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Original Article

Comparison of efficacy of 17% ethylenediaminetetraacetic acid, 0.2% chitosan nanoparticles, and QMIX2in1 in smear layer removal at apical third of root canal, using endovac system irrigation system – An *in vitro* scanning electron microscope study

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ABSTRACT

Aims: To compare the smear layer removal efficacy of 17% ethylenediaminetetraacetic acid (EDTA), 0.2% Chitosan nanoparticles, and QMIX 2 in 1 at apical third of root canal system, using Endovac system (Kerr, Switzerland) irrigation system and analyzed with the scanning electron microscope (SEM).

Materials and Methods: Forty-five extracted mandibular single-rooted premolar noncarious human teeth were selected. The samples were randomly divided into three groups: Group 1: Irrigated with 1 ml of 17% EDTA, Group 2: With 0.2% Chitosan nanoparticles, and Group 3: With QMix2in1 (Dentsply Sirona, USA); 15 teeth in each group as final irrigant. The root canals were sequentially cleaned and shaped till 0.30 mm/0.09 taper and were irrigated with 1 ml of 5% sodium hypochlorite and 1 ml of 0.9% saline, after introducing each file into the canal. Endovac system (Kerr, Switzerland) system was used as delivering unit for all irrigation solutions with separate syringes. The tooth samples were sectioned and analyzed under SEM. The data obtained were analyzed using the Chi–square test.

Results: All three irrigation solution in Group 1, Group 2, and Group 3 removed smear layer. Group 3 showed a significant difference in smear layer removal from the root canal system than Group 1 and Group 2.

Conclusions: The final irrigation with QMix2in1 (Dentsply Sirona, USA) solution aids in better smear layer removal at the apical third of the root canal system, using Endovac system (Kerr, Switzerland) irrigation system.

Keywords: Apical third, bridge model, chitosan, ethylenediaminetetraacetic acid, endo vac system, irrigation device, irrigation solution, nanoparticles, sodium hypochlorite, negative pressure, pendant model, pressure altering device, qmix, root apex, scanning electron microscope, smear layer, smear plug, sodium hypochlorite

INTRODUCTION

The main objective of root canal treatment is shaping and cleaning of the root canal system, followed by

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three-dimensional obturation to prevent reinfection or any other pathophysiological problems.[1] Shaping and cleaning of root canals using hand and rotary instruments to cut the dentinal walls, leads to shattering of mineralized tissues rather shredding or cleaving, producing a considerable amount of debris on the root canal surface. The debris consists of organic and inorganic contents, mostly collagen matrix, as well as microbial contents. This layer of debris spread over the instrumented root canal surface is termed as smear layer.[2] Smear layer is a loosely adherent structure that can lead to leakage and harbor's bacteria which may raise pathological concerns.[3,4] Furthermore, the penetration of intra-canal disinfectant such as sodium hypochlorite (NaOCl) or other intracanal medicaments is restricted into the dentinal tubules.[3] It also prevents the complete adaptation of obturating material to the dentinal walls. Hence, the smear layer itself may be infected or be a hindrance to the obturating materials. Therefore, the removal of smear layer has been advised using various irrigation activation techniques and irrigants.

Over many decades, there has been a search for an ideal irrigant that is capable of dissolving both the organic and inorganic portions of dentin and also effectively clear the smear layer and debris till the apex of the tooth. The Endovac system (Kerr, Switzerland) system (Sybron-Endo, Kavo Kerr Switzerland) is a pressure altering device, which efficiently pulls the irrigation solution toward the apex and removes the debris from the root canal system. It consists of a multiport adapter, a master delivering tip and a macrocannula and microcannula. The multiadapter port is attached to a hi-vac suction of the operating dental chair. The master delivering tip aids in delivering the irrigation solution into the pulp chamber and a small suction tube is attached along with it which maintains the solution levels. The macrocannula removes the gross debris, whereas the microcannula removes the micro-debris. The micro cannula is a 28G needle with 12 laser-drilled microholes, each with less than 100 microns in size at the end of the needle. The micocannula is placed 2–3 mm short of the working length. The irrigant along with the debris is pulled through the microholes creating a vortex like cleaning at the root apical third. Clogging of micro cannula often encountered.[5]

