

Original Article

Effect of Chitosan on Sealer Penetration into the Dentinal Tubules

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INTRODUCTION

The outcome of root canal therapy depends on the efficient administration of chemomechanical preparation. In this process, root canal instrumentation and irrigation should be carried out jointly and meticulously to disinfect the entire root canal system.^[1] The smear layer might act as a physical barrier that limits the antibacterial effects of intracanal medicaments and root canal sealers.^[2] Moreover, the removal of the smear layer maximizes the retention of root canal filling.^[3] Locally, further sealer penetration (SP) is supposed as an indicator that the smear layer was removed in such an area.^[4] The penetration of root canal sealer enables to entomb residual bacteria^[5] and also enables to transfer the

ABSTRACT

Objective: The objective of this study is to compare the effectiveness of final irrigation with chitosan, ethylenediaminetetraacetic acid (EDTA), and citric acid (CA) on a resin-based sealer (AH plus sealer [Dentsply DeTrey, Konstanz, Germany]) penetration into dentinal tubules using confocal laser scanning microscopy. **Materials and Methods:** Seventy recently extracted human mandibular premolars were instrumented and irrigated with sodium hypochlorite (NaOCl), then divided into four groups according to the final irrigation regimen used: (1) the EDTA group: 17% EDTA + 2.5% NaOCl, (2) the CA group: 10% CA + 2.5% NaOCl, (3) the chitosan group: 0.2% chitosan + 2.5% NaOCl, and (4) the control group: 2.5% NaOCl. All teeth were obturated using the cold lateral condensation technique with gutta-percha and AH Plus sealer labeled with fluorescent dye. The apical 2 mm of specimen was discarded, and slices were obtained for apical, middle, and coronal thirds of the root with 1 mm intervals. Maximum, mean, and percentage of sealer penetration (SP) inside tubules were measured using confocal laser scanning microscopy. **Results:** The percentage of SP was significantly higher in chitosan, EDTA, and CA group than control group for coronal thirds ($P < 0.05$), whereas there was no significant difference among all groups for middle and apical thirds. Chitosan and EDTA showed increased mean values of SP depth for middle thirds ($P < 0.05$). In all sections, the maximum depth of SP was significantly lower in EDTA group than other groups ($P < 0.05$). **Conclusions:** Chitosan, EDTA, and CA significantly improved the percentage of SP for coronal thirds.

KEYWORDS: Chelating, chitosan, confocal laser scanning microscopy, sealer penetration, smear removal agent

antibacterial effect of the sealer into the dentin tubules.^[6]

Several chelating solutions, including organic acids such as citric acid (CA), maleic acid and inorganic acids such as ethylenediaminetetraacetic acid (EDTA), phosphoric acid have been used to remove the smear layer.^[7,8] Although EDTA is one of the most widely used chelating molecules, it has some limitations and disadvantages as root canal irrigant. Many studies revealed that EDTA was not effective in smear layer

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removal in the apical third of the root canals.^[9-11] In addition, longer contact time with EDTA may cause loss of dentinal surface and reduction in microhardness of dentinal walls.^[12] Therefore, researchers seek an alternative to EDTA solution because of its erosive and toxic side effects on dentinal and periapical tissues.^[13]

AH plus, is an epoxy resin-based root canal sealer, that kills bacteria beyond its penetration depth.^[6] Although the flow and penetration of the sealer is not completely blocked, the smear layer's effect can be described as limiting.^[14] Various chelating agents have been recommended by researchers for effective removal of the smear layer.^[7,11,15] In a previous study,^[13] 0.2% chitosan removed the smear layer as effectively as 15% EDTA and 10% CA from the middle and apical thirds of the canal. Prabhu *et al.* suggested 5% and 7% maleic acid as alternatives to the routine use of 17% EDTA.^[16] There is a gap in the literature, which does not discuss whether 0.2% chitosan may provide greater SP than other chelating agents.

