Original Article

A Comparative Scanning Electron Microscopy Evaluation of Smear Layer Removal with Chitosan and MTAD

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ABSTRACT

The aim of the present study was to compare the efficacy of chitosan and MTAD for the smear layer removal from the root canal through a scanning electron microscope (SEM). Thirty teeth were randomly divided into three groups according to the final irrigants: 0.2% chitosan, MTAD, saline (control group). After the mechanical preparation, the samples were irrigated with saline (control group), 0.2% chitosan and MTDA respectively. Then, the samples were split and the smear layer at the apical, middle, and coronal thirds of each root canal was imaged using SEM. The statistical analysis was performed using the Kruskal-Wallis test and the Mann-Whitney U test ($\alpha = 5\%$). The difference between chitosan and MTDA was statistically significant in the apical region ($p < 0.05$), no significant difference was obtained in the coronal and middle regions in these two experiment groups ($p > 0.05$). The control group exhibited the lowest efficacy in smear layer removal in all regions. Thus, from the result of the present study, we may conclude that chitosan was more effective in smear layer removal than MTAD especially in the apical third. **Context:** Irrigation, which serves a variety of purposes including antibacterial action, tissue dissolution, cleaning and chelating, plays a centric role in the final success of root canal treatment. Thus, more and more attention has been put on the improvement and development of various irrigation techniques or systems. **Aims:** The aim of the present study was to compare the efficacy of chitosan and MTAD for the smear layer removal from the root canal through scanning electron microscopy (SEM). **Settings and Design:** Thirty single-canal premolars were instrumented with rotary-files and then, randomly assigned to test groups which were irrigated with chitosan and MTDA, and control group was treated with saline. Thereafter, the efficacy of smear layer removal was evaluated by SEM. **Materials and Methods:** Thirty teeth were randomly divided into three groups according to the final irrigants: 0.2% chitosan, MTAD, saline (control group). After the mechanical preparation, the samples were irrigated with saline (control group), 0.2% chitosan and MTDA respectively. Then, the samples were split and the smear layer at the apical, middle, and coronal thirds of each root canal was imaged using SEM. **Statistical analysis used:** Kruskal-Walli test and Mann-Whitney U test **Results:** The difference between chitosan and MTDA was statistically significant in the apical regions ($p < 0.05$), no significant difference was obtained in the coronal and middle regions in these two experiment groups ($p > 0.05$). The control group exhibited the lowest efficacy in smear layer removal in all regions. **Conclusion:** Thus, from the result of present study, we may conclude that chitosan was more effective in smear layer removal than MTAD, especially in the apical third.

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INTRODUCTION

Irrigation, which serves a variety of purposes including antibacterial action, tissue dissolution, cleaning and

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Key Messages: Chitosan was more effective in smear layer removal than MTAD, especially in apical third. In consideration of its low production cost and its value of environmental protection, the chitosan is advised to be used in the endodontics as irrigation.

Keywords: *Endodontics, chitosan, SEM*

chelating, plays a centric role in the final success of root canal treatment.[1] Thus, more and more attention has been put on the improvement and development of various irrigation techniques or systems.[2]

So far, the most commonly used method of smear layer removal is the chemical method using various chemical agents. Moreover, an ideal irrigant should eliminate both organic and inorganic content from all canal surfaces without harmful erosive effects on dentine. Ethylenediamine tetra-acetic acid (EDTA), the most widely used irrigant, was reported to be effective in removing the smear layer from the root dentine walls.[3] However, long time application of EDTA (> 1min) may cause inadvertent erosion of the peritubular dentine[4] and it also showed limited antibacterial activity. Moreover, environmental concerns have also led researchers to seek alternatives to EDTA, as the overuse of this compound has considerably increased its concentration in rivers and lakes. In addition, EDTA is not originally found in nature and considered to be a pollutant.[5] Thus, numerous chemical agents were introduced to eliminate the smear layer and proposed as an alternative to EDTA.[6]

The introduction of MTAD (Dentsply, Tulsa Dental, Tulsa, OK, USA) (mixture of tetracycline, acid and detergent) represented an advancement in endodontic irrigation research. It was reported that MTAD removed most of the smear layer when used as an irrigant.[7] However, some organic remnants of the smear layer were still scattered over the canal walls.[8]While in Andrabi's study, the results showed that MTAD was the most effective chemical agent for smear layer removal, especially in the apical third of the root canal when compared with EDTA.[9] This indicated that MTAD could be considered as a better alternative to EDTA.

