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Effect of different irrigation regimens on smear layer removal in human root dentin an *in-vitro* study

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ABSTRACT

Introduction: Aim of the study is to compare the efficacy of 5% NaOCl with 17% EDTA, 18% Etidronic Acid, 9% Etidronic Acid and 0.2% Chitosan as different protocols of irrigating solutions for smear layer removal using scanning electron microscope. **Methodology:** Forty extracted human teeth were collected and stored in saline. Single rooted teeth with complete, mature root apices without any anatomic variation having straight patent root canal extracted for periodontal cause, were included in the present study. Teeth where then divided into four groups with 10 teeth in each group. Access opening followed by Bio-mechanical Preparation was done and teeth were irrigated as follows (n=10 per group).Group 1: 5% NaOCl during instrumentation, 17% EDTA after instrumentation (3min),Group 2: 5% NaOCl during instrumentation, 9% Etidronic acid after instrumentation (5min),Group 3: 5% NaOCl during instrumentation, 18% Etidronic acid after instrumentation (3min),Group 4: 5% NaOCl during instrumentation, 0.2% Chitosan solution after instrumentation (5min).After the irrigation of specimens longitudinal sectioning of specimens was done and subsequently smear layer removal ability will be evaluated.**Results:** All irrigants tested, removed smear layer effectively form the apical third. EDTA (Group 1) showed comparatively better results than 9% Etidronic Acid (Group 2), 18% Etidronic Acid (Group 3) and 0.2% Chitosan (Group 4) at the apical third. **Conclusion:** There is no significant difference between 17% EDTA, 9% Etidronic Acid, 18% Etidronic Acid, and 0.2% Chitosan in the ability to remove smear layer.

Keywords: Efficacy, chitosan, bio-mechanical , *in vitro*

Introduction

Thorough debridement of root canals is essential to accomplish successful endodontic treatment. However, it is impossible to create a sterile space in infected root canals with mechanical preparation alone because of the complex anatomy of root canal systems.

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With both current nickel-titanium instrumentation systems and traditional stainless-steel hand instruments almost half of the root canal walls are left unprepared. Therefore, irrigation of root canal is essential as it allows for cleaning beyond the mechanical preparation. Ideally, all microorganisms, necrotic tissue remnants, and the smear layer that is created during bio-mechanical preparation should be removed. Smear layer removal facilitates opening of dentinal tubules for intracanal medication action and allow better adhesion of the root canal filling material. Therefore, endodontic treatment should not be limited to the removal of pulp remnants and the widening of the root canal, but also

focus on smear layer removal [1-4] Various chealting agents have been suggested for smear layer removal like EDTA,citric acid, maleic acid, etidronic acid etc., however most of them were found to reduce the hardness of dentin and weaken it. In the recent years chitosan nanoparticles have also been suggested for irrigation as they have dual benefit of chelating as well as antimicrobial properties.Since there are no studies comparing the smear layer removal ablity of etidronic acid and chitosan the present study was planned. This study was done to evaluate the efficacy of17% EDTA, 18% Etidronate, 9% Etidronate and 0.2% Chitosan in smear layer removal[5-9].

Aim

This present study was conducted to compare the efficacy of 5% NaOCl with 17% EDTA, 18% Etidronic Acid, 9% Etidronic Acid and 0.2% Chitosan as different protocols of irrigating solutions for smear layer removal using scanning electron microscope.

Materials and Methods

Solutions

Solutions of 5%(wt/vol) NaOCl (Prime dental products), 9% and 18% Etidronic acid (Tokyo Chemical Industry Co., Ltd. 6-15-9 Toshima, Kita-Ku, Tokyo, Japan) were prepared using pure chemicals dissolved in deionized water. 0.2 g chitosan oligosaccharide (Sigma Life Science, SIGMA-ALDRICH, Co., USA) was added to 100 mL of 1% Acetic acid solution, and the mixture was stirred until completely dissolved for preparing 0.2% chitosan.The 17% EDTA solution (Dent Wash, Prime Dental Products Pvt. Ltd. E-8, Shree Arihant Compound, Thane – 421302, India) was bought from the local dental dealer. All solutions were stored at room temperature in airtight dark containers between experiments.

