Removal of smear layer by 0.2% chitosan, apple cider vinegar and EDTA solution after root canal instrumentation using SEM: *An in - vitro study.*

Abstract

Introduction: Smear layer removal is mandatory in root canal treatment and this must be carried out before obturation.

Aim: To get a successful result in root canal treatment, there must be thorough debridement with smear layer removal. This study uses different irrigants to evaluate the smear layer removal and evaluation is done using by scanning electron microscopy (SEM),.

Materials and Methods: Forty maxillary human canines were instrumented and the final irrigation was carried out with 0.2% chitosan, apple vinegar and 15% EDTA. The smear layer removal evaluation in middle and apical thirds was done after longitudinal sectioning of the roots. The roots were then examined under SEM. The statistical analysis of the scores obtained after cleaning was done using the Kruskal–Wallis test.

Results: There was statistically significant difference between 0.2% chitosan and the other solutions with regard to smear layer removal.

Conclusion: The smear layer removal from middle thirds was more and less in apical thirds with chitosan (0.2%), EDTA (15%) and apple cider vinegar.

Keywords: Smear layer, Chitosan, EDTA, Apple Cider vinegar, scanning electron microscope.

1. Introduction

A successful endodontic treatment is not based on doing good work alone but also on wide clinical experience, treatment protocols, guidelines recommendations. Also it must be based

on current evidence based dental medicine. All root canal treatments must carry out complete debridement protocol[1]. As the anatomy of the root canal systems is complex, mechanical preparation alone is not sufficient in providing clean root canals [2].

Colonization of microorganisms in the root canal[3] is enhanced by the presence of smear layer. It also impaires the irrigants action[4.] Smear layer also blocks the penetration of sealing cements through the dentinal tubules [5]. For proper removal of debris, it is mandatory to irrigate the root canal as these not only help in killing microorganisms, but also flushing debris, and removal of the organic and inorganic contents [6]. Even though chemical, ultrasonic, and laser techniques are considered the current methods in removing the smear layer, none of it has been really effective individually. So it has not received universal acceptance [7]. The ideal root canal irrigation systems must be non-caustic, non-toxic and low levels of anaphylactic reactions. This was stated by a research that was carried out from major review articles and current evidences [8].

There are many irrigants for smear layer removal but the most widely used is ethylenediaminetetraacetic acid (EDTA). It acts on the inorganic material [9, 10, 11]. EDTA is a biocompatible and unnatural amino acid with a pH 7 that is used as a root canal irrigant in both primary and secondary cases; it has no antibacterial effect. EDTA has the potential to restrain the growth and kill microbes by chelating with metallic ions[12].

As EDTA is considered to be a contaminant and not originate in nature, the search for more biocompatible material continues [11]. Several acids like citric acid and apple cider vinegar, have also been evaluated and these are considered as weak acids[11, 13, 14].

Chitosan is a naturally occuring polysaccharide. It is available in different forms: film, fiber, bead, powder, or as nanoparticles. It is obtained from the shells of crabs and shrimp[15].. It is also the most abundant substance after cellulose[16]. It is biocompatible, bioadhesion, biodegradable and has antimicrobial activity[17], broad spectrum antimicrobial properties. in extreme acidic conditions, chitosan also has high chelating characteristics with metal ions [18]. The antimicrobial properties of chitosan was seen on E. faecalis by combining the properties of chitosan and calcium hydroxide [19] and evidence on the smear removal and conditioner was given by the same study. This was done by combining the property of chitosan-acetate solution, [19].

Applications of chitosan has been seen mainly in the areas of medicine and pharmaceuticals (antibacterial and antitumour agent, drug carrier, wound healing accelerator), biotechnology (enzyme and cell carrier, chromatography resin), environment (water treatment), agriculture (seed preparation), cosmetics and food (iron and calcium absorption accelerator, fibre source) [20]. In dentistry, the antifungal effect against Candida albicans was demonstrated with 2% chitosan gel containing 0.1% chlorhexidine [21]. When used as intracanal medication it has shown to enhance the release of calcium ion when added to calcium hydroxide paste [22].

For a long time, vinegar has been used for infected wound treatments. Due to its medicinal properties it was indicated as an antiseptic agent [23]. Apple cider vinegar has proven antimicrobial action, reduces dentinal microhardness[24] in addition to removing the smear layer [11, 25]. Apple cidar is composed of citric, acetic, lactic, formic, succinic (succinate), and tartaric acids with small quantities of alcohol obtained from the fermentation process. Acetic (5%) and maleic (0.35%) acids showed the highest acid concentrations of the vinegar [26].

