Effect of Different Final Irrigation Solutions on Dentinal Tubule Penetration Depth and Percentage of Root Canal Sealer

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Abstract

Introduction: The purpose of this study was to evaluate the effects of different solutions used for final irrigation on sealer penetration into dentinal tubules. **Methods:** Thirty-two recently extracted human mandibular premolar teeth were treated with sodium hypochlorite (NaOCl) irrigation. The samples were divided into 4 groups according to the final irrigation solution used: (1) the EDTA group: 17% EDTA + 2.5% NaOCl, (2) the maleic acid (MA) group: 7% MA + 2.5% NaOCl, (3) the citric acid (CA) group: 10% CA + 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 26 sealer (Dentsply; DeTrey, Konstanz, Germany) labeled with fluorescent dye. The teeth were sectioned at distances of 2, 5, and 8 mm from the root apex. Total percentage and maximum depth of sealer penetration were measured using confocal laser scanning microscopy. **Results:** The Kruskal-Wallis analysis results showed that there was a significant difference in the percentage and maximum depth of sealer penetration among all groups in all sections ($P < .05$).The coronal sections in each group showed a significantly higher percentage and maximum depth of sealer penetration than did the apical and middle sections ($P < .05$). **Conclusions:** Final irrigation with EDTA, MA, and CA after the use of NaOCl affected sealer penetration. However, there was no significant difference between these experimental groups (EDTA, MA, and CA) in all sections. *(J Endod 2012;38:860–863)*

Key Words

Citric acid, dentinal tubule, irrigation, maleic acid, sealer

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The purposes of root canal treatments are to eliminate microorganisms from the root canal system and to prevent re-contamination. Because of the complex anatomy of the root canal system, which includes lateral canals, ramifications, and deltas, it is impossible to complete disinfection of the root canal using instrumentation alone (1). Irrigation is a critical compliment to instrumentation because it removes bacteria, debris, and necrotic tissue present in the smear layer (2). The removal of the smear layer is still a matter of debate. Previous studies showed that the smear layer hinders the penetration of intracanal medicaments and sealers into the dentinal tubules (3), thus protecting bacteria within the dentinal tubules (4). Chelating agents, such as EDTA, citric acid (CA), maleic acid (MA), phosphoric acid, and combinations of EDTA and NaOCl have been used to remove the smear layer (5, 6).

Instrumentation and irrigation cannot be considered separately. The terms typically used for the combination of instrumentation and irrigation are chemomechanical preparation or biomechanical preparation (7). The presence of microorganisms has been observed, even after thorough chemomechanical preparation of the root canal system (8, 9). Therefore, it is essential to create a fluid-tight seal in the root canal system using core-filling material and root canal sealer (10). Sealer is used to fill spaces between the core materials and the canal walls and between the gutta-percha points (11). Furthermore, sealer penetrates into dentinal tubules, entombing residual bacteria (3, 12). Therefore, sealer penetration might serve as an indicator of the extent to which the smear layer was removed (13).

Several studies have compared the effectiveness of EDTA, CA, and MA in smear layer removal. Ballal et al (5) reported that a final irrigation with 7% MA is more effective than that with 17% EDTA in removing the smear layer from the apical third of the root canal system. However, to the best of our knowledge, no studies have examined how different irrigation solutions affect sealer penetration. The aim of this study was to evaluate the effect of different final irrigation solutions on the percentage and the maximum depth of sealer penetration into dentinal tubules at the apical, middle, and coronal areas of root canals. We tested the hypothesis that the percentage and maximum depth of sealer penetration would change with the use of different final irrigation solutions (EDTA, MA, and CA).

