The influence of calcium hydroxide on adaptation and root canal penetration in teeth filled with methacrylate-based resin sealer

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ABSTRACT

Objective: The aim of this study was to evaluate, by confocal laser scanning microscopy (CLSM), the effect of $Ca(OH)_2$ dressing on the depth of dentinal tubule sealer penetration and the percentage of sealer-dentin adaptation in the coronal, middle, and apical thirds of teeth filled with methacrylate-based resin sealer (Epiphany). **Methods:** A total of 30 extracted single-rooted human mandibular incisors were instrumented up to file #40.04 (ProFile), with 1.0% NaOCl as irrigating solution. The teeth were randomly divided into three groups (n = 10): GI = no $Ca(OH)_2$ dressing (control group); GII = $Ca(OH)_2$ for 14 days + removal with saline solution and #40 K-file; and GIII = similar to Group II plus 17% EDTA for dressing removal. The Epiphany sealer was labeled with Rhodamine B dye, and all canals were filled with the Epiphany system. Three sections of each tooth were sectioned and viewed under 5X and 40X magnifications. **Results:** ANOVA and Tukey tests showed significantly lower values of sealer penetration depth in the apical third compared to the middle and coronal thirds ($p < 0.05$). The deepest sealer penetration was observed in the coronal and middle thirds of Group II. Groups II (93%) and III (86%) had the highest percentage of adaptation compared with Group I (78%) ($p < 0.05$). **Conclusions:** $Ca(OH)_2$ favors the depth of methacrylate-based sealer penetration and the percentage of sealer-dentin adaptation in teeth filled with the Epiphany system.

Keywords: Calcium hydroxide. Confocal laser scanning microscopy. Fluorescent dye. Root canal filling.

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Introduction

The tridimensional filling of cleaned and shaped root canals is one of the main steps in endodontic therapy.[1] The objective of root canal filling is to avoid coronal leakage of oral bacteria or their by-products to prevent recontamination of the root canal system. In infected root canals, $Ca(OH)_2$ therapy aids disinfection and assists the re-establishment of periapical tissues.[2]

The Epiphany obturation system (Pentron Clinical Technologies, Wallingford, CT, USA) consists of an adhesive compound material with Resilon, a self-etching primer and a dual-curable resin sealer. The manufacturer claims that this system can bond to canal walls, creating a "monobloc," which consists of the chemical adherence of the Epiphany sealer to the Resilon cones and the root canal walls, thereby resulting in hybridization of intra- and intertubular dentin.[3,4] Previous studies have shown that Resilon fillings resist bacterial leakage compared with gutta-percha fillings.[3,4,5] Wang et al,[6] in a microbial leakage model, stated that the sealing ability of the Epiphany system was not influenced by $Ca(OH)_2$. However, Barbizam et al[7] stated that the dressing could adversely affect the adhesion of the Epiphany sealer to root canal walls.

The sealer-dentin interface provided by the Epiphany system[8] and the average depth of sealer penetration[9] have been evaluated by confocal microscopy and have shown a high percentage of adaptation and deep sealer penetration into dentinal tubules. Another study,[10] which used environmental scanning electron microscopy in canals treated with $Ca(OH)_2$, indicated good adhesion with the Resilon-Epiphany sealer, with some gap-free regions and good tag formation. Conversely, Gesi et al[11] reported poor bond strength at the Epiphany sealer-root canal wall interface. De Deus et al,[12] who used CLSM, also confirmed that the quality of the interfacial adaptation of the Epiphany root filling was compromised, even in teeth with simple anatomical features.

$Ca(OH)_2$ dressing has been applied to enhance disinfection after the root canal is cleaned and shaped.[2,13] However, residual $Ca(OH)_2$ may remain and influence the sealing ability and adaptation of the sealer in dentinal tubules, either due to blocking the entry[14] to the dentinal tubules or remaining on the root canal walls.[15,16]

Thus, the aim of this *in vitro* study was to evaluate, by confocal laser scanning microscopy (CLSM), the influence of $Ca(OH)_2$ dressing on the percentage of sealer-dentin adaptation to root canal walls and the average depth of sealer penetration into dentinal tubules filled with the Epiphany system. The null hypothesis is that $Ca(OH)_2$ dressing favors interfacial adaptation of sealer to root canal walls and penetration depth of the Epiphany system into dentinal tubules.

