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Research

A Comparative Evaluation of Smear Layer Removal by Using 0.2% Chitosan, 17% Edta, 10% Citric Acid as Final Root Canal Irrigation: Invitro A Scanning Electron Microscopic Study

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Abstract

Introduction: Mechanical instrumentation produces an irregular amorphous smear layer in the root canal system. Thus, the need to evaluate the irrigating solutions and their effect on smear layer removal.

Aim: The present study aimed to evaluate and compare the efficacy of 0.2% chitosan,17% ethylenediaminetetraacetic acid (EDTA), 10%citric acid in the removal of smear layer from the root canal system.

Materials and methodology: 45 mandibular premolars with single rooted tooth were selected. Crown down technique was used with rotary protaper next instruments and irrigation intermittently with 3% sodium hypochlorite after every instrument used. Depending on the final irrigation solution, the samples were divided randomly into three groups(n-15) group 1-0.2% chitosan, group 2-17% ethylenediaminetetraacetic acid (EDTA), group 3-10% citric acid. Teeth were then decoronated and sectioned longitudinally. Scanning electron microscope examination of canals was done for evaluating smear layer at different levels at X2000.

Result: The present study showed no statistically significant difference between 0.2% chitosan and 17% EDTA for smear layer removal from different tooth levels but there was statistically significant with 10% citric acid.

Conclusion: Final irrigation using 0.2% chitosan remove smear layer similar to 17% edta, 10% citric acid showed least effective in smear layer removal. However, the efficacy of removing the smear layer is greater when a low concentration of 0.2% chitosan was used.

***Keywords:** Smear Layer, Edta, Irrigating Solution, Chitosan, Citric Acid*

Introduction

Success in endodontic therapy depends on three-dimensional cleaning shaping and obturation with an adequate seal of the root canal system. [1]

The action of endodontic instruments on dentinal walls creates an irregular layer of debris on dentinal walls, known as the smear layer. It is an amorphous, irregular structure containing inorganic debris and organic materials such as odontoblastic processes, vital pulp tissue, necrotic debris, bacteria or bacterial products. This prevents the penetration of root canal irrigants, intracanal medicaments, and sealers into the tubules. [2]

Complete debridement of smear layer removal is an asset and could help to achieve a successful root canal treatment outcome. Effective cleaning of the canal system requires the use of various methods to remove the smear layer namely chelation agent, ultrasonic, and laser. [1]

None of the methods remove the smear layer throughout the length of the canal. A combination of methods helps in achieving higher smear layer removal. In common endodontic practice, dual irrigants are often used as an initial and final rinse to overcome the disadvantages of using a single irrigant. [3]

Till date, various chemicals irrigants have been used. These include antimicrobial agents (e.g., NaOCI, Chlorhexidine gluconate, MTAD), Oxidizing agents (e.g.H_2O_2), Chelating agents (e. g., EDTA, HEBP), Acids (citric acid, Maleic acid. etc) and natural agents (e.g., Triphala, chitosan, propolis). Combinations like Qmix, Tetraclean, and Smearclear were used, at present, Sodium hypochlorite is the most popularly used irrigant during root canal preparation, due to its antimicrobial action and ability to dissolve biofilm components and organic remnants but not inorganic components, thus it alone is incapable of removing the smear layer. Therefore, a chelating agent like ethylenediaminetetraacetic acid (EDTA) and chitosan have been suggest for removal of the smear layer during endodontic treatment. [4] [2]

Ethylenediaminetetraacetic acid (EDTA) is an artificial amino acid, biocompatible with pH 7 that is used as the most common chelating agent for root canal irrigants. The main feature of EDTA solution is its ability to chelate with metallic ions required for microbe cell envelope growth, which can eventually lead to bacterial death, even though it has no antibacterial effect. 15-17% EDTA can dissolve calcium from dentine within 5 minutes at approximate depths of 20-30 µm. [5]

Many weak acids, like citric acid found in citrus fruits, have the property of removing calcium ions from dentin, forming calcium citrate. Previous studies reported that 1% and 10% citric acid promoted greater decalcification of dentin than 17% EDTA. [6]

