EFFECT OF 0.2 % CHITOSAN IN ENDODONTIC SMEAR LAYER REMOVAL: SEM STUDY

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ABSTRACT

Background and Objectives: to evaluate the efficacy the effect of 0.2% chitosan in endodontic smear layer removal using the scanning electron microscope. Materials and Methods: twenty four single rooted mandibular premolars teeth with a single root canal and a closed apex were randomly divided into two groups of 12 teeth each. Cleaning and shaping of the canals were done using hand and rotary instrumentation up to size 50, 0.06 taper. All the specimens were irrigated using 3% hypochlorite between each file size. Group 1 (G1) specimens were irrigated with final flush of 17 % EDTA. Group 2 (G2) specimens were irrigated with final flush of 0.2% Chitosan. The teeth were sectioned longitudinally and prepared for evaluation by scanning electron microscope. Results: Data obtained from the study was subjected to statistical analysis using Mann Whitney test. 17 % EDTA and 0.02% Chitosan effectively removed smear layer from coronal and middle one third. There were no statistical significant difference in removal of smear layer between G1 and G2. Both the groups failed to remove smear layer completely from apical third. Interpretation and Conclusion: Under the limitations of the present study following conclusions were drawn, chitosan effectively removed smear layer from the coronal and middle third of the root canal in very less concentration. Hence, it can be used as an adjunct during instrumentation in efficient cleansing of the root canal system.

KEYWORDS: Chitosan, rotary instrumentation, sodium hypochlorite, root canal system, scanning electron microscope.
INTRODUCTION
Effective root canal treatment relies upon the root canal system being completely cleaned and disinfected, trailed by the complete obturation of the root canal space. Mechanical instrumentation alone will not completely eradicate bacteria from Root canal. Endodontic Smear layer produced while instrumentation contains tooth structure and some inorganic contents which are non specific.[1] The organic components may comprise of reacted coagulated proteins, necrotic or viable pulp tissue, odontoblastic processes, saliva, blood cells, and microorganisms. Smear plugs created by pushing smear into dentinal tubules up to 40 microns deep, can embalm bacteria and prevent adequate cleaning of the root canal system.[2] The decision to remove the smear layer in endodontic treatment has proponents and detractors. Many in-vitro studies have shown that removal of the smear layer increases dentin permeability and its removal has been the subject of many investigations of how it may affect the root canal seal quality.[3]

Distinctive irrigants have been utilized to uproot the smear layer. Sodium hypochlorite is an irrigant solution used universally in root canal treatment because of its bactericidal properties and capacity of dissolution of organic tissues; however Sodium hypochlorite has not been appeared to be viable in removing the smear layer.

Some irrigants like citric acid, phosphoric acid, maleic acid and EDTA have been accounted for as suitable for clearing the smear layer. While, studies have demonstrated that combined use of NaOCL and EDTA cleared the smear layer only partially.[4]

EDTA is a standout amongst the most broadly utilized irrigant for elimination of endodontic smear layer. It responds with calcium particles presents in dentine results in chelation. It improves dentine decalcification at normal profundities of 20–30 µm in 5 min. Since EDTA has its own particular disadvantages, hunt down for more biocompatible irrigants than EDTA, which is less unsafe impact on periapical tissues proceeds.

Chitosan is a naturally occurring polysaccharide, which has been utilized different field of dental exploration due to its alluring properties like biocompatibility, biodegradability, bio adhesion[4]. It has extraordinary chelating capacity for different metal particles and has been connected widely in distinctive modern purposes. Chitosan is acquired by the deacetylation of chitin, which is found in shells of Crustaceans and has turned out to be biologically convincing for distinctive applications as a result of its bounty in nature and low assembling.
Applications for chitosan are being seen more in the fields of medicine and pharmaceuticals. Chitosan is likewise an antibacterial and antitumour specialists, drug carrier, wound mending quickening agent, protein and cell transporter, chromatography tar, water purifier, iron and calcium retention quickening agent.\textsuperscript{[5]}