Material and Methods

This study was reviewed and approved by the Ethics Committee on Human Research (protocol #57/2008) of Universidade de São Paulo (USP), School of Dentistry, Bauru, São Paulo, Brazil. A total of 30 mandibular single-rooted central incisors were stored in saline solution containing 0.1% sodium azide. With the aid of an optical light microscope (DF Vasconcelos, São Paulo, Brazil), under 8X magnification, the absence of cracks and apical resorption was verified. Periapical radiographs revealed the presence of a single canal. Access openings were made with a #56 bur (Henry Schein, Melville, NY, USA). A #10 K-file was placed until just visible at the apex, and 1 mm was subtracted to establish the working length. Root canal shaping was then performed with the ProTaper system (Dentsply Maillefer) up to a F2 instrument, and complemented with Profile (Dentsply Maillefer) 35/0.4 and 40/0.4 instruments at the working length. Complementary hand files were applied by the balanced force technique[17] up to a #40 K-file at the working length. Irrigation procedures were carried out by the use of 2 mL of 1.0% sodium hypochlorite for each file used. To remove the smear layer, all canals were irrigated with 3 mL of 17% EDTA for three minutes, followed by 2 mL of 2.0% sodium hypochlorite for one minute. For all groups, a final rinse of 5-mL saline solution was used according to the manufacturer's instructions, and all root canals were dried with sterile paper points. The 30 teeth were randomly divided into three groups, according to the presence or absence of $Ca(OH)_2$ dressing: GI = no $Ca(OH)_2$ dressing (control group); GII = $Ca(OH)_2$ dressing (14 days) and removal with saline solution and a hand file; and GIII = $Ca(OH)_2$ dressing (14 days) and removal with saline solution, a hand file, and 17% EDTA. The dressing was placed in the canals by means of a Lentulo spiral.

One tooth from each group was randomly selected, and its dressing was labeled with fluorescein isothiocyanate dye (FITC) (Sigma-Aldrich, St. Louis, MO, USA) for confocal analysis to verify the presence of $Ca(OH)_2$ paste in dentinal tubules (Fig 1). After 14 days stored at approximately 37 °C and in 100% humidity, the dressing was removed. In Group II, the dressing was removed with 5 mL of saline solution and a #40 K-file at the working length. In Group III, the dressing was removed similarly to Group II, but with 17% EDTA left for three minutes.

Subsequently, all canals were irrigated with 5 mL of saline solution and dried with sterile paper points. The sealer was then mixed according to the manufacturer's instructions. To allow for analysis by CLSM, the sealer was labeled with Rhodamine B dye (Sigma–Aldrich) in 0.1% concentration determined by a precision analytic balance (Mettler Toledo, Jersey City, NJ, USA). The sealer was inserted by means of a Lentulo spiral up to 2-3 mm short from the apical foramen, and a 40/.04 Resilon point (Resilon, Resilon Research LLC, Madison, CT, USA) coated with sealer was inserted into the canal.

The rest of the canal was filled by lateral compaction technique with a #20 NiTi endodontic finger spreader (Dentsply Maillefer Instruments SA, Ballaigues, Switzerland), inserted 2-3-mm short from the working length, and fine-to-medium-sized accessory Resilon cones. Excess Resilon was removed by means of a heated plugger, and vertical compaction was performed at the orifice level. The specimens were light-cured for 40 seconds. The cervical portions of the specimens were sealed with a provisional filling material (Coltosol, Coltène, Altstätten, Switzerland). Specimens were stored at 37 °C and 100% humidity for seven days.

Three horizontal 0.3-mm sections were cut at the 3-mm, 7-mm, and 10-mm levels from the apical foramen by means of an Isomet saw (Buehler, Lake Bluff, IL, USA), at 200 rpm, under copious irrigation (5 °C) to prevent frictional heat. The polishing of the samples was performed with a polishing machine (Politriz, Arotec, Cotia, São Paulo, Brazil). The dentin segments were examined by an inverted Leica TCS-SPE confocal microscope (Leica, Mannheim, Germany). The respective absorption and emission wavelengths for fluorescein and Rhodamine B were 488/530 nm and 532/580 nm. Dentin samples were analyzed under 5X and 40X magnification by oil lenses. Layers to be visualized were selected 10 μm below the sample outer surface.[18] We obtained the 5X images by scanning a single section, whereas the 40X images were taken from 10 sections with a size of 0.5 μm. The images were acquired with LAS-AF software (Leica), with a resolution of 1024 x 1024 pixels. Using the ruler tool of the LAS-AF software, we measured the depth of sealer penetration and recorded it at four standardized points from each 5X image, according to Bitter et al[18] and Gharib et al[8] (Fig 1). The canal wall served as the starting point, and sealer penetration into dentinal tubules was measured to a maximum depth of 2000 μm.