Chitosan is a natural polysaccharide molecule that is derived from the shells of crustaceans mainly shrimp, crabs, and other sea crustaceans obtained by the deacetylation of chitin molecule has got good bio-compatibility, bio-degradability, bio-adhesion, and non-toxicity. It has been tried for a variety of potential pharmacological applications such as antibacterial, anti-oxidant,anti-viral, and anti-inflammatory. [7] [8] [9]

The previous study reported by Del et al and Silva et al. showed that Chitosan has excellent chelation capacity due to its acidic pH, therefore the inorganic parts of smear layers are dissolved. [10] [7] However, literature is so limited on the chelating effective of Chitosan ability to remove the smear layer and calcium ions from dentin

Hence, the present study evaluates and compares the efficiency of 17% EDTA, 0.2%Chitosan, and 10% citric acid as a final irrigant to remove the smear layer after instrumentation using a scanning electron microscope (SEM).

Materials and Method

Forty-five single-rooted permanent mandibular premolars extracted for orthodontic purposes and periodontal reasons with mature root apex, similar anatomic characteristics without any cracks, caries, significant canal curvature, or defects within root portion were selected and confirmation of a single canal was carried out by buccolingual and mesiodistal angulated radiographs.

All samples were stored in 2.5% NaOCl for 15mins to remove organic debris. Surface soft tissue debris was removed and stored in a 10% formalin solution for disinfection, till they were used for the study purpose.

A coronal access cavity was prepared using a BR 41 round bur in a high-speed air turbine handpiece under water spray allowing direct access to canals. The canals were located with the help of a DG-16 endodontic probe.

A 10 K-file size was used to determine the working length by inserted into the canal until it was visible at the root apex and by subtracting 1mm from the length of the tooth. To mimic the clinical condition the apical foramen of each root were closed using the sticky wax and embedded in small plastic containers filled with soft polyvinyl siloxane impression material.

The instrumentation was initiated with hand files upto 20 size k-file placed into the canal. Coronal enlargement was done by one flare (25/0.04), with rotation speed 250-400 rpm, torque 3 N.cm followed by protaper next rotary files upto X 3 ((30/0.07) at 400rpm, torque 1.2Ncm.

Root canals were irrigated with 2ml of 3% NaOCL solution throughout the instrumentation and after using each file. A 27 -gauge side Vent needle was used to remove debris from the coronal and middle one -third and 30 gauge was used to remove debris from the apical one-third.

Finally, root canals were rinsed with 5ml of saline, and according to the final irrigating solution used for smear layer removal sample was randomly divided into three groups (n-15).

Group I–Canals irrigated with 5ml of 0.2% Chitosan for 3mins.

Group II – Canals irrigated with 5ml of 17% EDTA for 3mins.

Group III – Canals irrigated with 5ml of 10 % citric acid for 3mins.

All groups were activated ultrasonically with red (25/04) EndoActivator tip for the 30s and then flushed with 5 ml of distilled water to stop the action of irrigating solutions, dried and root canal orifices were placed with sterilized cotton pellets.

All samples were decoronated using a diamond disc to standardize a root length of 16mm. Longitudinal grooves were prepared in each root on the buccolingual surfaces without perforating the root canals and then split into two halves with a chisel and mallet. For each tooth, one half is selected and stored in a 2.0% glutaraldehyde aqueous solution till the SEM was carried out. Each section was dehydrated in the concentration of alcohol (70-90%) and dried.

The coded samples of each group were mounted on aluminum stubs with carbon tape, sputter-coated with gold to a thickness of 20-30nm, and the coronal, middle and apical parts were examined under SEM at X2000 magnification.

Scanning Electron Microscope Evaluation

The SEM images were scored blindly by two endodontists using qualitative evaluation of the smear removal in the dentinal tubules or surface of the root canal according to criteria by Torabinejad et al [11]:

Score 0 = smear layer totally removed with clean and open all dentinal tubules.