Teeth selection and preparation

Forty freshly extracted single rooted human mandibular premolars were collected. Inclusion Criteria were Permanent human single rooted teeth, mature root apices without any anatomic variation, straight patent root canal extracted for periodontal cause, Exclusion Criteria were teeth with caries, cracks, and with root dilacerations. After extraction, teeth were collected and stored at room temperature and used within 1 month. Teeth were decoronted to obtain a uniform length of 17mm for all samples using a diamond disk. Standard access cavities were prepared. The working length was determined with size No #15K stainless steel File (Mani, Inc., Japan).All the samples were instrumented using crown-down technique using Rotary Protaper files (Dentsply). Throughout instrumentation canals were irrigated using 2 ml of 5% NaOCl solution following instrumentation ,teeth were randomly divided into 4 groups with 10 teeth in each group according to the final irrigation protocol. Prepared Samples were divided in to experimental groups.

Experimental Groups

- Group 1: 5% NaOCl during instrumentation, 17% EDTA after instrumentation (3min)
- Group 2: 5% NaOCl during instrumentation, 9% Etidronic acid after instrumentation (5min)
- Group 3: 5% NaOCl during instrumentation, 18% Etidronic acid after instrumentation (3min)
- Group 4: 5% NaOCl during instrumentation, 0.2% Chitosan solution after instrumentation (5min)

Final irrigation was done with 5ml of distilled water for each sample; all root canals were dried with absorbent paper points (Dentsply). Two parallel longitudinal grooves were prepared on the buccal and lingual surfaces of each root using a diamond disc withoutcutting through the root canal. Roots were then split into two halves with a chisel and mallet. For each root, the half containing the most visible part up of the apex was conserved and coded.The coded specimens were then mounted on metallic stubs, gold sputtered and observed apical thirds under SEM for presence or absence of smear layer. After that photomicrographs were taken at x5000magnification at apical third (2 mm to apex) of each specimen.

Scores

Scores were given according to rating system developed by Torabinejad *et al*

- Score 1 = No smear layer: No smear layer was detected on the surface of the root canals and all the tubules were clean and open
- Score 2 = Moderate smear layer: No smear layer was observed on the surface of the root canal, but tubules contained debris
- Score 3 = Heavy smear layer: The smear layer covered the root canal surface and the tubules

Statistical analysis

Analysis was done by using SPSS version 16. A p-value of <0.05 was considered statistically significant. In order to find out any significant difference between the four groups; that is, Group 1, Group 2, Group 3 and Group 4, Kruskal-Wallis ANOVA test was carried out. There was no significant difference in the distribution of scores among the 4 groups (p=0.603)

Results

All irrigants tested, removed smear layer effectively form the apical third. EDTA (Group 1) showed comparatively better results than 9% Etidronic Acid (Group 2), 18% Etidronic Acid (Group 3) and 0.2% Chitosan at the apical third. Intragroup comparison showed no significant difference.

Table 1: Groups and p-values

	Group								p-value
	1		2		3		4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Smear	2.40	.52	2.50	.53	2.50	.53	2.70	.48	0.603; NS