Materials and Methods

Ethical clearance was obtained from the Ethical Committee (#1746) of Istanbul University, Istanbul, Turkey. Thirty-two recently extracted human mandibular premolar teeth with single canals, straight mature roots, and no caries or resorption were used in this study. Teeth were kept in 0.2% sodium azide solution at 4°C until use. The presence of a single canal was verified radiographically on 3 angulated films. All experimental procedures were performed by the same operator. The teeth were decoronated with a 0.3-mm microtome saw (Isomet Buehler, Lake Bluff, IL) to standardize the root length to 14 mm from the anatomic apex. Samples were divided randomly into 3 experimental groups and a control group. The working length was established by inserting a size 10 K-File (Mani, Inc, Tochigi Ken, Japan) into each root canal until it was just visible at the apical foramen and then subtracting 1 mm from this point. Chemomechanical preparation was performed with a step-back technique using K-Files (Mani, Inc). In the apical area, the canals were enlarged up to ISO size 40 and stepped back to ISO size 60. A Gates Glidden drill (Mani, Inc) size 2 to 3 was used to enlarge the coronal third of the root

TABLE 1. The Percentage (%) of Sealer Penetration into Dentinal Tubules at Apical, Middle, and Coronal Sections

Group	Apical third (mean ± SD)	Middle third (mean ± SD)	Coronal third (mean ± SD)
17% EDTA + 2.5% NaOCl	54.93 ± 20.50	75.39 ± 27.32	93.38 ± 11.24
7% maleic acid + 2.5% NaOCl	50.98 ± 29.38	65.73 ± 22.56	85.25 ± 25.06
10% citric acid + 2.5% NaOCl	53.93 ± 25.16	60.24 ± 26.01	84.90 ± 15.04
Control	26.01 ± 12.40	36.02 ± 9.47	59.65 ± 31.37

canal. Irrigation was performed with 1 mL 2.5% NaOCl solution (Norateks Chemical Industry, Istanbul, Turkey) after each instrument change.

The final irrigation sequence was as follows:

1. The EDTA group: 5 mL 17% EDTA (Norateks Chemical Industry, Istanbul, Turkey) for 1 minute followed by 5 mL 2.5% NaOCl for 1 minute
2. The MA group: 5 mL 7% MA (Norateks Chemical Industry, Istanbul, Turkey) for 1 minute followed by 5 mL 2.5% NaOCl for 1 minute
3. The CA group: 5 mL 10% CA (Norateks Chemical Industry) for 1 minute followed by 5 mL 2.5% NaOCl for 1 minute
4. The control group: 5 mL 2.5% NaOCl solution for 1 minute

All irrigation solutions were introduced into the canal using a 5-mL disposable plastic syringe (Ultradent Products Inc, South Jordan, UT) with a 30-gauge side-vented needle (KerrHawe Irrigation Probe; KerrHawe SA, Biggio, Switzerland) inserted to a depth that was 1 mm less than the working length. The roots canals were finally irrigated with 5 mL of distilled water for 1 minute and were then dried with paper points (Diadent Group International Inc, Chongju, Korea). All canals were obturated with AH 26 sealer (Dentsply; DeTrey, Konstanz, Germany) and gutta-percha using the lateral compaction technique. For fluorescence under confocal laser microscopy, AH 26 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 a .02 taper. Gutta-percha was applied using a size B endodontic finger spreader (Dentsply Maillefer) inserted 2 to 3 mm short of the working length. Excess gutta-percha was removed using a heated plugger. The access cavities were sealed with Cavit (3M; ESPE, St Paul, MN), after which the teeth were stored in an incubator at 37°C and 100% humidity for 24 hours to allow the sealers to set.

Each tooth was sectioned in a plane that was perpendicular to its long axis. Sections (500-μm thick) were produced with a slow-speed, water-cooled 0.3-mm microtome saw (Isomet Buehler). Sections were obtained at distances of 2, 5, and 8 mm from the root apex. Samples from each treatment group were then divided into apical sections (2 mm from the apex), middle sections (5 mm from the apex), and coronal sections (8 mm from the apex). All sections were polished with silicone carbide abrasive papers. The specimens were mounted onto glass slides and examined with a Leica TCS-SPE confocal microscope (Leica, Mannheim, Germany).

We evaluated images according to the method used by Gharib et al (14). First, each sample image was imported into Photoshop (Adobe Systems, Inc, San Jose, CA). In each sample image, the circumference of the root canal wall was outlined and measured with the Photoshop software measuring tool. Next, areas along the canal walls in which the sealer penetrated into dentinal tubules were outlined and measured using the same method. In cases with an isthmus connecting both canals, sealer penetration was measured to the beginning of the isthmus. The outlined distances were divided by the canal circumference to calculate the percentage of the area of each canal wall covered by sealer in that section. For measuring the depth of penetration, the point of deepest penetration was measured from the canal wall to the point of maximum sealer penetration.