Score 1 = smear layer exists only in the opening of the dentinal tubules.

Score 2 = smear layer covered the root canal surface and opening of dentinal tubules.

Statistical Analysis

Comparing the mean scores of smear layer removal between different areas of root were calculated by one-way analysis followed by intragroup comparison using Kruskal-Wallis test and Mann-Whitney U test for individual group comparisons. P values < 0.05 were considered as statistically significant.

Result

The results of the Kruskal–Wallis test for intragroup comparison shows that there is a significant difference in the removal of smear layer in all section of the root canals of all the groups with p-value <0.001 at coronal third, <0.001 at middle third, and <0.001 at apical third(table 1, graph 1).

Table 1: Descriptive statistics (Mean±S.D) of smear layer removal scores and one-way analysis using the Kruskal-Wallis Test.

Sample	Chitosan	EDTA	Citric Acid	p value	Significance
coronal	1.06±0.25	1.2±0.14	1.81±0.40	<0.001	Significant
Middle	1.6±0.50	1.6±0.50	2.46±0.51	<0.001	Significant
Apical	2.06±0.25	2.33±0.48	2.86±0.35	<0.001	Significant

Mann–Whitney test was done for intergroup comparison of the irrigants for establishing which irrigant has better smear removal ability. In our study, the mean difference for removal of smear layer from the whole root canal between 0.2% chitosan and 17% EDTA came out as not statistically significant (P > 0.05) (TABLE 2) but the statistically significant difference with citric acid (P < 0.05) (table 3, table 4) following order was observed.

Chitosan=EDTA<citric acid

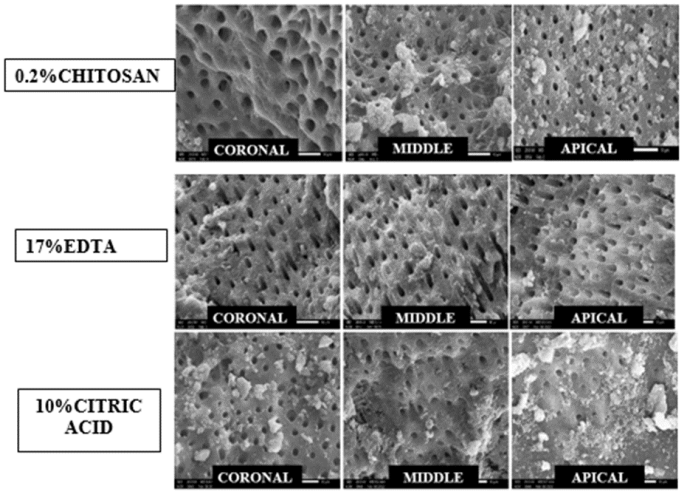

Figure 1: *Illustrates the indicative scanning electron microscopic images of root canal walls treated with the experimental solutions at various thirds of the root canal system.*

Table 2: Mann-Whitney test for intergroup comparison between 0.2%chitosan and ethylenediaminetetraacetic acid.

Group comparison		Mann-Whitney U value	p value	Significance
CORONAL	0.2%CHITOSAN	97.500	0.539	Not significant
	17%EDTA			
MIDDLIE	0.2%CHITOSAN	112.50	0.004	Not Significant
	17%EDTA			
APICAL	0.2%CHITOSAN	82.500	0.217	Not Significant
	17%EDTA			

Table 3: Mann-Whitney test for intergroup comparison between 0.2%chitosan and citric acid.

Group comparison		Mann-Whitney U value	p value	Significance
CORONAL	0.2%CHITOSAN	30.000	<0.001	significant
	10%Citic acid			
MIDDLIE	0.2%CHITOSAN	36.000	<0.001	Significant
	10%Citric acid			
APICAL	0.2%CHITOSAN	22.500	<0.001	Significant
	10%Citric acid			

Table 4: Mann-Whitney test for intergroup comparison ethylenediaminetetraacetic acid and 10% citric acid.