Different irrigation solutions were analyzed for their smear layer removal efficacy of the instrumented root canal. Ethylenediaminetetraacetic acid (EDTA) is a widely used chelating agent. Seventeen percent of EDTA is found to be efficient in the removal of smear layer.[6]

Chitosan which has gained interest by researchers in the recent past for its drug-delivering capacity, and antimicrobial[7] efficacy has also shown chelating[8] effects. Chitosan is a linear polysaccharide composed of D-Glucosamine obtained from the shells of the shrimps and other crustaceans.[9] Chitosan acts on the inorganic portion of the smear layer favoring its removal by the formation of complexes with metal ions due to adsorption, ionic exchange, and chelation and is responsible for elimination of the dentin calcium ions. It is found that chitosan does not alter the dentinal stability and is much lenient toward dentin than 17% EDTA.

QMix2in1 (Dentsply Sirona, USA) (Dentsply Tulsa); an antimicrobial root canal irrigant, that contains a mixture of a bisbiguanide – as an antimicrobial agent, a poly-amino-carboxylic acid as a calcium-chelating agent, saline, and a surfactant have been found to be more effective than BioPure MTAD.[10] Scientific literature search show limited evidence for the usage of nanoparticle chitosan as root canal irrigant. Therefore, the aim of the study is to compare the smear layer removal efficacy of 17% ethylenediaminetetraacetic acid, 0.2% Chitosan nanoparticles and QMIX 2in1 at apical third of root canal system, using Endovac system (Kerr, Switzerland) irrigation system and analyzed with scanning electron microscope (SEM).

MATERIALS AND METHODS

Sample teeth selection and preparation

Forty-five extracted mandibular single-rooted premolar noncarious human teeth were selected. The tooth length selected for this study was 21 mm ± 2 mm. Conventional access cavity preparations were done with high-speed nonend cutting small round burs (MANI, Germany). A #10 size K-file (M-access, Dentsply Malliefer) was inserted into the canal till the apex and the working lengths were determined. Coronal enlargements were done with using Protaper Gold orifice shaper (Dentsply Malliefer). The canals were instrumented using ProTaper Gold files (Dentsply Malliefer) from S1 to F3 using X-Smart endomotor (DENTSPLY Malliefer) according to manufacturer's instructions. The apical enlargement was accomplished till 0.30 mm/0.09 taper. For each group, the root canals were irrigated with 1 ml of 5% of NaOCl (Prime) for 1 min and 1 ml 0.9wt% of saline following instrumentation; hence, a total of 8–9 ml of irrigating solution were used for flushing.

De-coronation of teeth was not done as Endovac system (Kerr, Switzerland) irrigation system (Kerr, Switzerland) as this device requires intact pulp chamber to create negative pressure. The minor delivering tip is placed 2–3 mm short of the working length.

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Nano-chitosan powder preparation

Five g of nano-Chitosan powder of particle size 80 nm was prepared and supplied by NANO-SHELL Ltd., Chandigarh according to the following specification. 0.5 g of Chitosan of molecular weight 100,000–300,000 was dissolved in 1000 ml of 2% acetic acid and stirred for 30 min. Subsequently, 100 ml of this solution was added to 40 ml of tripolyphosphate, stirred for 2 h at ambient temperature, and then centrifuged at high speed. Nano-CS was isolated and rinsed with distilled water and freeze dried.[11]

Preparation of nano-chitosan solution

To a stirring of 100 ml distilled water 2 mg of nano chitosan were added. 2% of acetic acid v/v, was then added drop by drop till the powder particle dissolved. A solution of 0.2% chitosan solution was prepared to be used as an irrigant.[8] Fresh solution was prepared and was stored in a glass bottle.

Methodology

The teeth were randomly divided into three groups of 15 teeth each, GROUP 1: 17%EDTA (Smearoff, Deor) (POSITIVE CONTROL) GROUP 2: 0.2%CHITOSAN NANOPARTICLES, GROUP 3: QMix2in1 (Dentsply Sirona, USA) (Dentsply Malliefer, USA), respectively, according to the type of final irrigant used after instrumentation. The mode of irrigation activation employed in all study groups was Endo-Vac system (Kerr, Switzerland). After instrumentation, the canals were flushed with 5–6 ml of NaOCl and saline. Moreover, for final flushing, 1 ml of 17% EDTA (Smearoff, Deor) in group 1, 0.2% chitosan nanoparticles in Group 2 and QMix2in1 (Dentsply Sirona, USA) in Group 3 each for 1 min, by Endovac system (Kerr, Switzerland) irrigation system.

