Original Article

Effect of Different Irrigating Solutions on Depth of Penetration of Sealer into Dentinal Tubules: A Confocal Microscopic Study

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Abstract

Aim: The aim of the study was to evaluate the effect of different irrigating solutions used in final irrigation on depth of sealer penetration into dentinal tubules. **Materials and Methods:** Thirty recently extracted, human mandibular premolar teeth with single canals were randomly divided into two groups, and one of the two irrigants was used in each group - Group A (Chitosan) and Group B (Ethylenediaminetetraacetic acid). All the teeth were obturated with gutta-percha and AH 26® sealer labeled with fluorescent dye. The teeth were sectioned at distances 2, 5, and 8 mm from the root apex. Maximum depth of sealer penetration was measured using confocal laser scanning microscopy. **Statistical Analysis:** Statistical analysis used One-way analysis of variance and *t*-test. **Results:** At coronal third depth, the sealer penetration was greater in ethylenediaminetetraacetic acid (EDTA) group; however, depth of sealer penetration was greater at apical third in chitosan group. **Conclusion:** Final irrigation with EDTA and chitosan after the use of sodium hypochlorite affected sealer penetration.

Keywords: *Chitosan, ethylenediaminetetraacetic acid, sealer penetration*

Introduction

Creation of a sterile space is impossible in infected root canals using mechanical preparation alone due to the complex anatomy of root canal systems.[1] Almost half of the root canal walls were left unprepared during the mechanical preparation with traditional stainless steel hand instruments and current nickel–titanium instrumentation systems.[2] When dentin is planed by endodontic instruments, a smear layer forms. Smear layer containing bacteria or bacterial products might provide a reservoir of irritants. Thus complete removal of the smear layer would be consistent with elimination of irritant from the root canal.[3] This marks irrigation, an essential step in the root canal preparation.[4] There was a significantly greater pattern of reduction of bacteria when NaOCl was used as an irrigant, compared with sterile saline.[5] The most widely used irrigant for smear layer removal is ethylenediaminetetraacetic acid (EDTA). It aids in cleaning of root canal by acting on the inorganic material (calcium ions) in dentin resulting in chelation of calcium. Thereby, it promotes the decalcification of dentin. Within 5 min, a depth of 20–30 μm can be decalcified. As the concentration of EDTA is increased in lakes and rivers, because of its overuse researchers are looking for alternatives. EDTA is also considered as a pollutant as it is not normally found in the nature.

The search for better biocompatible solutions which have less detrimental effects on periapical tissues is still going on. Chitosan is a natural polysaccharide which is biocompatible, biodegradable, and bioadhesive which lacks toxicity. It chelates various metal ions in acidic conditions and hence got popular in industrial areas for removal or recovery of metal ions. It is derived from deacetylation of chitin, which is found in shrimp and crab shells (Kurita, 1998). It has become popular due to its abundance in nature and low production costs.[6] Most of the studies, using chitosan as irrigating solution, have focused on antimicrobial activity with very little literature comparing the efficacy of the same with EDTA on smear layer removal and depth of sealer penetration.

The goal of thorough canal obturation can be achieved not only by removing the root

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canal debris and contaminates but also by achieving high adaptability of the filling materials.[7] Sealer is used to fill spaces between the core materials and the canal walls and penetrates into dentinal tubules entombing residual bacteria. Sealers have an antibacterial effect which is even more helpful as they can penetrate into the dentinal tubules and control the bacteria located in there, if not kill them. The above reason makes it really important that the percentage of the sealer/dentin interface that is covered by the sealer and the degree of tubule penetration by the sealer is as much as possible. Sealer penetration, therefore, could serve as an indicator to know the extent of smear layer removed.[8,9]

Based on the above information, this study was designed to know the effect of different final irrigating solutions on the maximum depth of sealer penetration into dentinal tubules at the coronal, middle, and apical areas of the root canal.

Materials and Methods

Thirty recently extracted human mandibular premolar teeth with single canals were used in this study. Preoperative radiographs were obtained in the mesiodistal and buccolingual directions to interpret the presence of calcifications, root curvatures, and a number of root canals. All the teeth were decoronated at cement-enamel junction, and working length was determined with the help of #10 k-file. Chemomechanical preparation was performed in a step-back technique using K-Files (Dentsply-Maillefer, Ballaigues, Switzerland) and 2.5% NaOCl as an irrigant after the use of each file.

In the apical area, the canals were enlarged up to size #40 and stepped back to size #60. The teeth were randomly divided into two groups according to the final irrigant employed – Group A (Chitosan-Panvo organics Pvt. Ltd., Chennai, India) and Group B (EDTA – Endo-Cleanse, Roydent Dental, Johnson, TN, USA). The final irrigation sequence was as follows:

1. Chitosan group: 5 ml of 0.2% chitosan followed by 5 ml of 2.5% NaOCl for 1 min
2. EDTA group: 5 ml of 17% EDTA followed by 5 ml of 2.5% NaOCl for 1 min.

The root canals were finally irrigated with 5 ml of distilled water and then dried with paper points. Obturations of the canals were done with AH 26® sealer (Dentsply-DeTrey, Konstanz, Germany) and gutta-percha in lateral compaction technique. Nearly 0.2% fluorescent Rhodamine B isocyanate (Sigma– Aldrich, India) was added to AH 26® sealer for fluorescence under confocal laser microscopy. Excess gutta-percha was removed, and access cavities were sealed with Cavit, after which the teeth were stored in an incubator at 37°C and 100% humidity for 24 h to allow the sealer to set. Each tooth was sectioned horizontally at a distance of 2, 5, and 8 mm from the root apex. Sections were made at a thickness of 100 μ. The specimens were then mounted onto glass slides and examined with a Leica TCS-SPE confocal microscope (Olympus FV 100, Germany). Depth of penetration was measured from the canal wall to the point of maximum sealer penetration. Statistical analysis was performed using one-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$.