Chitosan is a natural polysaccharide, obtained from crab and shrimp cells by the deacetylation of chitin.^[17] Previous studies^[18,19] have proven chitosan's positive biological traits such as biocompatibility, biodegradability, bioadhesion, and lack of toxicity. Chitosan has been used in medicine and pharmaceuticals as a drug carrier, wound healing accelerator, and antibacterial and antitumor agent.^[20] Ballal *et al.*^[21] showed that in root canals, prolonged calcium hydroxide ion release occurs through the addition of chitosan to calcium hydroxide paste. Furthermore, Silva *et al.*^[13] indicated that a 0.2% chitosan solution was as effective as EDTA and CA with higher concentrations (15% EDTA and 10% CA) at removing the smear layer.

To the best of our knowledge, there have been no studies examining the effect of different chelating solutions with chitosan on SP into the dentinal tubules. The aim of this study was to compare the effect of final irrigation with chitosan, EDTA, and CA, on SP into the dentinal tubules using confocal laser scanning microscopy (CLSM). The hypothesis of this study is that there is no difference between the effects of chitosan and other chelating agents on SP into the dentinal tubules.

MATERIALS AND METHODS

Ethical clearance was obtained from the Ethical Committee of Mersin University, Mersin, Turkey (number: 2016/323, Clinical Research Ethics Committee dated October 6, 2016). Seventy recently extracted human mandibular premolar teeth with single canals, straight, mature roots, and no caries or resorption were selected and used in this study.

The presence of a single canal was verified with three angulated radiographs. Teeth were cleaned to remove all adherent debris and kept in 0.2% sodium azide at 4°C until use. All experimental procedures were performed by the same operator. The teeth were decoronated with a 0.3-mm microtome saw (Metkon Instruments Inc., Bursa, Turkey) to standardize the root length to 12 mm from the anatomic apex. A size of 15 K-file (G-Star; Golden Star Medical, Guangdong, China) inserted into each canal until its tip was just visible at the apical foramen and working length (WL) was established 1 mm short of this length. Cleaning and shaping were performed to the WL with a crown-down technique using ProFile rotary instruments (Dentsply Tulsa Dental, Tulsa, OK, USA). Each canal was shaped to a size of 40.06. A total of 10 ml (ml) of 2.5% sodium hypochlorite (NaOCl) (pH = 11.5) were used for irrigation between instruments. After chemomechanical preparation, the teeth were randomly divided into three experimental groups of 20 teeth each and control group of 10 teeth:

1. The EDTA group: 5 ml 17% EDTA (Norateks Chemical Industry, Istanbul, Turkey) for 1 min followed by 5 ml 2.5% NaOCl for 1 min
2. The CA group: 5 ml 10% CA (Norateks Chemical Industry, Istanbul, Turkey) for 1 min followed by 5 ml 2.5% NaOCl for 1 min
3. The chitosan group: 5 ml 0.2% chitosan for 1 min followed by 5 ml 2.5% NaOCl for 1 min
4. The control group: 10 ml 2.5% NaOCl solution for 1 min.

The 0.2% chitosan solution was prepared by diluting 0.2 g of chitosan (Sigma-Aldrich, St. Louis, MO, USA) in 100 ml of 1% acetic acid, and the mixture was stirred for 2 h using a magnetic stirrer. All irrigation solutions were introduced into the canal using a five-milliliter disposable plastic syringe (Ultradent Products Inc., South Jordan, UT, USA) with a 30G side-vented needle (KerrHawe Irrigation Probe, KerrHawe SA, Biggio, Switzerland). The irrigation needle was placed as deep as possible into the canal without binding to the canal wall but not closer than 2 mm from the WL. The roots canals were finally irrigated with 5 ml of distilled water for 1 min and then dried with paper points (Diadent Group International Inc., Cheongju, Korea). All canals were obturated with AH Plus sealer (Dentsply; DeTrey, Konstanz, Germany) and gutta-percha using cold lateral compaction technique. For fluorescence under confocal laser microscopy, the AH Plus sealer was mixed with 0.1% fluorescent rhodamine B isothiocyanate (Bereket Chemical Industry, Istanbul, Turkey). Sealer was applied with a size 40 master cone (Diadent Group International Inc.), and the root canals were filled

