Effect of Calcium Hydroxide Dressing on the Dentinal Tubule Penetration of 2 Different Root Canal Sealers: A Confocal Laser Scanning Microscopic Study

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Abstract

Introduction: The purpose of this study was to evaluate the effect of calcium hydroxide ($Ca[OH]_2$) dressing on the dentinal tubule penetration of epoxy resin–based sealer (AH 26; Dentsply Maillefer, Ballaigues, Switzerland) and tricalcium silicate–based sealer (BioRoot RCS; Septodont, Saint Maurdes Fosses, France). **Methods:** Fifty-two single-rooted mandibular premolars were used. Four samples were assigned as the positive control. Twenty-four samples received $Ca(OH)_2$ labeled with rhodamine B, whereas the rest did not. $Ca(OH)_2$ was removed with passive ultrasonic activation and copious irrigation 2 weeks later. Samples were further subdivided into 2 groups, and root canal fillings were performed with a single ProTaper F4 gutta-percha cone (Dentsply Maillefer) combined with 1 of the tested sealers labeled with fluorescein green. After 2 weeks, samples were transversely sectioned at the apical, middle, and coronal levels. The penetration depth and percentage were evaluated via imaging software. Statistical analysis was performed using Kruskal-Wallis, Siegel Castellan post hoc, and Mann-Whitney *U* tests at $P = .05$. **Results:** The mean dentinal tubule penetration depth and percentage values were lowest in the apical third for both sealers. BioRoot RCS showed higher penetrability in all thirds compared with AH 26 ($P < .05$) despite $Ca(OH)_2$ dressing remnants ($P < .05$). $Ca(OH)_2$ placement resulted in a shorter dentinal tubule penetration depth with BioRoot RCS statistically in the middle and coronal thirds ($P < .05$), whereas it did not affect the percentage ($P > .05$). **Conclusions:** Passive ultrasonic activation and copious irrigation were insufficient in removing $Ca(OH)_2$ from root canals. BioRoot RCS presented higher dentinal tubule penetration than AH 26 even in the presence of $Ca(OH)_2$ residues. $Ca(OH)_2$ remnants decreased both dentinal tubule penetration depth and the percentage of the tested sealers; however, a more drastic effect was observed for AH 26. *(J Endod 2018;■:1–6)*

Key Words

Calcium hydroxide, confocal laser scanning microscopy, dentinal tubule penetration, root canal sealer

Significance

It might be advantageous to fill the root canal with hydrophilic sealer such as calcium-silicate-based sealer instead of hydrophobic epoxy resin-based sealer following $Ca(OH)_2$ removal for ensuring higher penetration of root canal sealer into the dentinal tubules.

The main purpose of root canal treatment is to eliminate the existing infection and to protect the decontaminated tooth from future microbial invasion. Numerous devices and materials have been introduced to remove the sources of infection from the root canal system, including various mechanical instrumentation techniques, irrigation regimens, and intracanal medicaments (1). Calcium hydroxide ($Ca[OH]_2$) has been used extensively in endodontic therapies for the disinfection of infected root canals since Hermann introduced it in 1920 (2). Different biological properties of $Ca(OH)_2$, such as antimicrobial activity, tissue dissolving ability, inhibition of tooth resorption, and hard tissue formation, have been evaluated, and its widespread use in root canal treatment has been associated with periradicular healing and few adverse reactions (3). However, $Ca(OH)_2$ has some limitations. According to the current data, there are no available methods or irrigants that can remove all $Ca(OH)_2$ remnants from the root canal (4, 5), and as a result these remnants have different effects on the bonding capability of root canal sealers to dentin (6), the penetrability of sealers into dentinal tubules (7–9), and the sealability of root canal sealers (10). The complete and predictable removal of $Ca(OH)_2$ dressing before root canal filling is critical and could be directly related to the outcome of treatment (4).

The most preferable obturation materials are gutta-percha and different types of root canal sealers. Root canal sealers, unlike gutta-percha, are able to penetrate into dentinal tubules, isthmuses, and accessory canals (7–11). Sealer penetration into dentinal tubules might provide entombing of microorganisms in these areas, far away from nutrition (12, 13). Variations in the physical and chemical properties

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such as particle size, solubility, viscosity, and surface tension of sealers could influence the amount of penetration depth (11). Epoxy resin–based sealers such as AH 26 (Dentsply Maillefer, Ballaigues, Switzerland) and AH Plus (Dentsply Maillefer) are accepted as a gold standard and are used during testing of different specifications of newly manufactured root canal sealers (11, 14, 15). BioRoot RCS (Septodont, Saint Maurdes Fosses, France) is a powder/liquid hydraulic tricalcium silicate–based sealer marketed recently and recommended for the single-cone technique or cold lateral condensation root filling (16). The powder contains tricalcium silicate, povidone, and zirconium oxide; the liquid is an aqueous solution of calcium chloride and polycarboxylate. BioRoot RCS has bioactivity with calcium release, strong alkalizing activity, apatite-forming ability, and adequate radiopacity (16). It was reported that AH Plus penetrated deeper compared with BioRoot RCS (15).

The aim of this study was to evaluate the effect of $Ca(OH)_2$ dressing on the dentinal tubule penetration of an epoxy-based sealer (AH 26) and a tricalcium silicate–based sealer (BioRoot RCS). The null hypothesis was that there is no difference between the 2 sealers regarding penetrability parameters observed after the removal of $Ca(OH)_2$ dressing under a confocal laser scanning microscope (CLSM).

