Successful root canal treatment is aimed for complete removal of microorganisms by meticulous chemomechanical preparation followed by three-dimensional sealing of the endodontic system.[1] Chemomechanical preparation includes shaping by mechanical removal of dentin and cleaning by chemical dissolution of organic tissues and disinfection of microorganisms. However, shaping of the root canal can be well achieved by instrumentation but effective cleaning of the entire root canal system remains a challenge.[2]

Mechanical preparation inadvertently forms an amorphous layer termed as "smear layer" on the root canal walls which contains microorganisms too.[1] McComb and Smith were the first researchers to describe smear layer on the surface of instrumented root canal walls.[3] Smear layer from the dentinal tubules has the ability to protect the bacteria from irrigation or intracanal medicament thus avoiding complete disinfection of the infected canals and thorough sealing of the entire root canal system.[4] Thus, removal of smear layer from the root canal walls is highly necessary for long-standing endodontic success.[1]

The ideal requirements of an irrigant includes – should be an effective disinfectant with adequate lubrication and flushing action, possess an antibacterial property, have the ability to dissolve organic and inorganic tissue, nontoxic to surrounding tissues and not weaken the tooth structure.[5] It should also have low surface tension and should retain its effectiveness with dental hard tissue and with other irrigants when mixed with it. However, none of the available irrigants possess all these properties together.

At present, sodium hypochlorite (0.5%–6.15%) and ethylenediaminetetraacetic acid (EDTA) (15%–17%) are the two most

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commonly used irrigants. Sodium hypochlorite has the ability to dissolve organic tissue whereas EDTA serves as an inorganic solvent.[6] EDTA is a chelating agent for inorganic divalent cations including calcium ions forming calcium chelates.[2] Many other irrigants have been introduced which have got the smear layer removing ability one of which is Q-MIX™ 2 in 1 (Dentsply Tulsa Dental Specialities, Tulsa, OK, USA). Q-MIX™ 2 in 1 is composed of a polyaminocarboxylic acid chelating agent, a bisbiguanide antimicrobial agent, a surfactant, and deionized water.[6]

Phytic acid (IP6, inositol hexakisphosphate), is a major storage form of phosphorus in plant seeds and bran that contributes in a variety of cellular function. It has got multiple negative charges, making it an effective chelator of multivalent cations such as calcium (Ca^{2+}), magnesium, and iron. It has also got the cariostatic and antiplaque effect.[7] Phytic acid has got the ability to remove smear layer and was proved to be less cytotoxic and more biocompatible as compared to EDTA.[8]

Hence, the aim of this study was to compare the smear layer removing ability among 17% EDTA, Q-MIX, and phytic acid.

The null hypothesis tested is that there is no difference in smear layer removing ability among 17% EDTA, Q-MIX, and phytic acid.

Materials and Methods

Preparation of tooth root model

Thirty intact human single-rooted permanent mandibular premolar teeth having a single canal and fully developed apices, indicated for extraction due to orthodontic/periodontal reasons were selected for the study. The extracted teeth were scaled to remove debris, calculus, and rinsed with sodium hypochlorite to remove organic tissue and then stored in distilled water. A carbide disc was used to remove the clinical crown of each tooth and to standardize the root length at 13 mm. Subsequently, #10 K-file (Mani Inc., Japan) was inserted beyond the apex to confirm patency; 1 mm was subtracted from this length to establish the length to which the canals would be instrumented. The canals were enlarged, and a glide path established with hand instruments to a size #15 K-file (Mani Inc., Japan). In the presence of 3% NaOCl (Vishal Dental Products, India), nickel-titanium universal rotary pro Taper was used to shape the canal to an apical size of 25/0.06 (F2) (Dentsply Maillefer, Switzerland). Instruments were rotated at 350 rpm and allowed to progress without applying apical pressure to the established length, which was 1 mm short of the apex. Between each file, the canals were rinsed with 0.2 ml of 3% sodium hypochlorite and normal saline (Baxter Pvt. Limited, India) using 30-G side vented needle (Max-I-Probe™, Dentsply, New Delhi, India).