The percentage of adaptation was considered as the region where the Resilon-Epiphany sealer adhered to the canal walls. Areas with gaps or where the Resilon cone came into direct contact with the root canal wall were considered as nonadaptation zones. We estimated the percentage of adaptation by calculating the ratio of the circumference of the canal (root canal perimeter) to the gap-containing regions. These data were averaged to obtain a single measure for each section for the three groups.

First, to calculate the percentage of sealer penetration around the root canal walls, we imported each image into the Image Tool software V.3 (UTHSCSA, Houston, TX, USA) and measured the circumference of the root canal wall (Fig 1A). Thereafter, the areas along the canal walls with sealer between the dentin wall and Resilon cones were outlined and measured by the same methods (Fig 1B). Subsequently, the percentage of the root canal wall with sealer adaptation in that section was established.

To evaluate the dentinal tubules with fluorescence from the Rhodamine dye, marked by the sealer, we used the orthogonal section tool (Z optical section) of the LAS software (Leica) in representative sections, with the 40X oil lens with additional 3X zoom, to confirm the content of the dentinal tubules.

Statistical analysis of depth penetration and percentage of adaptation of root canal sealers was determined for each level of the root canal by analysis of variance (ANOVA) followed by Tukey test. The significance level was established as 5%. All tests were analyzed by GraphPad prism V5.0 software (GraphPad, La Jolla, CA, USA).

Results

Tables 1 and 2 shows the mean depths of sealer penetration and the percentages of sealer adaptation.