Statistical analysis was performed using the Kruskal-Wallis nonparametric test for comparisons between groups. The Wilcoxon signed rank test (also a nonparametric analysis) was used for comparisons within groups. The level of significance was set at $P < .05$.

Results

The Kruskal-Wallis analysis results showed that there was a significant difference in the percentage of sealer penetration among all final irrigation groups at the apical, middle, and coronal sections ($P < .05$). The control group showed a significantly lower percentage of sealer penetration than the other groups in all sections ($P < .05$). There was no significant difference in the percentage of sealer penetration between all experimental groups in all sections. The highest percentage of sealer penetration was found in the coronal section. There was no significant difference between the apical and middle sections (Table 1).

There was a significant difference in the maximum depth of sealer penetration among all final irrigation groups in all sections ($P < .05$).The Wilcoxon signed rank test showed a significantly lower maximum depth of sealer penetration in the apical area than in the coronal area. There were significant differences between the apical and middle sections in all experimental groups but not in the control group. Sealer penetration in the control group was significantly lower than that in all experimental groups ($P < .05$) (Table 2). Figure 1*A* to *C* shows representative patterns of sealer penetration around root canal walls.

Discussion

The penetration of root canal sealer into the dentinal tubules is clinically important. Many studies have reported the penetration of sealer into root canal dentin (15–21). Scanning electron microscopy (SEM) (3, 18), light microscopy (15, 16), and confocal laser scanning microscopy (CLSM) (13, 17, 19, 20) have been used to analyze sealer penetration. In the present study, CLSM was used because this technique has several advantages over SEM. CLSM does

TABLE 2. The Maximum Depth of Sealer Penetration (in micrometers) into Dentinal Tubules at the Apical, Middle, and Coronal Sections

Group	Apical third (mean ± SD)	Middle third (mean ± SD)	Coronal third (mean ± SD)
17% EDTA + 2.5% NaOCl	620 ± 510	1270 ± 260	1550 ± 320
7% maleic acid + 2.5% NaOCl	560 ± 510	1250 ± 430	1560 ± 670
10% citric acid + 2.5% NaOCl	530 ± 280	1090 ± 380	1310 ± 330
Control	170 ± 80	420 ± 330	690 ± 580

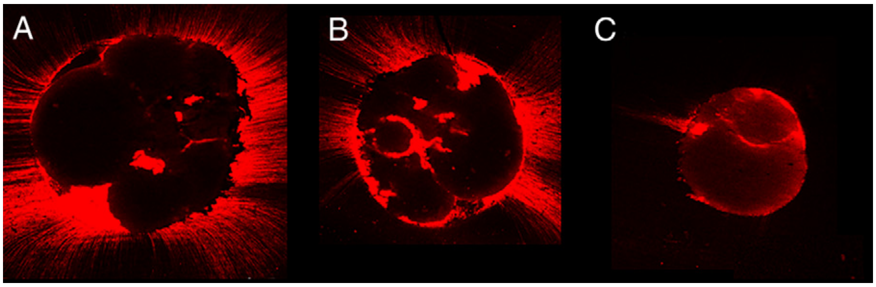

Figure 1. Representative confocal laser scanning microscopic images from the CA group. *A*, *B*, and *C* show the coronol, middle, and apical thirds of root canal, respectively.

not require any special specimen processing, and observations can be made under near normal conditions. The preparation of samples for CLSM also tends to produce fewer artifacts than does sample preparation for SEM.

The results of the present study showed that in all experimental groups the percentage area covered and the maximum depth of the sealer penetration were better in the coronal thirds than in the apical thirds of root canals. These results are in agreement with those of other studies (13, 15, 16, 17, 19). This may be a result of better removal of the smear layer in coronal thirds than in apical thirds of root canals. There are more dentinal tubules in the coronal area, and the diameters of the tubules in the coronal area are larger than those in the apical area (22).