**MATERIALS AND METHODS**

Twenty four extracted maxillary and mandibular single-rooted noncarious human teeth were used for this study. Teeth with previous coronal restorations or root canal treatment were excluded. The teeth were randomly divided into 2 groups of 12 teeth each according to the type of irrigants used during and after instrumentation. After preparing a conventional access preparation for each tooth, a K-file (size 10 or 15) was used to determine the working length by penetrating the apical foramen and pulling back into the clinically visible apical foramen. The working length of each tooth was 21 to 25 mm. Each canal was instrumented using a combination of passive step-back and rotary 0.06 taper nickel titanium files. The apical foramen of each tooth was enlarged to a size 30 file. Sterile distilled water was used as an intracanal irrigant in 12 root canals. Irrigants were delivered using a 30-G side-vented needle inserted to 1 mm above the apical seat. To determine the effect of experimental and control solutions as a final rinse on the surface of instrumented root canals, the canals were treated with 5 ml of one of the following solutions 1) 17 % EDTA ,2). 0.2% Chitosan.

The roots were grooved and spited longitudinally into two halves. All specimens were fixed in buffered formalin for 24 hours. The specimens then were dehydrated in a graded series of ethanol solutions, critical point dried, attached to coded stubs, and coated with gold. The specimens were examined under a Scanning electron Microscope for debris and smear layer coverage.

Three photographs filmed at $\times1000$ and $\times2000$ were taken randomly at the coronal, middle, and apical level. Each field was graded from 0 to 3 according to Rome et al\textsuperscript{[6]} as follows.

0 = No smear layer, dentinal tubules open, free of debris.
1 = Root canal surface covered with residue only at the opening of the dentinal tubules.
2 = Root canal surfaces with a thin covering of residue on dentinal tubules with visible tubules only in a few regions.
3 = Heavy smear layer, outlines of dentinal tubules totally covered with smear layer.
RESULTS

Removal of smear layer from the surfaces of root canals revealed the presence of more abundant and larger dentinal tubules in the coronal third of root canals compared with those seen in the middle and apical thirds of the root canal system. The dentinal tubules in the apical third of the canals were smaller and fewer than those observed in the rest of the root canals. In addition, removal of the smear layer showed the presence of many lateral canals in the apical thirds of the root canal systems. There was no statistical significant differences among two groups (Table 3, Table 4). Maximum Smear layer was removed from Coronal third followed by middle third and apical third of the root canal (Graph 5, Graph 6).

Table 3: Mean score for smear layer at coronal third, middle third, and apical third using Mann Whitney test.

<table>
<thead>
<tr>
<th>Sub group</th>
<th>N</th>
<th>Chitosan</th>
<th>EDTA</th>
<th>Mann Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean(SD)</td>
<td>Mean(SD)</td>
<td>Median(Q1-Q3)</td>
</tr>
<tr>
<td>Coronal 3rd</td>
<td>12</td>
<td>0.58(0.51)</td>
<td>0.33(0.49)</td>
<td>1(0-1)</td>
</tr>
<tr>
<td>Middle 3rd</td>
<td>12</td>
<td>1.08(0.66)</td>
<td>0.83(0.57)</td>
<td>1(1-1.75)</td>
</tr>
<tr>
<td>Apical 3rd</td>
<td>12</td>
<td>2.75(0.45)</td>
<td>2.33(0.49)</td>
<td>3(2.25-3)</td>
</tr>
</tbody>
</table>