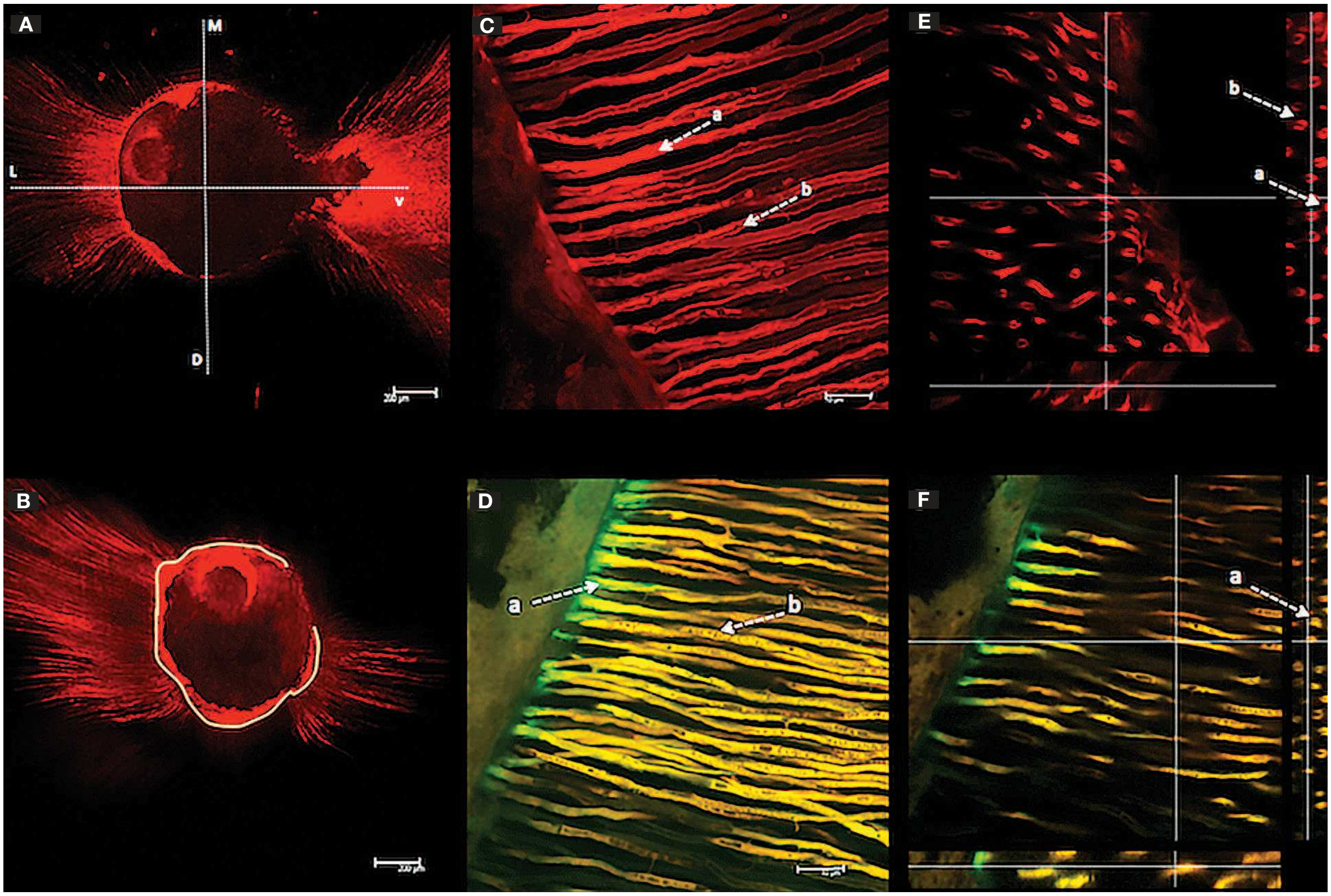

Figure 1. A, **B**) Confocal laser scanning microscopy images, 5X lens. **A**) The four points used for evaluation of the depth of sealer at four standardized points (V, L, M, D). **B**) Analysis of adapted zones to canal walls. **C** and **D**) Confocal laser scanning microscopy images, 40X lens. (**C**) Different patterns of complete tubule fillings, complete (**a**) and peripheral (**b**). **D**) $Ca(OH)_2$ on the canal walls (**a**) and filling of tubules with sealer and dressing (**b**). **E** and **F**) Orthogonal section tool (Z optical section) in confocal microscopy. **E**) Confirmation of the different patterns complete (**a**) and peripheral (**b**) dentinal tubules filled with sealer. **F**) Indicates the presence of $Ca(OH)_2$ and sealer in dentinal tubules (**a**).

Table 1. Means and standard deviations (SD) of sealer penetration depths (SPD) in micrometers.

	Group I	Group II	Group III
Coronal	$997.9 \pm 263.5^{a,AB}$	$1287.8 \pm 246.1^{a,B}$	$769.8 \pm 301.9^{a,A}$
Middle	$774.5 \pm 297.9^{a,A}$	$1217.6 \pm 197.1^{a,B}$	$668.4 \pm 208.9^{a,A}$
Apical	$339.8 \pm 134.1^{b,A}$	$287.7 \pm 167.9^{b,A}$	$252.1 \pm 173.6^{b,A}$

Lowercase superscript letters indicate statistically significant differences among thirds in the same groups. Uppercase superscript letters indicate differences among groups in each third ($p < 0.05$).

Table 2. Means and standard deviations (SD) of the percentages of gap-free regions in all thirds and evaluated groups.

	Group I	Group II	Group III
Coronal	$78 \pm 8.8^{a,A}$	$98 \pm 4.0^{a,B}$	$88 \pm 8.6^{a,C}$
Middle	$80 \pm 12.3^{a,A}$	$91 \pm 8.3^{a,B}$	$87 \pm 5.4^{a,AB}$
Apical	$75 \pm 8.0^{a,A}$	$91 \pm 9.7^{a,B}$	$84 \pm 13.9^{a,AB}$

Lowercase superscript letters indicate statistically significant differences among thirds in the same groups. Uppercase superscript letters indicate differences among groups in each third ($p < 0.05$).

The depth of sealer penetration was significantly lower in the apical thirds than in the coronal and middle thirds ($p < 0.05$). Sealer penetration in the middle third was significantly deeper in Group II than in Groups I and III, and in the coronal section only of Group III ($p < 0.05$). The highest percentage of adaptation was observed in Group II, followed by Group III, with statistically significant differences among all groups in the cervical portion ($p < 0.05$). In the apical portion, statistically significant difference occurred between Groups I and II ($p < 0.05$). The 40X CLSM images showed different sealer and dressing patterns inside the dentinal tubules, with complete or partial filling of sealer and dressing (Fig 1C and 1D). The $Ca(OH)_2$ group analysis indicated the simultaneous presence of sealer and dressing (Fig 1F, a).

Discussion

The null hypothesis was accepted because $Ca(OH)_2$ dressing favored interfacial adaptation and dentinal tubule sealer penetration depth of the Epiphany system.

CLSM has been applied to evaluate the penetration and interfacial adaptation of root canal sealers. This methodology offers panoramic visualization of sealer adaptation and penetration into dentinal tubules.[8,9] At high magnification (40X), the confocal software is able to build 3D images to facilitate visualization of the filling patterns inside dentinal tubules. Rhodamine B was used in minimal quantities (0.1%). The labeling of the sealer with Rhodamine B favored the analysis of depth penetration and adaptation to the canal walls, as seen in previous studies.[8,9]

Hence, a complete view of sealer adaptation and penetration into the root canal and dentinal tubules can be easily confirmed at higher magnifications. In this case, we observed different patterns of sealer penetration, including total and peripheral filling of the dentinal tubules.