Group comparison		Mann-Whitney U value	p value	Significance
CORONAL	17%EDTA	45.00	0.004	significant
	10%Citic acid			
MIDDLIE	17%EDTA	36.000	<0.001	Significant
	10%Citric acid			
APICAL	17%EDTA	52.500	0.011	Significant
	10%Citric acid			

Discussion

One of the greatest challenges in endodontic therapy is the procedure of rendering a complex root canal system, completely cleaning both organic and inorganic debris, thereby creating a healthy environment for the tooth to achieve maximal healing. [11]

Rotary or hand instrumentation cuts root dentin to produce mineralized tissues that are shattered and ill-defined or cleaved to produce a considerable amount of debris and smear layer. The current trend of routinely using rotary instruments to prepare root canals results in more effective cutting and results in the generation of more volume of smear in all the thirds of the root canal. [12] [13]

NaOCl solution is the most commonly used gold standard in endodontic irrigants. It is known for its antibacterial activity and for its capacity of dissolving organic tissue in the root canal. NaOCl alone did not remove both organic and inorganic solvents of the smear layer and this required the use of chelating agents. [14]

Chelating agents improve the biomechanical debridement during root canal therapy by removing the smear layer as well as demineralizing and softening dentin. Mozayeni et, Isabel Mello et al studies reported the use of chelating solutions as part of the final irrigation regimen to remove the smear layer. [15] [12]

Calt and Serper demonstrated that the efficiency of such chelating agents depends on many factors, such as penetration depth of the material, the root length, and diameter of the canal, application time, pH, and the concentration of materials. [16]

Hence, In this study, we have evaluated and compared the efficacy of three chelating solutions using 0.2%CHITOSAN, 17%EDTA, and 10%CITRIC ACID as a final irrigating agent for 3mins to remove the smear layer from the coronal, middle, and apical section of the root canal. The results obtained in our study are in agreement with previous studies by P. V. Silva [9], A. M. Darrag [5], M. Sarkees[17] A. Del[18] that 0.2% low concentration chitosan and 17%Edta showed had not been the statistically significant difference for smear layer removal in all the position of the root canal system.

The chelating capacity of chitosan on root canal dentin has been assessed by P. V. Silva [7], which showed that a CNPs-based solution for 3 minutes effectively removed the smear layer from the root canals. This mechanism was explained in two theories. The first theory states that chitosan chain binds to the same metallic ion even with different amino acid groups (Chemical bridge model). The second theory stated that "the free-arm model" where only one amino group of the structure of the substance were binding, which forms metal ion "anchored" to the amino group. [7]

Several dimers chitin chain constitutes a chitosan polymer. Like the Edta molecule, the chitin dimer shows pairs of free electrons in 2 nitrogen atoms are validating elements for the metal and chelating agent interaction (ionic action). In an acidic medium, the amino groups present in the bipolymer are protonated, resulting in an overall position charge (-NH3+). This form is responsible for the attraction to other molecules for adsorption to occur. The formation of complexes between Chitosan and metal ions most probably is due to the process of ion exchange, and chelation. [6]

In our study 10%citric acid solution when used showed less smear layer removal when compared to 0.2% Chitosan and17% EDTA where citric acid is a weak acid. The pH of 10% citric acid is 1.8 whereas the pH of 17% EDTA is 7 and chitosan is 7.4. Hence 17% EDTA and chitosan are more acidic and capable of dissolving smear layer more than citric acid [14]. The chelating action of citric acid probably becomes greater as its concentration increases.

Future studies are required to assess the action of chitosan on dentine microhardness, degree of erosion, sealer penetration, and restorative materials, as well as to evaluate the effects on periapical tissues.

Conclusion

Within the limitation of this investigation, the present study concludes that Chitosan exhibits similar smear layer removing efficacy in different regions of the root canal with EDTA in much lower concentration than other irritants. Thus, Chitosan can be used as a final irrigant as an alternative to replace EDTA due to many advantages such as cost-effectiveness, effective chelating action on root dentin, antibacterial activity, healing of wounds, and bioadhesive nature.

Conflict of Interest

The authors declare no conflict of interest.

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