Statistical methods

The mean and standard deviation of depth of sealer penetration of samples were obtained for each group and comparisons were performed using one-way ANOVA and *t*-test.

Results

Despite the results showing nonsignificant difference in depth of sealer penetration at coronal and middle third between Group A (chitosan) and Group B (EDTA), a statistically significant difference was observed at apical third ($P < 0.05$). In both experimental groups, the maximum depth of the sealer penetration was better in the coronal third than in the apical third of root canals [Table 1].

Discussion

The results of the present study showed that the maximum depth of the sealer penetration was better in the coronal thirds than in the apical thirds of root canals in all experimental groups [Table 1]. This might be a result of better removal of the smear layer in coronal thirds than in apical thirds of root canals. The number of dentinal tubules in the coronal third is more in number with a larger diameter than those in the apical area.[8] EDTA is effective in smear layer removal from both coronal and middle thirds but not from the apical third.[10] A 1 min application of 7% maleic acid followed by 2.5% NaOCl is an effective final irrigant for the removal of the smear layer from the root canal system in the apical third, which is a crucial area for the disinfection of the root canal system than that

Table 1: Intergroup comparison of depth of sealer penetration between two groups at three different levels by Independent *t*-test

	Mean	SD	*t*	*P*	Inference
Coronal					
Chitosan	941.36	277.95	−0.77	0.45	NS
EDTA	1005.57	166.93			
Middle					
Chitosan	711.57	87.44	−1.01	0.32	NS
EDTA	751.57	126.72			
Apical					
Chitosan	490.48	158.22	2.21	<0.05	S
EDTA	379.09	114.02			

EDTA: Ethylenediaminetetraacetic acid; SD: Standard deviation; NS: Not significant; S: Significant

of 17% EDTA.[11,12] This may be due to the higher surface tension of 17% EDTA when compared with maleic acid. Galler *et al.* found that irrigation with EDTA may lead to exposure of growth factors in dentinal tubules which might optimize the conditions for cellular differentiation, tissue formation, and regeneration.[8] The search for more biocompatible solutions than EDTA, aiming at reducing its harmful effect on periapical tissues continues.

Atomic absorption spectrophotometry with flame analysis of 0.2% chitosan disclosed a calcium ion concentration of 104.13 mg, with no significant difference when compared with 15% EDTA (121.80 mg). When two materials have a similar chelating effect, then the cost-effective agent with less concentration should be preferred. It is known that the efficiency of a chelating agent depends on several factors including application time, pH, concentration, and amount of the solution.[13] In addition, the relationship between the concentration of the chelating agent and the application time seems to be important since it was found that highly concentrated solutions applied for a long period, cause roughness of dentin surface.[14] To avoid these detrimental effects with the use of high concentration solution, lower concentration solution was used (0.2% chitosan) in the present study.

According to the results of the present study, the depth of sealer penetration was highest with 0.2% chitosan at apical third and showed statistically similar results with 17% EDTA at coronal and middle third [Table 2]. A volume of 0.2% chitosan solution, even in such a low concentration, was capable of removing smear layer and provides statistically similar results to those of the solutions with higher concentrations.[6] Chitosan polymer is hydrophilic which favors intimate contact with root canal dentin and is adsorbed to root canal wall. In addition, it has a large number of free hydroxyl and amino groups that make it cationic in nature that is responsible for the ionic interaction between the dentin calcium ions and the chelating agent. Moreover, in an acid medium, the amino groups present in the polymer are protonated, resulting in attraction to other molecules for adsorption to root dentin to occur and were capable of being delivered to deeper location of dentinal tubules.[15] In addition, chitin polysaccharide, the precursor to chitosan, is the most abundant substance in nature after cellulose.[16] The production cost of chitosan is considered to be low, making its use ecologically attractive. Depth of penetration of sealer is also influenced by its chemical and physical properties.

Table 2: Mean values of depth of sealer penetration in two groups at three different levels by one-way analysis of variance

Variables	Parameters	Minimum	Maximum	Mean	SD
Chitosan	Coronal	586.90	1862.43	941.36	277.95
	Middle	543.43	856.98	711.57	87.44
	Apical	265.87	764.76	490.48	158.22
EDTA	Coronal	856.43	1562.83	1005.57	166.93
	Middle	589.99	989.79	751.57	126.72
	Apical	241.86	642.98	379.09	114.02

EDTA: Ethylenediaminetetraacetic acid; SD: Standard deviation

Sealer may be drawn into tubules due to the capillary action and not by hydraulic forces created during root canal filling. Among the sealers tested, AH 26® sealer appeared to have the most optimal tubular penetration and adaptation to the root canal wall. To observe the penetration under confocal laser scanning microscope, labeling the sealer with Rhodamine B is essential. According to American Dental Association specifications, the sealer labeled with 0.2% Rhodamine B did not show changes in its flow. Future studies are required to investigate in detail the physical, chemical, and biological properties of chitosan to verify its benefits and consequences to humans.

Conclusion

Within the limitations of the present study, it can be concluded that the depth of penetration of sealer at the apical third is greater with chitosan when compared to EDTA and is statistically significant. There is no significant difference between EDTA and chitosan in terms of the depth of penetration of sealer at the coronal and middle thirds. Chitosan (0.2%) can be indicated as a better root canal irrigant alternative to EDTA owing to its biocompatible, biodegradable, and bioadhesive nature.

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Conflicts of interest

There are no conflicts of interest.

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