Irrigation groups

Following final instrumentation and rinsing with NaOCl, the canals were irrigated with 3 mL of distilled water. The specimens were divided randomly into three groups of ten teeth each and final irrigation protocol was followed Group 1 (n = 10): with 1 ml/min of 17% EDTA (Prime Dental Products, India), Group 2 (n = 10): with 1 ml/min of QMIX™ 2 in 1 (Dentsply Tulsa Dental Specialities, Tulsa, OK, USA), Group 3 (n = 10): with 1 ml/min of phytic acid[8] (Tokyo Chemical Industry Co., Ltd, New Delhi, India). To ensure adequate and even distribution of the solutions, the roots were irrigated with 30-gauge side vented needle with an apical-coronal motion to within 1 mm of working length. Finally, the root canals were rinsed thoroughly with 5 ml sterile distilled water to remove any excess solution and dried using sterile absorbent paper points.

Scanning electron microscopy

For scanning electron microscopy (SEM) analysis, two longitudinal grooves were prepared on the buccal and lingual surfaces of each root using a diamond disc, avoiding penetration into the canal. The roots were split into two halves with a chisel and hammer in corono-apical axis and were coded according to groups. Specimens were then dried with ascending concentrations of ethanol (25%, 50%, and 75% for 20 min, 95% for 30 min and 100% for 60 min). The specimens were then dried overnight inside a covered glass vial and then sputter-coated with gold and observed under SEM. After general evaluation of the canal wall, three SEM photomicrographs were taken at magnification of ×1000 for the evaluation of smear layer at the center of the coronal, middle, and apical thirds of each specimen [Figures 1-3]. Three calibrated examiners viewed the SEM photomicrographs, analyzed independently and in a blind manner, to grade the removal of smear layer from root canal walls using the 5-point scoring system by Hülsmann *et al*.[9] and the results were tabulated.

- Score 1: No smear layer and all dentinal tubules open
- Score 2: A small amount of smear layer and some dentinal tubules open
- Score 3: Homogenous smear layer covering the root canal wall and only a few dentinal tubules open
- Score 4: Complete root canal wall covered by homogenous smear layer, no open dentinal tubules
- Score 5: Heavy, nonhomogenous smear layer covering the complete root canal wall.

The collected data were subjected to two-way analysis of variance to compare the means between the groups at three levels. Tukey's *post hoc* test was performed to find the interrelationship between different groups at significance level $\alpha = 0.05$.

Results

The statistical parameters: mean, standard deviation along with median of smear layer removal scores were

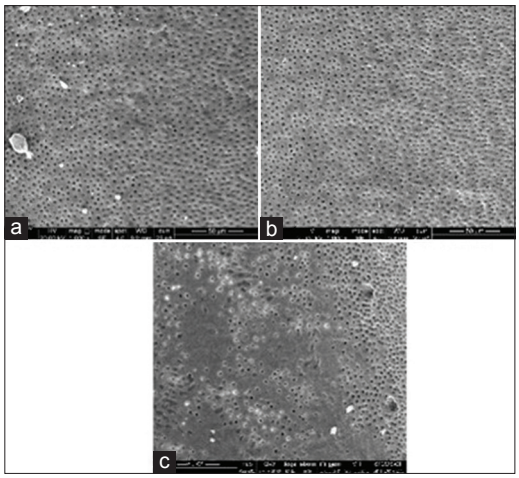

Figure 1: (a) SEM image of 17% EDTA at coronal third of root section. (b) SEM image of 17% EDTA at middle third of root section. (c) SEM image of 17% EDTA at apical third of root section. SEM: Scanning electron microscope; EDTA: Ethylenediaminetetraacetic acid

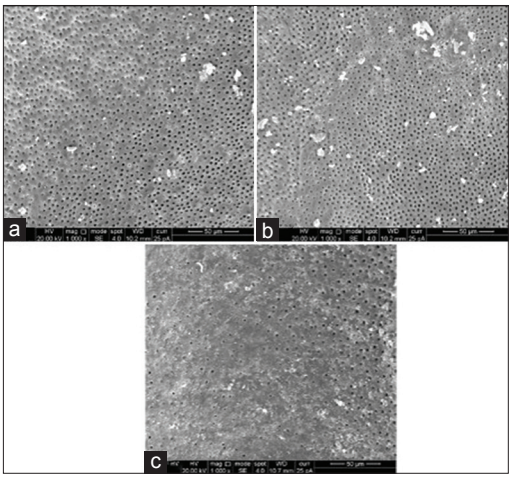

Figure 2: (a) SEM image of Q-MIX at coronal third of root section. (b) SEM image of Q-MIX at middle third of root section. (c) SEM image of Q-MIX at apical third of root section. SEM: Scanning electron microscope