with accessory gutta-percha size 20 cones with .02 taper. Gutta-percha was applied using a size B endodontic finger spreader (Dentsply Maillefer, Ballaigues, Switzerland) inserted 2–3 mm short of the WL. Excess gutta-percha was removed using a heated plugger. After the access cavities of the teeth were sealed with Cavit (3M; ESPE, St. Paul, MN, USA), specimens were stored in an incubator at 37°C and 100% humidity for 72 h to allow the sealers to set.

Seventy roots were embedded in clear self-cured resin (Kemdent, Swindon, UK) using a cylindrical split mold of 17 mm in diameter and left to polymerize overnight. Then, 1.2 mm slices were cut using a low-speed diamond saw under water cooling. Each slice was evaluated with a digital caliper (Teknikel, Istanbul, Turkey) to an accuracy of 0.001 mm. The apical 2 mm of each specimen was discarded and slices were obtained by sectioning apical, middle, and coronal thirds of the root at 1-mm intervals.

Sealer penetration

Two hundred and ten sections from 70 roots with root canal filling were then polished with silicon carbide abrasive paper. All specimens were mounted onto glass slides and examined under CLSM (Zeiss LSM 700, Oberkochen, Germany) at $\times 10$ magnification. First, each sample image was imported into ImageJ software (National Institutes of Health, Bethesda, MD, USA) [Figure 1a]. To calculate exact distance and percentage values, images were calibrated according to scale on CLSM image. The process tab was selected on the ribbon, and the find maxima option was used to prevent possible loss of data. Find maxima tool settings were set as noise tolerance at 1000 value and output style as maxima within tolerance. Then, the output image was exported as a tagged image file format (TIFF) file to be altered later in image manipulation software [Figure 1b].

An image manipulating program (GIMP, GNU Image Manipulation Program version 2.8) was used to superimpose acquired CLSM images, the ImageJ find maxima tool applied raw images and the custom-prepared circular ruler template. Three layers were superimposed with a transparency value of 30%. To standardize the measurement of the canal walls, the custom-prepared circular ruler template was placed at the center of the root canals in each section [Figure 1c]. Three-layered images were exported as a single-layered image and saved as a TIFF file.

In each sample image, the circumference of the root canal wall was outlined and measured with the ImageJ software measuring tool (National Institutes of Health, Bethesda, MD, USA). Then, areas along the canal walls

in which the sealer penetrated into dentinal tubules were outlined and measured using the same method. In each section, the percentage of the area of each canal wall covered by sealer was calculated by dividing the outlined distances to the canal circumference measurements. To calculate the mean percentage of SP, an eight-sectioned, custom-prepared circular template was used as described before.^[22] Penetration depths were measured using the ImageJ straight-line measuring tool at eight standardized points starting from the inner side of canal wall, then the average of the sum of these measurements was recorded as the mean depth of SP. The measurements were repeated at apical, middle, and coronal slices of the roots.^[22] In each sample image, the point of deepest penetration was measured from the canal wall to the point of maximum depth of SP.

Statistical analyses were performed using IBM SPSS Statistics (Armonk, NY, USA) version 22, and $P < 0.05$ were considered statistically significant. Sample size (n), median (M), 25th percentile (Q₁), and 75th percentile (Q₃) were used as descriptives. Normality of numeric variables was evaluated by the Shapiro–Wilk normality test and Q-Q plots. Comparisons among groups in SP (mean, maximum, and percentage penetration) were performed using the nonparametric Kruskal–Wallis test and the Dunn–Bonferroni test was used for the *post hoc* comparisons of the data. Within each group, SP at apical, middle, and coronal levels was analyzed using the Friedman test and multiple comparisons were performed using the Student–Newman–Keuls test.