Erosive effect of irrigating solutions is not acceptable. It should should eliminate both organic and inorganic portions of the smear layer and have gentle effect on dentin surfaces [27, 28]. This study aims to evaluate using scanning electron microscopy (SEM), the smear layer removal efficacy of Chitosan, 15% EDTA and Apple cider vinegar,. The null hypothesis was that EDTA performed better than the test groups.

2. Materials and Methods

2.1 Sample selection and preparation

Forty maxillary human canines were removed from storage in a 0.1% thymol solution at 9 °C and washed in tap water. Access cavities were created using round burs with a high-speed handpiece under continuous water cooling. LA Axxess® drills (size 45, 0.06 taper; size 35, 0.06 taper and size 20, 0.06 taper; Sybron Endo Corporation, Orange, CA, USA) were used for coronal canal preparation. A K-file (size 10), (Dentsply Maillefer, Ballaigues, Switzerland) was used to determine the working length (WL). The K-file was introduced into the canal in passive motion till the file tip was visible at the apex. From the total length, 1mm was substracted and the remaining length was noted as the desired working length (WL).The anatomical diameter was determined by introducing successively larger K-files to the pre-established WL until resistance was felt upon removal of the file.

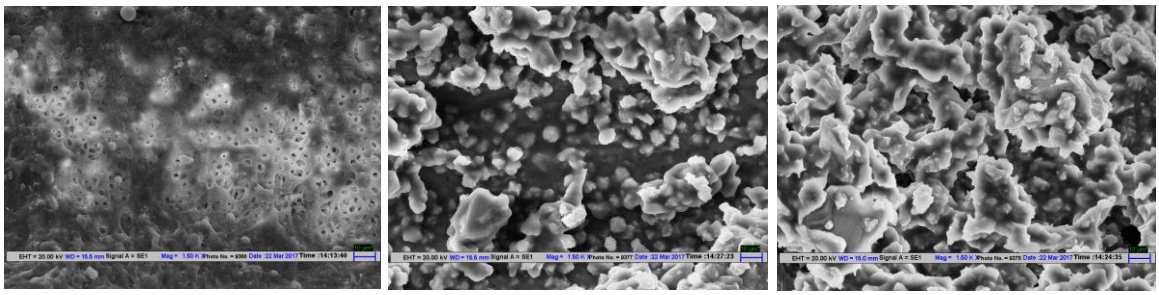
The teeth were prepared upto ProTaper F5 using X-Smart electric motor (Dentsply Maillefer) according to a crown-down technique. After every instrumentation, the canals were irrigated with 1% sodium hypochlorite (1 ml). Deionized water (20 ml) was used to irrigate the canals inorder to remove loose dentine chips. The canals were finally dried with absorbent paper.

2.2 Distribution of teeth and final irrigation

Random distribution of the teeth were done into 4 groups (n = 10), according to the type of final irrigation for smear layer removal: Chitosan (0.2%), EDTA (15%), Apple cider vinegar and control group (without final irrigation).

2.3 SEM Analysis

Two grooves (diametrically opposed) were made in the teeth under cooling using metallic discs. The teeth were then split into halves (vertically) using a bi-bevel chisel. The side with less irregularities, was selected. Each specimen was measured lengthwise with a digital calliper from the apex to the cement-enamel junction for delimitation of the root thirds. Then, starting from the apex, the points corresponding to ½ and⅙ 6of the root length were demarcated to indicate the half of the middle and apical thirds, respectively. These areas were used for the SEM analysis. SEM micrographs (magnification of 1.5Kx) were obtained using scanning electron microscope and examined by three endodontic specialists with respect to the amount of smear layer remaining on the dentine walls. Scores from 1 to 5 were attributed according to the following scoring system modified from Takeda et al. (1998) [29]: (i) smear layer covering the entire surface, (ii) smear layer partially covering the surface and few visible tubules, (iii) about half of the surface with smear layer and half with open tubules, (iv) smear layer covering a small amount of surface; and visible tubules, (v) absence of smear layer on the surface.



1 2 3

Fig 1: SEM Micrographs (original magnification 1.5Kx) of the root canal walls after irrigation. Smear layer removal of a root canal after final irrigation with 1. 0.2% chitosan 2. Apple cider vinegar 3. EDTA (Better Smear layer removal with chitosan than apple vinegar and EDTA.