Materials and Methods

This study was approved by the Noninterventional Clinical Research Ethics Board of Hacettepe University, Ankara, Turkey (GO 17/782-13). Fifty-two mature single-rooted, single-canaled, round-shaped mandibular premolars without calcifications, cracks, and fractures in roots were selected. Teeth were decoronized with a diamond disc under water cooling to standardize a length of 16 mm. The working length was established 1 mm short from the root apex. Four samples randomly chosen represented the positive control group, and the remaining 48 samples were randomly assigned into 2 experimental groups (n = 24/each group).

Sample Preparation

Positive Control Group. Four root canals were prepared up to F3 with the ProTaper System (Dentsply Maillefer) according to the manufacturer's instructions. Irrigation was performed with 2 mL 2.5% sodium hypochlorite (NaOCl) at each change of instrument, and a final rinse was performed with 5 mL 17% EDTA for 3 minutes. Then, canals were irrigated with 5 mL distilled water and dried with paper points. The canals were filled with $Ca(OH)_2$ dressing mixed with 0.1% rhodamine B (Sigma-Aldrich, St Louis, MO). The dressing was prepared by mixing $Ca(OH)_2$/rhodamine B powders with distilled water. The powder/liquid ratio of the dressing was 1:1 (7). The prepared dressing was placed into the root canals using a size #30 Lentulo spiral (Dentsply Maillefer). The coronal openings of the root canals were sealed with a small cotton pellet and temporary filling material (META Biomed Co Ltd, Cheongju, Korea) to avoid leakage. The specimens were stored at 37°C in 100% humidity for 2 weeks (17). The positive control group samples were prepared to examine the penetration ability of $Ca(OH)_2$ labeled with rhodamine B.

Negative Control Group. Twenty-four root canals were prepared up to F4 with the ProTaper System according to the manufacturer's instructions with no prior intracanal $Ca(OH)_2$ placement. Irrigation was performed with 2 mL 2.5% NaOCl at each change of instrument, and a final rinse was performed with 5 mL 17% EDTA for 3 minutes and 5 mL 2.5% NaOCl.

$Ca(OH)_2$ Group. Twenty-four root canals were prepared up to F3 with the ProTaper System and filled with $Ca(OH)_2$ dressing as in the positive control group. After the incubation period, 24 samples were instrumented with ProTaper F4 and #40 H files (Dentsply Maillefer) in order to remove $Ca(OH)_2$ mechanically, and samples were irrigated with 5 mL 17 % EDTA and 5 mL 2.5 % NaOCl. Passive ultrasonic irrigation was also performed with a Satelec P5 Newtron XS ultrasonic system handpiece (Acteon Group, Merignac, France) equipped with a smooth wire at 1 mm short of the working length as described previously (5).

Filling of Samples

Root canals of samples were irrigated with 5 mL distilled water and dried with paper points, except in the positive control group. Samples in the negative control and $Ca(OH)_2$ groups were further divided into 2 subgroups (n = 12) according to the root canal sealer used, AH 26 or BioRoot RCS. Each sealer was mixed with 0.1% fluorescein (Sigma-Aldrich). The fluorescein-sealer mixture was delivered into the canal with a Lentulo spiral carrier size 40 (Dentsply Maillefer). An F4 gutta-percha cone was entirely coated with a labeled sealer and then inserted into the root canal. A size B finger spreader (Dentsply Maillefer) was used to introduce accessory cones (which were also coated with a sealer) until complete obturation of the root canal. All procedures were performed by 1 observer (O.E.)

Confocal Laser Scanning Microscopic Analysis

After the final obturation, samples (n = 48) were stored for 15 days (37°C, 100% relative humidity) to permit complete setting of the sealers. Samples (N = 52) were embedded into resin blocks. Then, they were sectioned transversely with a cutting machine (Isomet 1000; Buehler, Lake Forest, IL), under water cooling at 2, 5, and 8 mm from the apex to represent the apical, middle, and coronal thirds, respectively. The slices were photographed under a CLSM (Zeiss LSM 510; Carl Zeiss, Jena, Germany) and a method of epifluorescence with wavelengths of absorption and emission for rhodamine B of 540/590 nm and for fluorescein of 536/617 nm.

The images were analyzed in the CLSM Image Browser (Carl Zeiss) to measure the longest penetration depth of sealer and the percentage of penetrated sealers into the dentinal tubules as shown in Figure 1. Measurements were performed by 1 observer who was blinded to the groups (E.U.O) and repeated 2 times for intrarater reliability.

Statistical Analyses

Statistical analysis was performed using SPSS Version 22 (IBM Corp, Armonk, NY). Because the data sets for sealers did not show a normal distribution with the Shapiro-Wilk normality test, the Wilcoxon signed rank test was used to compare intrarater reliability. Differences among the coronal, middle, and apical thirds of similar samples were compared using the nonparametric Kruskal-Wallis test followed by the post hoc Siegel Castellan tests. The Mann-Whitney U test was used to compare the effect of different sealers and the presence of $Ca(OH)_2$ dressing remnants in respective root canal thirds. The threshold for statistical significance was set at $P < .05$.

Results

The Wilcoxon signed rank test value was $P > .05$ for intrarater reliability; thus, the average values of measurements were used in statistical analysis. Tables 1 and 2 show the results of the present study. Figure 2 shows the representative confocal laser scanning microscopic images of the experimental groups.

Dentinal Tubule Penetration Depth

The highest measurement was obtained in the samples filled with BioRoot RCS and gutta-percha, whereas the lowest was obtained in