For the effective removal of the smear layer, treatment with chelating agents such as EDTA, MA, BioPure MTAD (Dentsply Tulsa, Tulsa, OK), and CA is recommended (5, 23, 24). Recommended concentrations and length of exposure vary with each agent. Chelating agents withdraw calcium from the inorganic calcium phosphate crystal lattice; this results in demineralization of the superficial dentin layer and exposure of collagen fibrils of the organic matrix. Cruz-Filho et al (25) reported that 15% EDTA and 10% CA solutions significantly reduced the microhardness of the most superficial dentin layer from the root canal lumen. However, Galler et al (26) found that irrigation with EDTA might optimize the conditions for cellular differentiation, tissue formation, and regeneration through the exposure of growth factors trapped in the dentin matrix. The combined application of EDTA and NaOCl is commonly used for the removal of smear layer from the root canal system. EDTA is effective in smear layer removal from the coronal and middle thirds but not from the apical third (5, 24). Prabhu et al (27) showed that 5% MA and 7% MA are alternatives to the routine use of 17% EDTA. A recent study showed that a final irrigation with 7% MA is more efficient than that of 17% EDTA in the removal of the smear layer from the apical third of the root canal system. This may be caused by the higher surface tension of 17% EDTA compared with MA (5).

According to the results of the present study, there was no significant difference in the percentage and maximum depth of sealer penetration among all experimental groups; therefore, the null hypothesis tested in this study cannot be rejected. Many factors may influence the percentage and maximum depth of sealer penetration. These factors include the effectiveness of the removal of the smear layer, the obturation technique, the physical and chemical properties of the sealer, and the anatomy of the root canal system. The present study focused on the effects of different final irrigation solutions on sealer penetration into dentinal tubules. It is important to note that the obturation technique influences the percentage of sealer penetration into the root canal walls (15). De Deus et al (28) reported that the core-carrier–based Thermafil technique and warm vertical condensation technique produced significantly deeper sealer penetration than the cold lateral condensation technique. These results are similar to those of Ordinola-Zapata et al (20) who studied the percentage of sealer penetration in roots obturated with the Thermafil and RealSeal (SybronEndo, Orange, CA) obturation techniques. The authors concluded that both thermoplastic carrier–based systems allow adequate penetration into the root canals. In the present study, we chose a commonly used technique for obturation, cold lateral compaction of gutta-percha with sealer. Wu et al (29) evaluated the effect of obturation techniques on sealer distribution and reported that the area of sealer-coated root canal wall in the coronal area was significantly higher when lateral condensation was used than when vertical condensation was used. Furthermore, there was significantly more sealer in the lateral canals obturated using lateral condensation as measured by the ratio of gutta-percha and sealer in the main and lateral root canals. In contrast, thermoplastic carrier–based systems produced lateral canals filled with significantly more gutta-percha (30, 31).

The chemical and physical properties of sealer also influence the depth of penetration (32). Mamootil et al (18) noted that the epoxy resin–based sealer AH26 and the methacrylate resin–based sealer EndoREZ showed deeper penetration than the zinc oxide eugenol–based sealer Pulp Canal Sealer EWT (Kerr, Sybron Dental Specialties, Romulus, MI). This might be explained by the capillary action in the dentinal tubules and the fact that AH26 requires a longer setting time than EndoREZ. Sealer may be drawn into the tubules by capillary action and not by hydraulic forces created during root canal filling. Balguerie et al (33) showed that AH Plus sealer appeared to have the most optimal tubular penetration and adaptation to the root canal wall among the sealers tested. This may be explained by the high flow rate of AH Plus sealer. In addition to the effects of the chemical and physical properties of sealer, residual moisture in the root canal after drying reduced the penetration depth of epoxy resin–based sealer (34). Labeling of sealer with rhodamine B is essential to observe penetration using CLSM. Pilot studies were performed to determine whether labeled sealer and unlabeled sealer had different physicochemical properties. It was concluded that the sealer labeled with 0.1% rhodamine did not show changes in flow according to American Dental Association specifications (1, 20).

Within the limitations of this study, we observed that a final rinse with 17% EDTA, 7% MA, or 10% CA after a rinse with 2.5% NaOCl influenced the percentage and maximum depth of the sealer penetration. In all experimental groups, the percentage and maximum depth of penetration were better in the coronal thirds than in apical thirds. Therefore, the null hypothesis that the percentage and the maximum depth of sealer penetration are affected by the use of different final irrigation solutions was accepted.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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