Table 4: Over all Comparison of scoring of smear layer at coronal third, middle third, and apical third by Chitosan and EDTA.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>N</th>
<th>Median (Q1-Q3)</th>
<th>Friedman Test</th>
<th>Coronal 3rd vs Middle 3rd</th>
<th>Coronal 3rd vs Apical 3rd</th>
<th>Middle 3rd vs Apical 3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chi-Square</td>
<td>z</td>
<td>p-value</td>
<td>z</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Coronal 3rd</td>
<td>1</td>
<td>1(0-1)</td>
<td>22.286</td>
<td>&lt;0.001*</td>
<td>-2.44</td>
<td>.01*</td>
</tr>
<tr>
<td></td>
<td>Middle 3rd</td>
<td>1</td>
<td>1(1-1.75)</td>
<td>21.350</td>
<td>&lt;0.001*</td>
<td>-2.12</td>
<td>.03*</td>
</tr>
<tr>
<td></td>
<td>Apical 3rd</td>
<td>1</td>
<td>3(2.25-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>Coronal 3rd</td>
<td>1</td>
<td>0(0-1)</td>
<td>21.350</td>
<td>&lt;0.001*</td>
<td>-2.12</td>
<td>.03*</td>
</tr>
<tr>
<td></td>
<td>Middle 3rd</td>
<td>1</td>
<td>1(0.25-1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apical 3rd</td>
<td>1</td>
<td>2(2-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Graph 5: Graph showing scoring of smear layer by Chitosan

Graph 6: Graph showing scoring of smear layer by EDTA

Figure 1: SEM MICROGRAPHS SCORE 0 = No smear layer. No smear layer on the surface of the root canals; all tubules were clean and open
Figure 2: SEM MICROGRAPHS SCORE 1 == Root canal surface covered with residue only at the opening of the dentinal tubules.

Figure 3: SEM MICROGRAPHS SCORE 2 = Moderate smear layer. No smear layer was observed on the surface of root canal, but tubules contained debris.

Figure 4: SEM MICROGRAPHS SCORE 3 = Heavy smear layer. Smear layer covered the root canal surface and the tubules
Figure 5: Group 1, Irrigated with a final flush of 17% EDTA. Coronal third of root canal wall appeared clean with no smear layer.

Fig 6: Group 2, Irrigated with a final flush of 0.2% Chitosan. Coronal third of root canal wall appeared clean with no smear layer.

**DISCUSSION**

Micro-organisms in the root canals are the prime causative factors in the development of pulp and periapical lesions.\(^{[16]}\) Eradication of the microbes is one of the significant goals for successful root canal treatment. It is compulsory to chemically debride teeth with complex internal anatomy that can be missed by instrumentation of the root canals. Consequently the utilization of irrigants during root canal treatment is of prime need.\(^{[9]}\)

The ability of an irrigating solution to remove smear layers from the coronal third, middle third, and the apical third of a canal wall depends on the aggressiveness of the irrigant and the manner in which the irrigant is delivered.\(^{[13]}\) For example, the presence of a vapor lock\(^{[19]}\) in a
closed-canal system precludes optimal delivery of an irrigant to the apical third of the canal wall. This variable was not examined in the present study because the objective was to evaluate irrigant effectiveness rather than the efficacy of canal irrigation. The bar charts in Figure 2 show that the efficacy of smear layer removal: coronal third > middle third > apical third. These results are consistent with the general findings from the endodontic literature that the apical third of the canal is more difficult to clean. When the contribution from different canal levels was taken into consideration, the effectiveness of smear layer removal with the respective final irrigant is in the following descending order.

Glyde > 0.2% Chitosan > Smear clear > QMix 2in1

Although the presence of a vapor lock in a closed-canal system may affect the efficacy of smear layer removal from the apical third of the canal well, the presence of a film of irrigant between the air bubble and the canal wall still permits some form of smear layer removal in a less efficient manner. On the contrary, the ability to clear debris from the canal walls is more dependent on the flow of the irritants and the manner in which the irrigant is agitated instead of the aggressiveness of the irritants. Because there is only limited flow of irrigants by manual delivery of an irrigant through a side-vented needle without additional agitation, it is not surprising that there are no differences in the five experimental groups in terms of clearing of debris from the canal walls. In the future, the efficiency of debris clearance from the canal space should be evaluated in a closed-canal system in conjunction with agitation devices such as sonic and ultrasonic agitation systems as well as devices that incorporate an apical negative pressure approach.