2.4 Statistical analysis

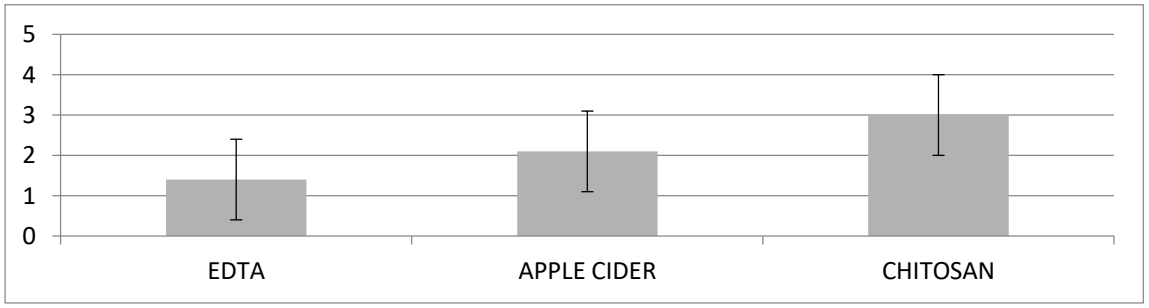
Analysis of the irrigants were done using Kruskal–Wallis test. A significance level of 5% was adopted.

3. Results

The smear layer removal in middle section of the tooth is presented in Table 1 and apical section of the root in Table 2. Limitation of this study, showed a comparability of results in Group I (EDTA), Group II (Apple cider vinegar) and Group III (Chitosan).The highest smear layer removal was observed in Group III(0.2% chitosan).

		N	Mean	±Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
MIDDLE SLA	EDTA	10	1.40	±.516	.163	1.03	1.77	1	2
	APPLE CIDER	10	2.10	±.876	.277	1.47	2.73	1	3
	CHITOSAN	10	3.00	±.943	.298	2.33	3.67	1	4
	Total	30	2.17	±1.020	.186	1.79	2.55	1	4

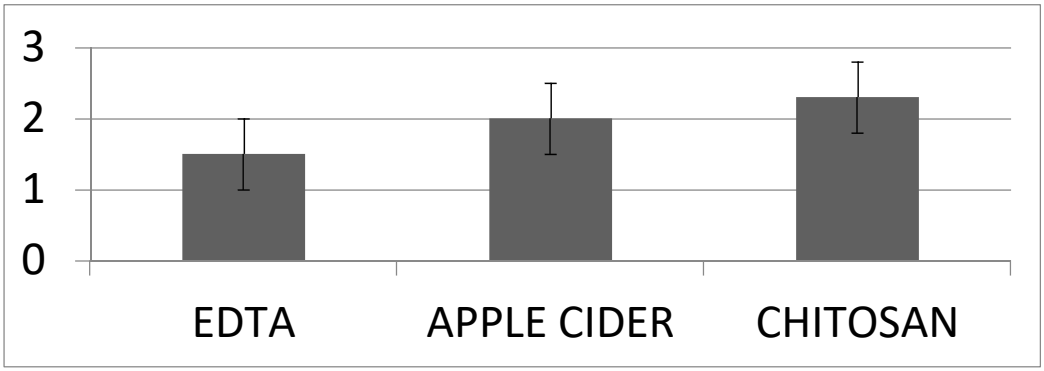
Table 1: Distribution of mean ± S.D. of scores of smear layer removal of three groups in Middle section of root



Graph :Distribution of mean ± S.d. of scores of smear layer removal of three groups in Middle section of root

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
APICAL	EDTA	10	1.50	±.527	.167	1.12	1.88	1	2
	APPLE CIDER	10	2.00	±.816	.258	1.42	2.58	1	3
	CHITOSAN	10	2.30	±.949	.300	1.62	2.98	1	4
	Total	30	1.93	±.828	.151	1.62	2.24	1	4

Table 2: Distribution of mean ± S.D. of scores of smear layer removal of three groups in apical section of root



Graph: Distribution of mean ± S.D. of scores of smear layer removal of three groups in apical section of root

KRUSKAL WALLIS TEST

Mean ranks of smear layer scores at the middle and apical levels of different groups

Ranks

	Group	N	Mean Rank
MIDDLE SLA	EDTA	10	9.10
	APPLE CIDER	10	15.20
	CHITOSAN	10	22.20
	Total	30	
APICAL SLA	EDTA	10	11.25
	APPLE CIDER	10	16.40
	CHITOSAN	10	18.85
	Total	30	

Test Statistics[a,b]

	MIDDLE SLA	APICAL SLA
Chi-Square	12.092	4.440
df	2	2
P value	.002*	.109**

a. Kruskal Wallis Test

*Significant p <0 .05, ** Not significant p > 0.05

Smear layer on the dentinal surfaces were checked by analysing the middle and apical root canal levels between groups. SEM analysis showed that, 0.2% chitosan had better smear layer removal in middle and apical third. This a followed by apple cider vinegar and 15% EDTA. So statistically, the p value in middle third is significant ($p < 0.05$) and in apical third is not significant ($p > 0.05$).

According to the Kruskal Wallis test, there were statistically significant differences between the middle and apical thirds for the comparison of individual specimens within groups.