RESULTS

Figure 2 shows representative images from each group at apical, middle, and coronal levels. Regarding the mean depth of SP [Figure 3a], there was a significant difference among all groups for middle thirds ($P < 0.05$), the chitosan and EDTA groups showed higher mean values of SP depth than CA and control group. However, in the middle thirds, there was no statistical difference between chitosan and EDTA group ($P > 0.05$), and also, no significant difference existed between CA and control group, ($P > 0.05$). Meanwhile, at apical and coronal

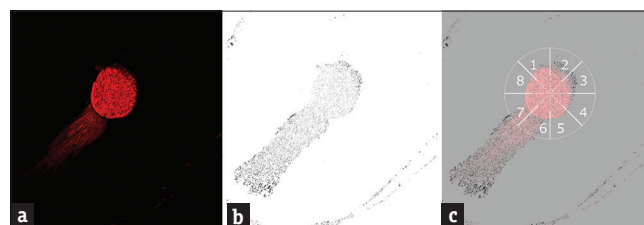


Figure 1: (a) Raw image, (b) processed image with find maxima tool in ImageJ, (c) three-layered image with the custom-prepared circular ruler template

levels, there was no significant difference among all groups regarding mean depth of SP ($P > 0.05$). Within the groups, in chitosan and control group, similar results were recorded for both apical, middle, and coronal thirds ($P > 0.05$). In EDTA group, values for mean depth of SP were significantly higher than those in apical thirds ($P < 0.05$). However, there

was no statistical difference both between coronal-middle thirds and coronal-apical thirds ($P > 0.05$). In CA group, higher mean depth of SP values were recorded for coronal and middle thirds than those for apical thirds ($P < 0.05$) whereas, there was no statistical difference between coronal and middle thirds ($P > 0.05$).

In all sections, the maximum depth of SP was significantly lower in the EDTA group than other groups ($P < 0.05$) while no significant difference was observed among chitosan, CA, and control group [Table 1]. Comparisons within the groups revealed that, in chitosan, CA and control groups, no significant difference was found among the coronal, middle, and apical thirds of the root canals regarding the maximum depth of SP. In EDTA group, values for maximum depth of SP were significantly higher in middle thirds than those in apical thirds ($P < 0.05$), whereas no statistical difference occurred both between coronal-middle-thirds and coronal-apical thirds ($P > 0.05$).

The percentage of SP in coronal thirds was significantly higher in the chitosan, EDTA, and CA groups than the control group ($P < 0.05$), while there was no statistically difference among the experimental groups for coronal levels ($P > 0.05$), and also, there was no significant difference among all groups for middle and apical thirds ($P > 0.05$). In CA group, for coronal thirds, the percentage of SP was significantly higher than apical thirds ($P < 0.05$) whereas no statistically

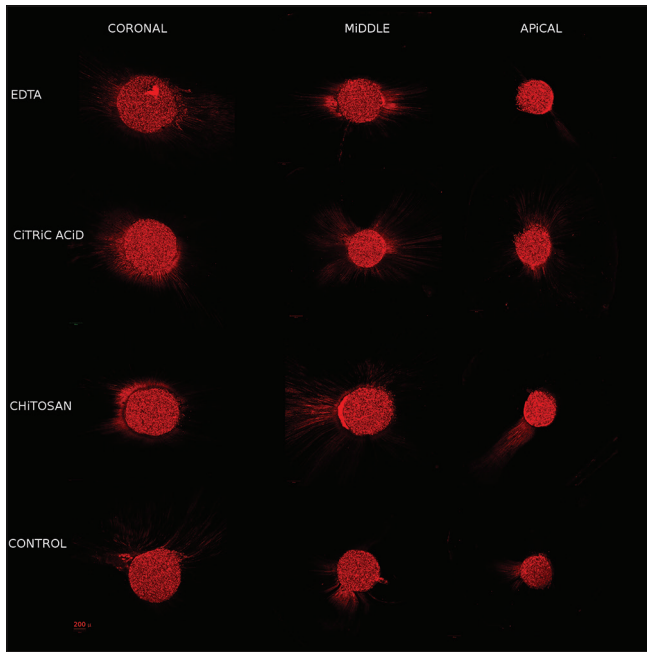


Figure 2: Representative confocal laser scanning microscopy images from each group at apical, middle, and coronal levels