Chitosan is a natural polysaccharide, derived from the deacetylation of chitin, it can be obtained from the natural world, such as shrimp and crab shells. So, it is abundant in nature and more environmental friendly compared to other solutions. It has attracted attention in dental research because of its biocompatibility, biodegradability, bio-adhesion and lack of toxicity. With regards to irrigant, Silva *et al*. [10] demonstrated that chitosan had similar smear layer removal capacity as that of EDTA, although it is associated with demineralization effect on root canal dentine. In addition, in the study of time dependent effects of chitosan on dentin structure, 3 min treatment of chitosan appeared to be efficient for removing the smear layer, while causing little erosion of dentin.[11]

Till recently, there has been no report on the comparison of the efficacy of smear layer removal using chitosan and MTAD, which were both suggested as alternatives to EDTA. Thus, the aim of the present study was to compare the efficacy of chitosan and MTAD for the smear layer removal from the root canal using a single, common protocol. The null hypothesis states that there is no difference in the efficiency of chitosan and MTAD with respect to smear removal.

Material and Methods

Thirty human single-rooted premolars, recently extracted for orthodontic reasons, were used for this study. Teeth with mature root apex, similar anatomic characteristics, and without significant canal curvature (degree of root curvature < 30) were selected. They were divided into one control group and two experimental groups, each containing 10 samples respectively.

The preparation of sample was according to a previous study.[12] After taken from the saline, the anatomical crowns of these teeth were decapitated, leaving behind an average root length of 12 mm. The working length was measured by subtracting 0.5 mm from length recorded when the tip of #10 K-file was visible at the apical foramen. Mechanical preparation of teeth was done by crown-down technique using ProTaper rotary files. During preparation, the canals were rinsed with 3 mL of 5.25% NaOCl after each file. The apical foramen of each tooth should be sealed with a soft wax to prevent solution from passing from the apical foramen. After the biomechanical preparation, the control group was rinsed with 5 ml of saline, experimental groups were rinsed with 5ml of 0.2% chitosan (Shanghai Jinsui Biological Technology Co.Ltd., 9012-76-4) and MTAD (Longly Biological Medicine Co.Ltd., Q/WHLL04-2013) respectively. The pH of 0.2% chitosan was 3.01, while the pH of MTAD was 5.02. The total time for final irrigation was 3 minutes for all solutions.

SEM preparation and analysis

The canals were dried with paper points and the apical foramens were sealed with a small cotton plug to keep the root canal clean. Two furrows were made on the

buccal and lingual aspects of each tooth and the teeth were longitudinally split into two halves with a bone chisel. The half containing major part of the apex was prepared for SEM evaluation. These specimens were immersed, for tissue fixation, in 2% gluteraldehyde with phosphate buffer (pH = 7.3) for 12 hours. The specimens were then washed with 20 mL of phosphate buffer for 15 min. Specimens were dehydrated in a graded ethanol series: 70%, 80%, 90% and 100% for 15 min each, except for 100% ethanol for 30 min. After that, the specimens were dried in Zero point dessicator (Samdri-PVT-3D, Tousimis, America). The specimens also had to be coated with a Palldium-gold film. Then, the specimens were ready for observation under a scanning electron microscope (SEM). Representative photomicrographs were taken from the coronal, middle and apical portions of the canals at different magnifications (×1000, ×2000).

Photographs were evaluated for presence of smear layer using the scoring system described by Takeda, *et al.* (1999) and modified by Prado, *et al.* (2011). Scoring of the images was done by three investigators who calibrated with Cohen's Kappa coefficient test.

Score 1: No smear layer and debris at all, with all tubules cleaned and opened

Score 2: A few areas covered by smear layer and debris, with most tubules cleaned and opened

Score 3: Smear layer and debris covering almost all the surfaces, with few tubules opened

Score 4: Smear layer and debris covering all the surfaces.

Statistical Analysis

The score data from SEM analysis was subjected to the Kruskal-Walli test and the Mann-Whitney U test (α = 5%). All analyses were performed on SPSS v. 21.