Scanning electron microscopy evaluation

After instrumentation, the teeth were sectioned buccolingually using a water-cooled diamond disc. They were then coated with gold and examined under SEM (JEOL-JSM-IT 200 with EDS, USA). A magnification of ×1000 was used to evaluate the teeth samples. SEM images were analyzed using following modified Takeda scoring criteria: [12]

- Score 1: No smear layer, with the tubules cleaned and opened
- Score2: Few areas covered by smear layer, with most tubules cleaned and opened
- Score 3: Smear layer covering almost all the surface, with few tubules opened
- Score 4: Smear layer covering all surfaces.

To eliminate the bias, smear evaluation of samples was performed by a blinded resident endodontic observer.

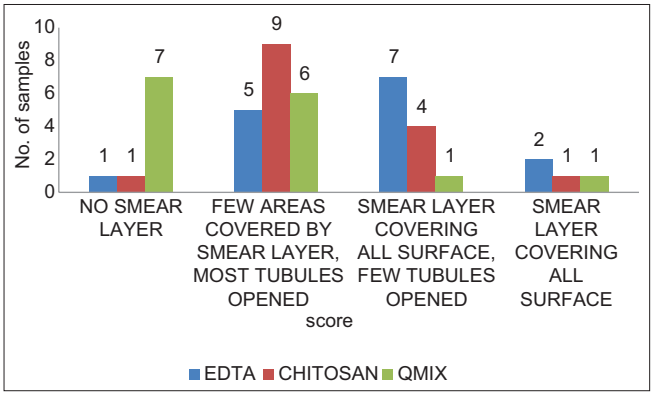

Figure 1: Graph depicting the distribution of samples based on the removal of smear layer while using three different irrigation systems

Statistical analysis

The data obtained were analyzed using SPSS V.20 (Armonk, NY, USA: IBM Corp) software. The Chi–square test was the test of significance used and significance was set at $P \leq 0.05$.

RESULTS

The results for the smear layer removal efficacy of the irrigant solutions are shown in Table 1. The results were tabulated in accordance with the SEM analysis using modified Takedas[12] scoring system. A statistically significant difference between the three groups was observed. Group 3 Qmix 2in1 exhibited significantly higher values than the other groups for smear layer removal efficacy. A significant P value of 0.026 with respect to removal of smear layer was observed.

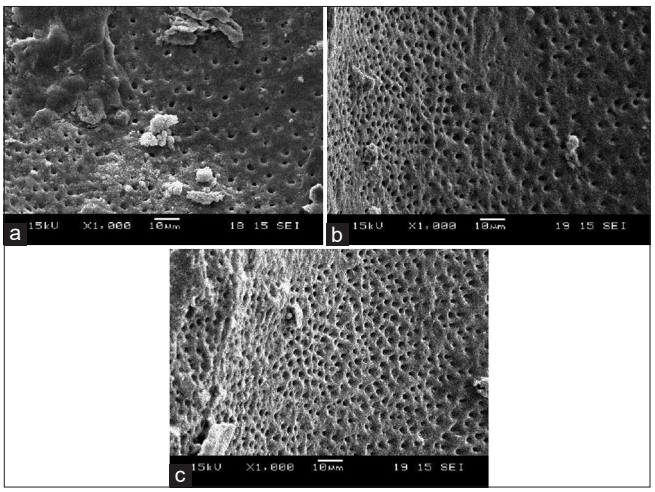

Figure 2: Scanning electron microscope images of three different groups for smear layer removal at the apical third of root canal are given in (a) Group 1: 17% ethylenediaminetetraacetic acid shows minimal opened dentinal tubules and presence of debris in (b) Group 2: Chitosan nanoparticles and (c) Group 3: QMIX2in1 shows opened dentinal tubules and negligible debris