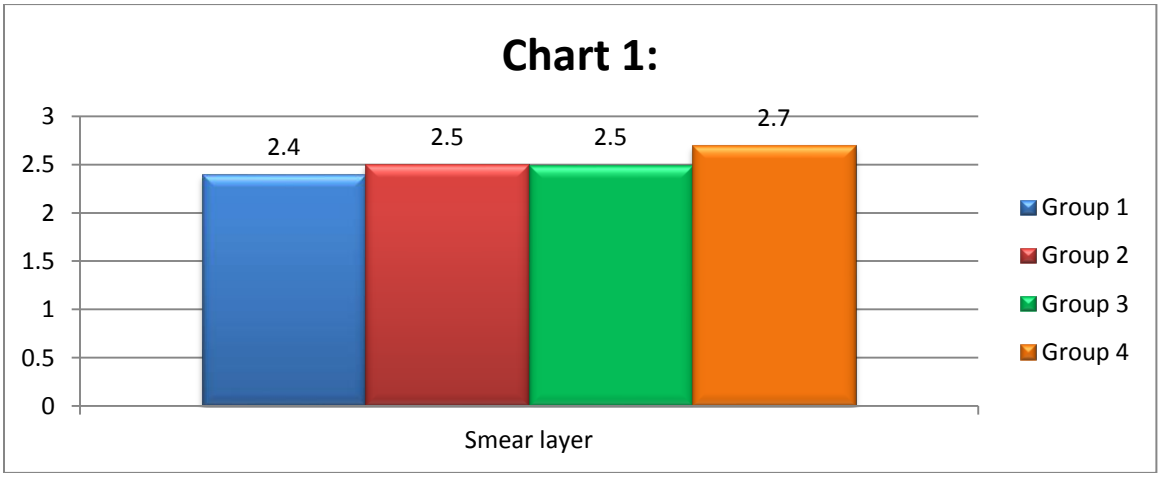


Fig 1: Groups versus smear layer

Discussion

The purpose of irrigating a root canal is twofold, firstly to remove the organic component, the debris originating from pulp tissue and microorganisms, and secondly in removing the inorganic component & the smear layer. Smear layer is composed of a superficial layer that is firmly adhered to the dentine surface, and a deep layer that is formed by smaller particles that are compacted into the dentinal tubules, making the deep layer difficult to remove. The first researchers to describe the smear layer on the surface of instrumented root canals were McComb & Smith. They suggested that the smear layer consisted not only of dentine as in the coronal smear layer, but also the remnants of odontoblastic processes, pulp tissue and bacteria. It has been demonstrated that the smear layer itself may be infected and may protect the bacteria within the dentinal tubules, it may be prudent to remove the smear layer in teeth with infected root canals and allow disinfection of the entire root canal system. The generation of a smear layer is almost inevitable during root canal instrumentation. Whilst a non-instrumentation technique has been described for canal preparation without smear formation, efforts rather focus on methods for its removal, such as chemical means and methods such as ultrasound and hydrodynamic disinfection for its disruption. Root canal preparation without the creation of a smear layer may be possible. A non-instrumental hydrodynamic technique may have future potential and sonically driven polymer instruments with tips of variable diameter are reported to disrupt the smear layer in a technique called hydrodynamic disinfection Current methods of smear layer removal include chemical, ultrasonic and laser techniques, none of which are totally effective or have received universal acceptance.

Irrigating solutions used in endodontics clean the dentin surface, and may interfere with the chemical structure of dentin, changing the calcium/ phosphorus (Ca/P) ratio of the surface.

The irrigation solutions might influence the physicochemical properties of human root canal dentin, including micro-hardness, permeability, solubility, wettability and roughness.

Specifically, when strong chelators are employed to completely remove the smear layer, the decalcification of the root canal wall is a side effect that could have a negative impact on canal sealability.

In this study 17% EDTA, 18% Etidronate, 9% Etidronate and 0.2% Chitosan were used.

Sodium hypochlorite

Sodium hypochlorite solution (NaOCl) is the most commonlyused irrigating agentin biomechanical preparation based on their excellent microbicidal activity and tissue-dissolving capabilities. The use of NaOCl solutions has been suggested as a strategy to remove the exposed collagen matrix in a process called deproteination, which restores the surface characteristics of untreated dentin.

Its capacity to remove smear layer from the instrumented root canal walls has been found to be lacking. Many methods concluded that use of NaOCl during or after instrumentation produces superficially clean canal walls with the smear layer present.

Consequently, it has been recommended that a sodium hypochlorite irrigant be used during instrumentation of the root canal to prolong disinfection and tissue dissolution time, and then a chelator solution be administered to clean the canal system of inorganic debris. Finally, sodium hypochlorite or another antiseptic can be applied to optimize disinfection.

NaOCl only removes the organic structure of the smear layer produced during mechanical instrumentation, therefore combining it with chelating agents is necessary to remove the inorganic phase of this layer.

Advantage that a sodium hypochlorite-etidronic acid combination could be used as a single irrigant during and after instrumentation so that a smear layer is never created. However, the chelating capacity of etidronic acid is relatively weak and it is not known whether its use results in root canals that are as clean as counterparts irrigated with NaOCl followed by EDTA.

Chelating agents

The most common chelating solutions are based on EDTA which reacts with the calcium ions in dentine and forms soluble calcium chelates. It has been reported that EDTA decalcified dentine to a depth of 20–30 lm in 5 min.

Calt and Serper reported that 1 and 10 minutes irrigation using 17% EDTA can efficiently remove smear layer; however, dentin erosion was detected following 10 minutes use of EDTA.In the present study we assessed the efficacy of 17% EDTA for 1 minute and results were in accordance with other studies.

The use of a combination of EDTA and NaOCL is commonly used for the effective removal of the smear layer from the root canal system. However,reports have also indicated that the use of EDTA and NaOCL may lead to dentinal erosion in the root canal wall.

As far as the study is concerned, there is no published study comparing 17% EDTA, 9% Etidronic Acid, 18% Etidronic Acid, and 0.2% Chitosan at the same concentration as there used in the present study.

Both 17% EDTA and 9% Editronic acid were equally effective in the apical third without any much statistical difference in removing smear layer.