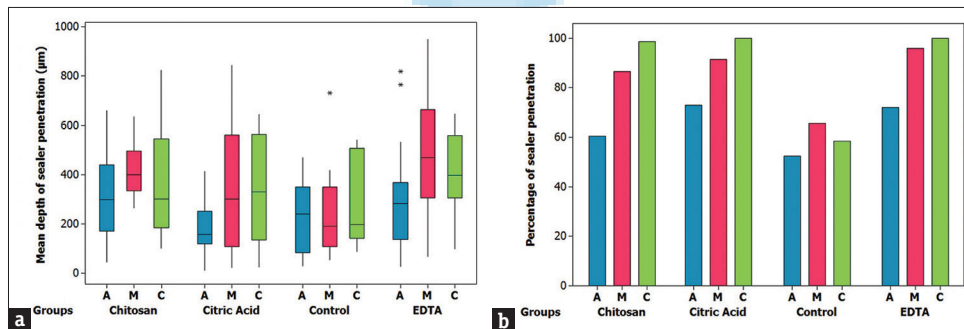


Figure 3: (a) Mean depth of sealer penetration for apical (A), middle (M), and coronal (C) levels. The median (line inside the box), minimum, and maximum values are presented. Outliers are represented with symbol (*). (b) The percentage of sealer penetration into dentinal tubules at the apical, middle, and coronal sections

Table 1: Maximum depth of sealer penetration in µm (median, 25%, 75% quartile values of the groups)

Group	Level			P
	Apical M (Q ₁ -Q ₃)	Middle M (Q ₁ -Q ₃)	Coronal M (Q ₁ -Q ₃)	
Chitosan	947 (454-1158) ^b	1045 (943-1226) ^b	1025 (779-1227) ^b	0.069
EDTA	302 (207-413) ^{A,a}	492 (329-663) ^{B,a}	424 (338-559) ^{A,B,a}	0.030
Citric acid	783 (452-937) ^b	937 (476-1180) ^b	895 (468-1091) ^b	0.486
Control	1135 (335-1349) ^b	887 (651-1426) ^b	960 (595-1139) ^b	0.717
P	<0.001	<0.001	<0.001	

Different groups are shown with different superscript letters. EDTA showed significantly lower depth values than other groups for each level. EDTA=Ethylenediaminetetraacetic acid

difference was observed between coronal-middle levels and middle-apical levels ($P > 0.05$). Both within the chitosan and EDTA group, the values of the percentage of SP were significantly higher for the coronal and middle thirds than for the apical thirds ($P < 0.05$) while, no significant difference was found between coronal and middle thirds ($P > 0.05$). In the control group, no significant difference was observed among the coronal, middle, and apical sections regarding the percentage of SP ($P > 0.05$) [Figure 3b].

DISCUSSION

In this study, CLSM was used for investigating dentinal adaptation of root canal filling materials. CLSM has several advantages over scanning electron microscopy (SEM), CLSM is a simple method^[23-25] that does not require any special specimen processing,^[24] allows observations to be made under normal conditions, and tends to produce fewer artifacts on images of samples than SEM.^[26]

In this study, a resin-based root canal sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany) and lateral condensation technique were used jointly. In previous studies, deeper penetration ability of AH Plus has been proven^[5,27], and as a result of this ability, better sealability was expected. After the mixing procedure, resin sealers are characterized with thin film structure that allows adequate flow and deeper penetration within the tubules.^[28] Clinicians generally prefer the lateral condensation technique because increased hydraulic forces occur throughout the process. In this manner, sealer can be pushed into the dentinal tubules in all.^[29] An easily detectable fluorescent dye, Rhodamine B, was used in low concentration (0.1%) to avoid impairing the physicochemical properties of the root canal sealer.