The primary motivation behind this examination was to assess the viability of an irrigant solution with contents equipped for cleaning and disinfecting the dentin, clearing the smear layer, opening the dentinal tubules and permitting the antibacterial agents to enter the whole root canal anatomy. The endodontic smear layer has been depicted as one that is framed amid instrumentation, comprising of dentin as well as necrotic and suitable tissue, including leftovers of odontoblastic procedures, pulp tissue and microorganisms. The smear layer assumes an essential part in the lateral sealing of the root canal, as a barrier that can meddle with attachment and infiltration of the root canal sealer into the dentinal tubules. Pashley et al had portrayed the smear layer as a permeable structure which was porous to even expansive molecules such as albumin.
EDTA is a standout amongst the most usually utilized chelating specialists which responds with the calcium ions in dentine and structures dissolvable calcium chelates. It has been accounted for that EDTA demineralize dentine to a profundity of 20–300mm in 5 min (von der Fehr & Nygaard-Ostby 1963); on the other hand, Fraser (1974) expressed that the chelating impact was verging on irrelevant in the apical end of root canal.[33]

The combined use of sodium hypochlorite and EDTA has demonstrated compelling in clearing smear layer shaped during root canal preparation (Goldman et al. 1982, Baumgartner & Mader 1987, Abbott et al. 1991).[24] 17 %EDTA follows up on the inorganic parts of the smear layer, causes the decalcification of peritubular and intertubular dentine, and leaves the collagen particle uncovered. In this manner, the utilization of NaOCl disintegrates the collagen, further aiding the passages to the intra dental tubules which are uncovered (Goldman et al. 1982, Baumgartner & Mader 1987) The 0.2% chitosan solution, even in such a low fixation, had the capacity to clear smear layer and give factually comparable results to those of the arrangements with higher focuses (15% EDTA and 10% citrus extract).

Ionic exchange, adsorption and chelation are presumably the mechanisms in charge of the arrangement of complexes among chitosan and metal particles. The sort of communication that happens relies on upon the particles included, the chemical structure of chitosan and the pH of the solution.[20] (Guibal et al. 2000, Rhazi et al. 2002). Two models are accounted for in the writing as could be allowed activity instruments. One of them, known as the bridge model, depends on the hypothesis that two or more amino groups of a chitosan molecule associated to the same metal ion(Blair & Ho 1981). The other model backings the hypothesis that only one amino group of the substance's structure is included in the coupling, that being the metal ion "attached" to the amino group.

CONCLUSION
Effective instrumentation and irrigation are prerequisites for successful endodontic treatment. Instrumentation of root canal leads to formation of smear layer in root canal walls. Smear layer formed alters the dentine permeability and effect adversely on adhesion of intracanal medicaments and obturation materials to the root canal wall. Removal of smear layer lead to better obturation and better treatment outcome. There are different irrigating solutions available for removing the smear layer from the root canal walls. EDTA is one of the most widely used irrigant used for the same. Aim of the present study was to evaluate effect of 0.2% Chitosan in removal of smear layer and its comparison with 17 % EDTA.
Within the limitations of the current study, it could be concluded that, combination of EDTA and Chitosan was found to be effective in the removal of intracanal smear layer. Chitosan could remove the smear layer as good as EDTA which is still considered as gold standard in clearing smear layer in a low concentration of 0.2 %. Both Chitosan and EDTA could not remove smear layer effectively from the areas of apical third. This could be because of insufficient cleaning of apical region or may be due to various anatomical complexities present in apical third. Additional properties of chitosan like antibacterial and antifungal effectiveness will addictively enhance its importance to be used as a root canal irrigant.

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