There was no significant difference between 17% EDTA and 18% Etidronic acid in smear layer removing ability in the apical third.

Etidronate is a member of the hydroxyethylidene bisphosphonate (HEBP) drug family for prevention of osteoclastic bone resorption in patients suffering from bone diseases such as osteoporosis, Paget`s disease.

Etidronate has been recently suggested as an alternative for other chelators because of fewer adverse effects on dentin structure.

Etidronate can even be mixed with NaOCl without interfering with the antimicrobial property of it. Zehnder *et al*. was the first investigator who used HEBP for SL removal.Irrigation with 2.5% NaOCl resulted in 5.5 ± 3.6 vol% accumulated hard-tissue debris compared with 3.8 ± 1.8 vol% when Etidronic Acid was contained in the irrigant ($P < .05$).Despite Etidronate being a chelator it has fewer adverse effect on dentin structure and when its mixed with NaOCl it does not interfere with its antimicrobial property. Hence we can consider Etidronate as an alternative irrigant. Chitosan is a natural polysaccharide, which has attracted attention in dental research because of its biocompatibility, biodegradability, bioadhesion and lack of toxicity.

17% EDTA and 0.2% Chitosan also showed no significant difference in smear layer removing ability in the apical third of the root.The 0.2% chitosan solution, even in such a low concentration, was able to remove smear layer and provide statistically similar results to those of the solutions with higher concentrations. Chitosan despite having chelating ability it has other advantages like biocompatibility, biodegradability, bioadhesion and excellent antibacterial activity. Hence we can consider 0.2% chitosan as an alternative irrigant. In the current study, all four irrigants were effective in removing smear layer and no significant difference was observed

between experimental groups. Traditionally been inspected using scanning electron microscopy. This method, however, is prone to bias, because it largely compares the amount of open dentinal tubules between groups. Dentine is a heterogeneous structure. In addition, it undergoes changes during ageing. It is possible, that in the studies on root canal smear layer that have been published so far, smear layer may not have been differentiated from sclerotic dentine.

Conclusion

According to the results of the present study there is no significant difference between 17% EDTA, 9% Etidronic Acid, 18% Etidronic Acid, and 0.2% Chitosan in the ability to remove smear layer.

Therefore, 9% Etidronic Acid, 18% Etidronic Acid and 0.2% Chitosan may be an appropriate alternative for EDTA.

All irrigation solutions have their limits and the search for an ideal root canal irrigant should continue.

Fig 1: Single root teeth

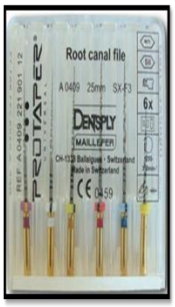


Fig 2: Rotary ProTaper Files

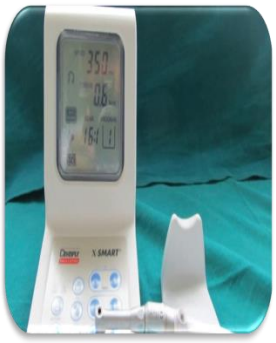
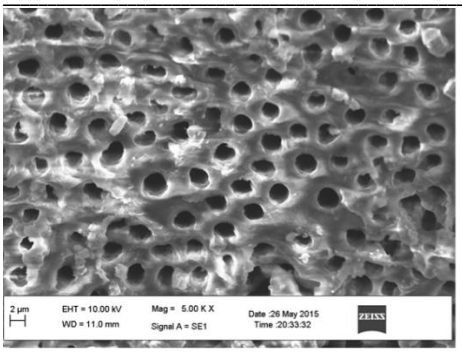