According to our results, in the coronal thirds, the percentage of SP values were significantly greater in all experimental groups (Group 1: 17% EDTA + 2.5% NaOCl; Group 2: 10% CA + 2.5% NaOCl; Group 3: 0.2% Chitosan + 2.5% NaOCl) than in the control group (2.5% NaOCl) ($P < 0.05$). In contrast, there was no significant difference between experimental groups and control group in the middle and apical thirds. The higher percentage of SP of experimental groups in coronal thirds might be explained by higher smear layer removal ability of chelating solutions in coronal thirds than in the middle and apical thirds. In addition, due to fact that larger number and diameter of dentinal tubules exist in coronal areas, chelating solutions in experimental groups may have induced significantly greater percentage of SP. In a previous study, a final rinse with 17% EDTA, 7% maleic acid, and 10% CA after a rinse with 2.5% NaOCl was compared with a

control group (2.5% NaOCl), and results showed that greater SP percentages were found in experimental groups in coronal, middle, and apical sections.^[14] In our study, 0.2% chitosan, like other chelating agents with higher concentrations (17% EDTA, 10% CA), affected SP in coronal thirds after the use of 2.5% NaOCl.

Our study also found that in each experimental group, coronal sections showed a significantly higher percentage of SP than apical sections. Our results are in accordance with previous studies.^[14,22,30] In a previous study, lateral condensation technique was associated with greater percentage of SP in the coronal area.^[31] Our findings may be related with the facts that, dentinal tubule orifices are denser and larger in the coronal thirds than apical thirds, tubular sclerosis begins within the apical area.^[32] In addition chelating agents in all experimental groups may not be conducted to apical thirds through conventional syringe technique. Many factors, including the filling technique, smear layer removal, flow properties of canal sealer, number and size of dentinal tubules, and anatomy of the root canal system,^[14,33] affect the percentage, maximum, and mean depth of SP.

All groups, including the control group, showed greater maximum depths of SP in all sections than the EDTA group. Ballal *et al.*^[21] stated that spreading of AH Plus sealer was reduced on root canal dentine when EDTA was used for final irrigation, and the situation was associated with the absence of a surfactant effect. García-Godoy *et al.* argued that EDTA caused a collapse of the dentin matrix structure, by this way, SP could be impeded and hybrid layer bonding formation could be damaged.^[34] In the same study, smear layer-covered dentine was associated with better hybrid layer quality and less potential for nanoleakage than those dentine exposed to EDTA. Our results are compatible with aforementioned study. Although the percentage of SP can be accepted as more reliable and relevant than the maximum depth of SP, the dilemma should be illuminated due to EDTA's widespread clinical usage.

In previous studies,^[35,36] the failure of conventional needles at disinfecting the apical thirds of root canals has been proven. Likewise, Kara Tuncer and Unal showed that an apical negative pressure system (EndoVac) was more effective than conventional irrigation for apical and middle thirds of the root canal.^[37] In this study, the tip of the needle was inserted as far as possible without binding to the canal; the maximum limit was established not closer than 2 mm to WL. Due to fact that the main focus of this study was to evaluate the efficacy of irrigants on SP rather than the type of the irrigation method, irrigation was carried out via side-vented needle and syringe.

CONCLUSIONS

Under the conditions of the study, chitosan, EDTA, and CA significantly improved the percentage of SP for coronal thirds. 0.2% chitosan showed similar effects on the percentage of SP with higher concentration of EDTA and CA. In further studies, the SP effect of chitosan and other chelating agents may be evaluated with an apical negative pressure system at certain time intervals.

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Conflicts of interest

There are no conflicts of interest.

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