4. Discussion

An important protocol in root canal therapy is removal of smear layer by the use of irrigation solutions and it must be in accordance with the benefits and consequences to the human beings as per laboratory studies [30]. One of the most commonly used techniques for this purpose is SEM (currently). So, the efficiency of 0.2% chitosan solution to remove the smear layer was evaluated using this technique [31]. SEM analysis showed that 0.2% chitosan even in such a low concentration, was able to remove smear layer as compared to15% EDTA and Apple cider vinegar. The result demonstrated in the present study revealed better smear layer removal in middle third ($p < 0.05$) than apical third ($p > 0.05$). As reported by many studies, the irrigating solutions used were not effective enough, in the apical region[32, 33, 34].

Factors such as pH, application time, concentration and amount of the solution are responsible for the efficiency of a chelating agent [35]. Also, the interrelationship between the the application time and concentration of the chelating agent must be considered since it was found that solutions when applied for a long period in high concentrations, give rise to dentin surface roughness[36]. But in this study, there's no standardization of the concentration of final irrigating solutions and application time. These were chosen according to the manufacturer's instructions and the findings of previous researchers[7, 36, 37, 38].

Da Silva *et al*, study showed the efficacy of EDTA in removing the smear layer. [39] Study by Spanó *et al*.[11] showed highest calcium ions concentration. Their study compared 15% EDTA with other chelating agents using SEM and atomic absorption spectroscopy. They stated that, better smear layer removal was observed with 15% EDTA. According to the study by Gu *et al*.[40] better smear layer removal and dentinal tubule opening was shown by EDTA as compared to NaOCl and NaCl. EDTA does not rely on high hydrogen ion concentration to complete decalcification. At neutral pH, it is very effective as a chelating agent. Also as pH decreases, with time, the efficacy of EDTA also decreases. This is due to the exchange of calcium from dentin by hydrogen [35].

Apple vinegar has therapeutic properties and the presence of malic acid makes it biocompatible [41]. It increases the organism resistance because it is one of the acids of the Kerbs cycle, which is a set of reactions responsible for production of energy in the cells. Due to its high mineral content (magnesium, calcium, phosphorus, sulphur, fluoride potassium, and silicon) it has remarkable medicinal potential [42]. Apple vinegar contains elements like beta-carotene, pectin, enzymes, amino acids and these elements affect the immune system by

attacking free radicals [42,43]. It also has bactericidal activity against E.faecalis and is also biocompatible [43].

As per the study assessed by Spanó et al.[11](2009) smear layer removal with various chelators such as (15% EDTA, 5% acetic acid, 1% NaOCl, apple vinegar, 10% citric acid, 5% malic acid, and 10% sodium citrate) were evaluated using SEM, and hence, concluded that EDTA and citric acid were the most efficient solutions. However, apple vinegar was not used as an irrigating solution in that study but rather as a chelator with 1% NaOCl for 5 min after chemomechanical preparation.

Chitosan (0.2%) removed the smear layer better than all chelating agents in the present study. As per the previous study the most effective combination considered for use on the root dentin was 0.2% chitosan solution applied for 3-5 min [44]. In a similar study, the chitosan properties were closely recognised as a better chelator with enhanced cleansing and chelating capabilities[45].Based on the mechanism involved in chelating with chitosan two theories were hypothesized according to some researchers. The first theory states that the identical metal ion on the chitosan chain is anchored by two or more amino groups [46]. This theory is based on the bridge model whereas, the second theory states that on the chitosan chain only one of the amino groups is bound to the metal ion. Further analysis claimed that ion exchange, chelation, and adsorption are a result of chitosan and metal ion complexes [47]. The pH of the solution, and the type and structure of chitosan interaction is responsible for chelation bond [48]. According to a recent study, chitosan proved to have more conditioning effects on radicular dentin.

At a pH of 3.2, chitosan (0.2%) had shown dentin reducing properties as revealed by some researchers [49]. Therefore, chitosan citrate can be considered an ideal conditioner for radicular dentin[51]. In the present study, the groups irrigated with chitosan showed better efficacy than EDTA and apple vinegar. Of the solutions used in this study, apple vinegar and 15% EDTA had a similar capacity to remove smear layer.

Thus, based on an extensive literature review and a comprehensive overview of conventional dental irrigants, chitosan-citrate can be considered as a new, novel, safe, and effective irrigant and conditioner for radicular dentin in endodontics. As demonstrated in the literature, application of 0.2% chitosan for 3 min. presented better smear layer removal with less erosion than EDTA [47].

So among the solutions used, the most effective for removing the smear layer was 0.2% chitosan for 3 min. Therefore the result suggests 0.2% chitosan to be used as an alternative to EDTA.

5. Conclusion

Within the limitations of this study, effective smear layer removal was shown by 0.2% chitosan, followed by apple cider vinegar and 15% EDTA. Better removal was seen in middle thirds and less in apical thirds of root canals.

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