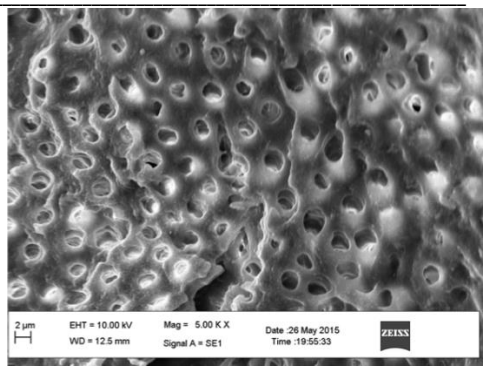
Fig 3: Dentsply X SMART Endo Motor

Fig 4: Gold Sputter

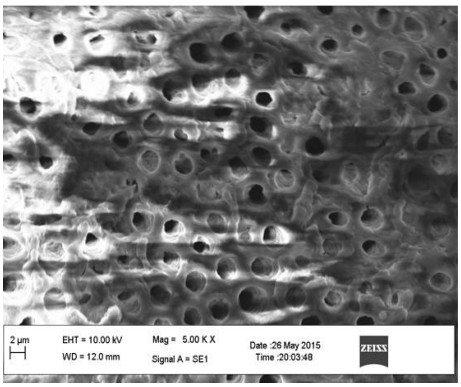
Fig 5: Scanning electron Microscope



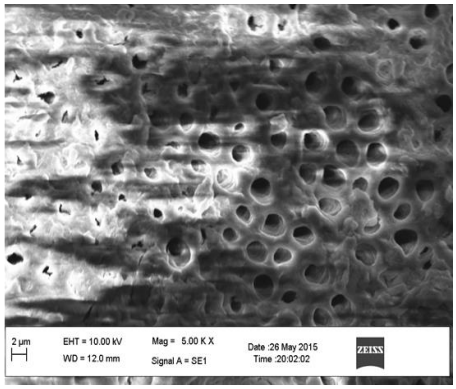
17% EDTA



18% Etidronic Acid



9% Etidronic Acid



0.2% Chitosan

Fig 6: SEM Pictures

The Photomicrographs were taken at x5000 magnification at the apical third (2mm to apex) of each specimen.

Table 2: The Comparison of mean SEM scores was done using Kruskal Wallis ANOVA

	Group								p-value
	1		2		3		4		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Smear	2.40	.52	2.50	.53	2.50	.53	2.70	.48	0.603; NS

The Comparison of mean SEM scores was done using Kruskal Wallis ANOVA in this study.

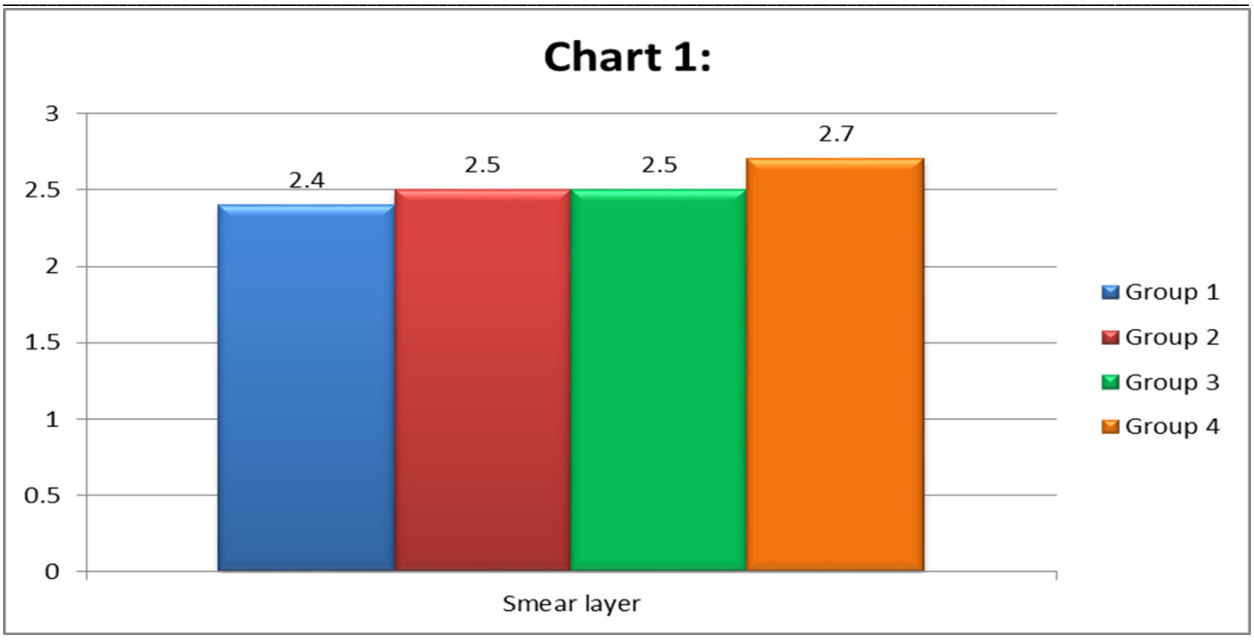


Fig 7:Mean smear layer removal by different irrigants

Group 1: 17% EDTA
Group 2: 9% Etidronic Acid
Group 3: 18% Etidronic Acid
Group 4: 0.2% Chitosan

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