Results

Representative images for each group are shown in Figure 1. Table 1 shows the mean smear scores (± SD) at coronal, middle, and apical thirds of the canals in each group. Pair-wise intergroup comparison of the three groups at the coronal, middle and apical third level showed that at the apical third , the best result was obtained in 0.2% chitosan group ($p < 0.05$). While at the coronal, middle third level, 0.2% chitosan and MTAD showed similar cleaning ability ($p > 0.05$), which were much better than the control group. Intra-group comparison revealed that in 0.2% chitosan group, the coronal third had lowest smear layer level ($p < 0.05$). In MTAD and the control groups, the canal walls at the coronal and middle third were much cleaner compared with the apical third ($p < 0.05$).

The same lowercase letters in the same row denote non-significant differences in the intragroup analysis. The same uppercase letters in the same column denote non-significant differences in the intergroup analysis ($p > 0.05$).

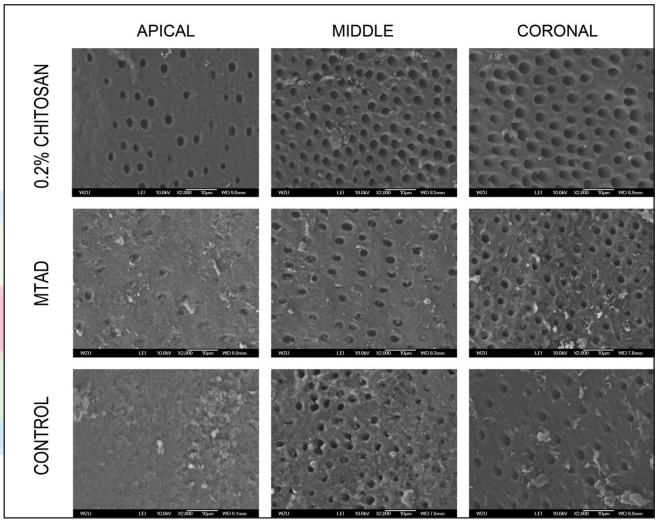

Figure 1: Representative images for each group (×2,000)

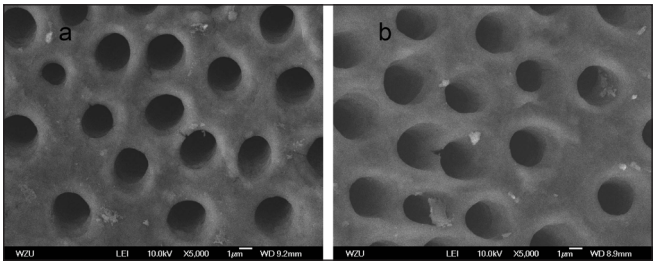

Figure 2: SEM micrographs for chitosan (a) and MTAD (b) (×5,000).

Table 1. Mean smear scores (± SD) in coronal, middle, and apical thirds of the canals in each group

Group	Middle 3rd				Means(SD)	Middle 3rd				Means(SD)	Apical 3rd				Means(SD)
	1	2	3	4		1	2	3	4		1	2	3	4	
0.2% Chitosan	6	4	0	0	1.40 ± 0.516^{aA}	2	6	2	0	2.00 ± 0.667^{bA}	1	3	6	0	2.50 ± 0.707^{bA}
MTAD	3	3	4	0	2.10 ± 0.876^{aA}	2	5	2	1	2.20 ± 0.919^{aA}	0	1	5	4	3.30 ± 0.675^{bB}
Saline	0	2	7	2	3.00 ± 0.667^{aB}	0	1	7	3	3.20 ± 0.632^{aB}	0	0	3	8	3.70 ± 0.483^{bB}

The same lowercase letters in the same row denote non-significant differences in the intragroup analysis. The same uppercase letters in the same column denote non-significant differences in the intergroup analysis ($p > 0.05$).

Discussion

The present SEM analysis revealed that 0.2% chitosan and MTAD were associated with similar smear layer removal patterns in coronal and middle thirds of root canal. However, at the apical third, the canal surfaces were cleaner in samples from chitosan. Thus, the null hypothesis was rejected.

As early as 1984, Yamada had already found that 17% EDTA was effective in cleaning the root canal wall.[13] While more and more studies found that EDTA had excessive erosion on dentine, resulting in loss of the peritubular dentin; the intertubular dentin reduced the flexural strength of the root canal. In addition, it was considered to stimulate the periapical tissues [14] and be an environmental pollutant.[5] So, the search for more biocompatible solutions than EDTA, aiming at minimizing its harmful effect on periapical tissues and environment continues.