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The descriptive statistical analysis is tabulated in Table 1. In Group 1, median was calculated as 3.00 with a standard deviation of 0.81650. Similarly, in Group 2, median was tabulated as 2.00 and with a standard deviation of 0.72735. In Group 3, median was tabulated as 2.00 and with a standard deviation of 0.88372. The frequency distribution [Table 2] shows that in Group 1 (17% EDTA), one sample recorded a score of 1 and five samples had a score of 2 and seven samples recorded a score of 3 and two samples recorded a score of 4. In Group 2 (0.2% Chitosan Np), one sample recorded a score of 1 and 9 samples had a score of 2 and four samples recorded a score of 3 and one sample recorded a score of 4. In Group 3 (Qmix2in1), seven samples had a score of 1 and six samples had a score of 2; and for score 3 and 4 had one sample each. On a cumulative basis, the scores can be concluded as group 3> group 2> group 1. Both Group 2 and Group 3 had better results on smear layer removal than Group 1. A graph depicting the distribution of samples based on the removal of smear layer while using three different irrigation systems is given in Figure 1. The SEM images of the three different groups are shown in Figure 2.

DISCUSSION

Root canal treatment focuses on thorough shaping and cleaning the root canals of the teeth. To achieve this goal over the years, lot of importance was focused on instruments and instrument design. However, instrumentation of root canal produces smear layer which adheres to the canal walls. The extension of this layer into dentinal tubules to a depth of 40 microns, often known as smear plug is also evident.[13] Various irrigation, methods, and systems were introduced in the recent past to remove the smear layer. An ideal irrigant, must flush out debris, dissolve tissue, as well as disinfect the root canal system,[14] and toxic free. It is important that the irrigants must contact and flow along the canal wall surfaces for effective action, especially in the apical part of root canals because of the typically challenging complexity of the root canal morphology.[15] This can be achieved either by activation of the irrigant or by means of delivering system. An effective irrigation delivery system is required for the irrigants to reach the working length. Such a delivery system should have adequate flow and volume of irrigant to the working length to be effective in debriding the root canal system.

Table 1: Descriptive statistical analysis

Descriptive statistics	Value
Group 1	
Median	3.0000
Mode	3.00
SD	0.81650
Group 2	
Median	2.0000
Mode	2.00
SD	0.72375
Group 3	
Median	2.0000
Mode	1.00
SD	0.88372

SD: Standard deviation

Another challenge in the irrigation process is the presence of vapor lock in a closed-canal system,[16] often encountered with NaOCl, may affect the efficacy of smear layer removal from the apical third of the canal wall. This efficacy problem is more dependent on the ability of flow of the irrigants and the manner in which they are agitated rather than on the aggressiveness or concentration of the irrigant solutions. The Endovac system (Kerr, Switzerland) is one such device that meets up with the above-mentioned issues for resolving the flow of the irrigants. It is a pressure altering device, which efficiently pulls the irrigation solution toward the apex and removes the debris from the root canal system. Nielsen and Craig Baumgartner[17] found that Endovac system (Kerr, Switzerland) showed significantly better debridement at 1 mm from working length compared with needle irrigation.

Seventeen percent EDTA is the most commonly used chelating solution in smear layer removal. It reacts with the calcium ions in the dentine and forms soluble calcium chelates.[18] It has been reported that EDTA decalcified dentine to a depth of 20–30 microns in 5 min (Von der Fehr and Nygaard-Ostby 1963). However, Fraser stated that the chelating effect was almost negligible in the apical third of root canals.[19] It was recommended that a higher concentration solution and the prolonged contact time in the root canal may increase the

Table 2: Distribution of samples between three groups based on scanning electron microscope scores

	SEM score				*P*
	Score 1: No smear layer	Score 2: Few areas covered by smear layer, most tubules opened	Score 3: Smear layer covering all surface, few tubules opened	Score 4: Smear layer covering all surface	
EDTA	1	5	7	2	0.026*
Chitosan	1	9	4	1	
QMix	7	6	1	1	
Total	9	20	12	4	

*$P<0.05$, EDTA: *Ethylene-di-amine-tetra-acetic acid*, SEM: Scanning electron microscope

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cleaning and eventually leading to increased de-mineralizing properties in the dentine.[20,21] Calt and Serper suggested that the application of EDTA should not be prolonged to more than 1 min during endodontic treatment.[21] EDTA efficiently removes the inorganic debris; on the other hand, NaOCl removes organic debris. Final rinsing with EDTA, which has potential of removing the smear layer, did not produce the expected smear free surfaces in the apical region of root canal using conventional methods.[18] In the present investigation, SEM images [Figure 2a] demonstrate that Group 1 had lowest score on smear layer removal at the apical third of root canal in agreement with previous reports.