Our results showed that the penetration depth of the sealer decreased from the coronal to the apical thirds. These findings are in agreement with those of previous studies and can be explained by the differences in numbers and diameters of dentinal tubules.[19,20,21] Sealer penetration may be influenced by dentin permeability,[22] filling technique,[23] and the physical-chemical properties of the sealer.[22] The type of sealer may influence its ability to penetrate dentinal tubules. The superior mean of sealer penetration in Group II may be explained by the potential of calcium hydroxide to reduce superficial tension, including some substances known to have high superficial tension, such as water;[24] thus, facilitating sealer penetration. This beneficial effect was decreased in Group III, which had the action of an acid, even though the use of EDTA is recommended to improve dentin hybridization of methacrylate-based sealers.[25] Other point to be considered is the fact that NaOCl and EDTA have high superficial tension similarly to water.[26] In addition, the hygroscopic properties of $Ca(OH)_2$ and propylene glycol used as a vehicle may have dehydrated the dentin; thus, improving sealer penetration.[27] A previous study[9] indicated that different types of material present distinct sealer penetration.

Nevertheless, some authors have reported that $Ca(OH)_2$ dressing can block the entrance of sealer into dentinal tubules.[14,15] Groups II and III showed deeper sealer penetration than the control group (Group I), particularly in the cervical and middle thirds of Group II. The dressing was beneficial: once combined with the Epiphany sealer, the $Ca(OH)_2$ paste did not inhibit sealer penetration. The use of 5.25% NaOCl solution associated with EDTA has been shown to be more effective in removing $Ca(OH)_2$ in the dentinal tubules and root canal walls,[15,28] even though residual dressing has been observed by scanning microscopy in these areas.[29] Saline solution and hand files were ineffective in removing $Ca(OH)_2$ for evaluation of its interference with sealer penetration and its adaptation to root canal walls. In this study, saline solution was chosen as a final rinse, according to the manufacturer's instructions. Similarly to our results, some studies have indicated that the presence or absence of $Ca(OH)_2$ dressing has not been shown to have adverse effects on the sealing ability of Resilon-Epiphany fillings.[6,28]

In the present study, using CLSM under 40X magnification, we observed the presence of fluorophores inside dentinal tubules and classified the patterns and the contents of fillings according to the fluorescence emitted and their coloring. The excitation and emission wavelengths for fluorescein are shorter and might overlap with those of Rhodamine B. To avoid some false positive signals,[30] this study used appropriate filters on the laser-scanning microscope, which enables each dye emission to be examined separately in the same sample, thereby greatly reducing image artifacts. Thus, we could observe that each dye was separately stimulated and then the green, red and orange colors could be viewed when there were calcium hydroxide, sealer or both, respectively. In Group I, the presence of sealer alone allowed us to notice, in red, the high intensity of fluorescence and complete filling of dentinal tubules. Lower fluorescence represented partial or incomplete filling of the dentinal tubules. Thus, in Groups II and III, $Ca(OH)_2$ was observed as green fluorescence. The overlaid images of the sealer and dressing indicated an exclusive zone of dressing in the root canal wall. Leakage studies indicated that $Ca(OH)_2$ dressing did not affect the sealing ability of the Resilon system.[6,16,28]

However, the presence of residual $Ca(OH)_2$ affected zinc oxide and eugenol sealer adaptation, leading to higher rates of leakage.[16] Our results indicated that the $Ca(OH)_2$ dressing favored the adaptation of the Epiphany sealer, because higher percentages of sealer adaptation were observed in Groups II and III. Babizam et al[7] stated that $Ca(OH)_2$ might affect adhesion strength in canals filled with Epiphany sealer. The results of Gesi et al[11] in canals without dressings are in agreement with theirs. In cases without $Ca(OH)_2$ dressing, the canals filled with the Epiphany system presented gap-free regions at the dentin wall and filing material interface.[3] However, Tay et al[10] confirmed the presence or absence of regions with gaps between the Epiphany sealer and root canal wall, corroborating the results of our Group I. In addition, they observed the presence of a hybrid layer along the dentin surface and around dentinal tubules.

Conclusion

$Ca(OH)_2$ dressing positively affected the depth of sealer penetration and percentage of sealer-dentin interface in root canals filled with Epiphany resin-based sealer.

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