Recently, MTAD has been introduced to dentistry as a final irrigant for smear layer removal. It has been proved to be effective in eliminating resistant micro-organisms and providing sustained antimicrobial activity.[15] Andrabi, *et al*.[9] demonstrated that MTAD was more effective than EDTA for smear layer removal, especially at the apical third of the root canal. This was in agreement with the results of the study by Paul, *et al*.[12] In addition, Torabinejad, *et al*.[7] indicated that NaOCl was needed as an irrigant to assist MTAD to remove the smear layer completely. From the current study, we clearly found MTAD was effective in the removal of smear layer compared with control group, however, for the apical third, MTAD showed limited cleaning capability. This result was not consistent with Andrabi's study. The different results might be attributed to differences in methodology. However, in Lotfi's study, they revealed a 2 min final rinse of MTAD was not able to eliminate the smear layer successfully because of the relative short time application of NaOCl for initial rinse in 10 minutes instrumentation periods.[16] This may well explain the relatively limited capability of MTAD in smear layer removal at the apical third of root canal in the present study.

However, as suggested by Lotfi, it seems that current irrigation solutions and techniques should be developed according to instrumentation time to achieve ultimate results[16] which is reasonable and feasible for its clinical usage.

Due to the high chelating ability for various metal ions in acidic conditions, chitosan was also suggested as a new solution for removal of smear layer after root canal instrumentation. In the present study, even in a low concentration (0.2%), chitosan promoted a more superior cleaning of the root canal walls than control group. In addition, chitosan was able to remove smear layer and provide similar results to the MTAD in the coronal and middle third of root canal, most importantly, chitosan exhibited more effective in the efficacy of smear layer removal at the apical third compared with MTAD. This information indicated that chitosan might be an excellent irrigant instead of EDTA.

The chitosan solution used in the present study was prepared using 1% acetic acid. According to previous study,[11] the smear layer removal capacity of chitosan was attributed to its own properties, instead of those of the 1% acetic acid. Thus, we could deduce that the chelating behavior of chitosan favored its smear layer removal. What's more, in our recent study, the irrigant was applied without agitation or ultrasonic, chitson showed good ability to remove the smear layer at the apical third while MTAD failed to achieve this result. This may be related to the different work action of irrigant on the smear layer.

On the other hand, Intra-group comparisons demonstrated that the chitosan was most efficient in removal of the smear layer at the coronal third, and had similar cleaning ability at the middle and apical thirds. While in MTAD and control groups, the results were different. We speculated that a larger canal diameter at the coronal and middle thirds exposed the dentin to a higher volume of irrigant, allowing a better flow of the solution, further improving the efficacy of smear layer removal. But with narrow space at the apical third, the canal walls could not be washed clean enough. Also, because of the large diameter at the coronal third, less smear layer was formed during the biomechanical preparation and was loosely attached to the canal wall with less pressure of the Protaper. So, the smear layer at the coronal third could be removed much easier than the apical third. At the middle third of the canal, a higher volume of irrigant went through the canal wall attributing to the much more powerful flush to the canal walls. This facilitiated the removal of the smear layer in a more thorough fashion than that of the apical third. The result that chitosan removed the smear layer of the middle and the apical third similarly, demonstrated that the smear layer removal ability of the chitosan was not just for the flush of the solution, but also mainly attributed to its chelating ability.

In our study, little erosion of dentinal tubules was observed in most samples irrigated with the chitosan and MTAD as shown in Figure 2. The prior irrigation with NaOCl during the biomechanical preparation might change the collagen matrix, while the chelation of the final irrigant caused the demineralization of the

dentin, these two functions combined together might cause progressive dissolution of dentin.[17] Moreover, the erosive effect of the irrigant depended on the concentration and the apply time of the solution. So, it was the most dominant for chitosan that it could remove smear layer adequately with much lower concentration than other irrigants. This further suggested that chitosan would be the excellent alteration of EDTA as irrigant.

Conclusion

Within the limitations of this investigation, we may conclude that chitosan was more effective in smear layer removal than MTAD. In consideration of its low production costs and its value of environmental protection, the chitosan is advised to be used in endodontics as an irrigant.

However, further studies are required on the depth of demineralization and the consequent micro-hardness loss, its influence on the bond strength of endodontic sealer to radicular dentin in order to provide more information on the clinical performance of chitosan.

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Conflicts of interest

There are no conflicts of interest

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