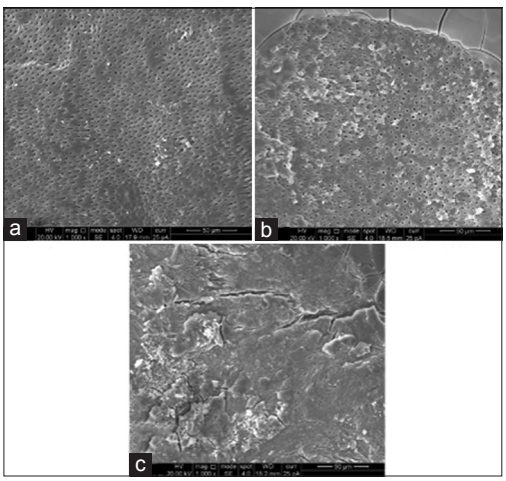

Figure 3: (a) SEM image of phytic acid at coronal third of root section. (b) SEM image of phytic acid at middle third of root section. (c) SEM image of phytic acid at apical third of root section. SEM: Scanning electron microscope

obtained for each group is shown in Table 1. The mean for 17% EDTA at coronal third of section was the lowest while that of phytic acid at the apical third was the highest.

Two-way ANOVA revealed a significant effect between the groups ($F_{2, 81} = 8.85$, $P < 0.00$) between the sections ($F_{2, 81} = 26.09$, $P < 0.00$) and a nonsignificant interaction for groups and sections ($F_{4, 81} = 1.05$, $P < 0.39$).

In Table 2, the *post hoc* test (Tukey HSD) for the groups revealed a significant difference between 17% EDTA and phytic acid ($P < 0.00$) whereas it was nonsignificant for 17% EDTA versus QMIX and QMIX versus phytic acid both ($P > 0.05$). On sectional level, there is a significant difference between coronal versus apical and middle versus apical ($P < 0.00$) and no difference between middle versus coronal ($P = 0.25$).

Bar chart shows the means and 95% confidence interval of smear layer removing score of 17% EDTA, Q-MIX, and phytic acid in coronal, middle, and apical sections of root canal wall [Figure 4].

Discussion

Chelating agents are the essential part of root canal treatment. Smear layer consist of dentin chips, necrotic tissue, including leftovers of odontoblastic procedures, pulp tissue, and micro-organisms. Smear layer acts as barrier and hinders the penetration of irrigants and root canal sealer within the dentinal tubules.[10] Thus, choice of irrigants should also be based on its smear layer removing ability.

Although NaOCl is the most commonly used irrigant in endodontic treatment which is known to dissolve organic

Table 1: Smear layer removal score of the groups at different levels of root canal in terms of mean±standard deviation (median)

Group (*n*=10)	Coronal	Middle	Apical
17% EDTA	1.57±0.28 (1.50)	1.60±0.54 (1.67)	2.70±0.60 (2.83)
Q-MIX	1.80±0.85 (1.50)	2.23±0.82 (1.67)	2.93±0.47 (3.00)
Phytic acid	2.00±0.85 (1.83)	2.50±1.29 (2.00)	3.90±0.89 (3.83)

SD: Standard deviation; EDTA: Ethylenediaminetetraacetic acid

Table 2: Pairwise comparison between the irrigants and sections using Tukey's *post hoc* test

Group	*P*
17% EDTA - phytic acid	0.0002***
QMIX - 17% EDTA	0.1681
QMIX - phytic acid	0.0518
Coronal - apical	0.000000***
Middle - apical	0.000002***
Middle - coronal	0.251897

Significance level used: $P<0.05$. ***Highly significant.
EDTA: Ethylenediaminetetraacetic acid