Recently, various authors have reported about the smear layer removal efficacy of chitosan nano-particles and its leniency toward the dentin stability. Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its bio-compatibility, bio-degradation, bio-adhesion, and lack of toxicity.[22] Alkaline chitosan is obtained by the deacetylation of chitin, which is found in crab and shrimp shells (Kurita 1998) and has become ecologically interesting for various applications because of its abundance in nature and low production cost.[9,18]

Chitosan has high chelating ability for various metal ions in acidic conditions and also has antimicrobial efficiency,[7] as well as osteoinductive properties. Two models proposed the chelation mechanism of chitosan: (1) the bridge model[23] and (2) the pendant model.[24] The former suggests that 2 or more amino groups of chitosan binds with the metal/calcium ion. The pendant model proposes that one amino group is utilized in the binding, while the other amino groups are linked to the metal/calcium ions like a pendant which results in the breaking of inorganic component in the smear layer, thus leading to weaken the smear layer. Furthermore, Chitosan induces the remineralization of exposed and demineralized dentine as the functional phosphate groups binds to calcium ions to form a layer of calcium phosphate. Chitosan also enhances the dentinal surface to resist degradation due to collagenases.

Very limited scientific evidence is available for the potency of 0.2% nanoparticled chitosan as root canal irrigant and its ability to remove the smear layer in the endovac system.

Dentsply Sirona USA introduced QMix2in1 (Dentsply Sirona, USA), which is an experimental antimicrobial root canal irrigant which was found to be better than MTAD BioPure.[10] QMix2in1 contains a biguanide, a surfactant and a chelator. The manufacturer claims that the irrigant solution do not precipitate considering the components. Dai *et al*.[8] reported in his study that teeth samples treated with QMix2in1 had more opened dentinal tubules than teeth samples treated with 17% EDTA. Elnaghy[25] also similar results that QMix2in1 removed the smear layer more efficiently than 17% EDTA and 2% Chlorhexidine with EDTA, based on the number of fully opened dentinal tubules and the efficacy of debris removal along the entire postspace. SEM images and analysis show that Group 3 [Figure 2c] had better score on smear layer removal followed by Group 2 [Figure 2] agreeing with earlier studies.

Our study also validates the results of studies conducted by several authors, on the smear layer removal efficacy of chitosan. Silva *et al*.[26] concluded that 15% EDTA, 0.2% chitosan, and 10% citric acid effectively removed smear layer from the middle and apical thirds of the root canal. In yet another study, Silva *et al*. concluded that irrigation of 0.2% chitosan for 3 min removed the smear layer adequately and caused less erosion than EDTA.[8] Darrag *et al*.[6] in their study concluded that 0.2%chitosan np solution was more efficient in smear layer removal than 17% EDTA, 10% citric acid and MTAD when used as final irrigating solution. In our study, Group 2 does show effective removal, but on comparative basis, it had low values than Group 3. *The difference in the results* may be because the chitosan nanoparticles were manually prepared and more research and standardization on the specification of the material as a chelating agent is required. Our study henceforth validates the previous studies with QMix2in1 and 0.2% chitosan nanoparticles showing high efficacy in smear layer removal at the apical third than 17% EDTA when used in conjunction with Endovac system irrigation system.

Within the limitations of this present investigation, it is observed that nano particled 0.2% chitosan show promise in removal of smear layer when employed with endovac system. Further, studies could be designed to evaluate this novel material for smear layer removal with other activated irrigation devices. Furthermore, further critical clinical *in vivo* interpretation for root canal and periapical healing can be studied to evaluate the use of this novel material as a root canal irrigant, (as these nano particles induces osteocytes and fibroblast).[22] On the other hand, Qmix 2in1 due to the combined chelation effect of bis biguanide and poly-amino-carboxylic acid with the activated penetration of surfactant has better action on the root canal walls in effectively removing the smear layer induced by the instrumentation at the apical third of root canal.

CONCLUSIONS

0.2% Chitosan nanoparticles delivered with enodvac system has scope for potential use in root canal irrigation in

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efficient removal of smear layer. The final irrigation with QMix2in1 (Dentsply Sirona, USA) solution aids in better smear layer removal at the apical third of the root canal system, using Endovac system (Kerr, Switzerland) irrigation system than 17% EDTA and 0.2% chitosan nanoparticles.

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Conflicts of interest

There are no conflicts of interest.

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