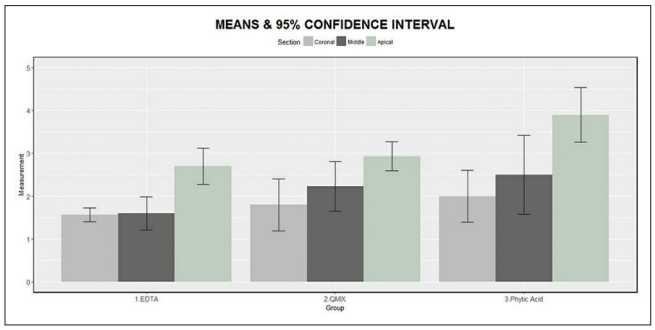

Figure 4: Bar chart showing means and 95% confidence interval of smear layer removing score of 3 irrigants in coronal, middle, and apical sections of the root canal wall

tissue, it does not have the ability to remove inorganic part of smear layer. Therefore, other irrigants were introduced.[3]

In this present study, 17% EDTA which is considered as gold standard in elimination of smear layer is used. This ability is due to the property of ionized EDTA to chelate calcium ions present in the dentin.[8] pH of 17% EDTA is on an average 7.3,[11] in which it is effective. But since, it has its own particular disadvantages – cytotoxicity (Koulaouzidou *et al*. 1999) and it inhibits the macrophage function, thus altering the inflammatory response in periapical lesions (Segura *et al*. 1997),[12] search for more biocompatible irrigants than EDTA, which has less severe impact on periapical tissues continues.[10] EDTA also lacks antimicrobial activity.[4]

QMIX is also a newer irrigant which was used in this study. It chelates the Ca^{+} ions from the dentin due to the presence of EDTA in it; it also contains chlorhexidine which gives it an antibacterial activity along with surfactant which reduces the surface tension and thus helps in better wetting of the root canal walls. pH is 7.5 and 8.[13] It has also shown the ability to remove the smear layer from the root canal walls from the previous studies.[5,6,10,14] Furthermore, QMix does not interact with remnant NaOCl to generate a precipitate if used as directed for the final rinse [Internal data on record with DENTSPLY Tulsa Dental Specialties, Tulsa, OK, USA] and its ability to penetrate into patent, smear-plug free dentin to kill bacteria present has been demonstrated using a novel model with potential significant clinical outcomes and implications.[6]

Another newer chelating agent which was used in this study was phytic acid. Nassar *et al*.[7,8] found phytic acid to be more effective in removing the smear layer from NaOCl-treated flat coronal dentin surfaces and instrumented root canals than EDTA and had no deleterious effect on pulpal cell. The pH of 1% phytic acid solution was around 1.2, which may be the reason for better calcium ion extraction. Nikhil *et al*.[15] evaluated in his study that phytic acid has the ability to reduce the dentin microhardness which was same as chitosan but lesser than EDTA.

In this study, the effectiveness of smear layer removal with the respective final irrigant is that there was statistically significant difference between Group 1 (17% EDTA) and Group 3 (phytic acid). The results shown are not in agreement with Nasser *et al*. study. This may be due to the pH of phytic acid in that study, which was 1.2, and this acidity, along with chelation ability, led to effective smear layer removal and Ca++ extraction. There was no statistical difference between Group 1 (17% EDTA) and Group 2 (Q-MIX) which was in agreement with Stojicic *et al*. study[5] and also between Group 2 (Q-MIX) and Group 3 (phytic acid).

The smear layer removal in the apical third of the canals was the least, followed by middle third, and good results were observed in the coronal thirds of the canals. The reason attributed for this would be the lesser number of dentinal tubules at the apical third, Paque *et al*. reported that dentin in the apical third of the root canal is sclerosed.[16] Thus, irrigants may not show much pronounced effect as seen in the coronal thirds of the canals as in agreement with the earlier studies.

Although phytic acid showed the least smear layer removal, more studies are required to see the effect of phytic acid in the presence of pH 1.2.

Conclusion

In this study, 17% EDTA showed promising results in coronal and middle third of root canal followed by QMIX and then phytic acid. However, further studies with larger sample size are required to see the efficacy of phytic acid in clinical scenario.

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Conflicts of interest

There are no conflicts of interest.

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