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Effect of ethanol-conditioned dentine on sealer penetration into dentinal tubules: a confocal microscopy study

Carlos Augusto de Morais Souto Pantoja[1], Diogo Henrique da Silva[1], Adriana de Jesus Soares[1], Caio Cezar Randi Ferraz[1], Brenda Paula Figueiredo de Almeida Gomes[1], Alexandre Augusto Zaia[1], José Flávio Affonso de Almeida[1,*]

[1] Department of Restorative Dentistry, Endodontic Division, Piracicaba Dental School, University of Campinas, Piracicaba, SP, Brazil.

Aim: The aim of this study was to evaluate the effect of ethanol-conditioned dentin on endodontic sealer penetration into dentinal tubules by confocal laser scanning microscopy (CLSM). **Methods:** Forty human maxillary anterior teeth were instrumented and divided into four groups (n = 10) according to the drying methods: 1) wet: vacuum only, 2) paper points: vacuum + absorbent paper points, (3) 70% ethanol: 70% ethanol (1 min) + vacuum + absorbent paper points, and (4) 100% ethanol: 100% ethanol (1 min) + vacuum + absorbent paper points. All root canals were filled with resin-based endodontic sealer. Four sections from each third (cervical, middle, and apical) were examined by CLSM. Root canal wall perimeter infiltrated by sealer, maximum depth of sealer penetration, percentage of penetrated area, and fluorescence intensity of rhodamine B were measured. Statistical analysis was performed by analysis of variance and Tukey's tests (α = 0.05). **Results:** No statistical difference was found when percentage of root canal wall coverage infiltrated by sealer were compared. The groups in which ethanol solutions were used presented greater depth of sealer penetration, higher percentage of penetrated area, and higher fluorescence intensity of rhodamine B ($p< 0.05$) when compared with the wet and paper point groups. Overall, 100% ethanol produced better results than 70% ethanol, except for rhodamine B intensity (cervical third). In addition, the absorbent paper points drying method behaved better than did vacuum only group, except for rhodamine B intensity (apical third). **Conclusion:** Ethanol-conditioned dentin improved the penetration of resin-based sealer into dentinal tubules, especially at the concentration of 100%.

Keywords: Microscopy, confocal. Dentin. Endodontics. Ethanol. Wettability. Resin cements.

Corresponding author:
José Flávio Affonso de Almeida
Assistant Professor, Department of Restorative Dentistry, Endodontic Division
Address: Piracicaba Dental School
University of Campinas
P.O. Box 52
13414-903, Piracicaba, SP, Brazil
Phone: 55 19 21065344
E-mail: jfa.almeida@gmail.com

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Introduction

Bacteria causing persistent endodontic infections are usually located in areas unaffected by instruments and antimicrobial agents, including anatomically complex areas of the root canal system, especially dentinal tubules[1,2]. Sealer penetration into these spaces is considered a desirable outcome. In addition to their antibacterial effect, sealers can penetrate into dentinal tubules, entombing residual bacteria[3,4]. However, many factors may influence the penetration of the filling material, such as the physicochemical properties of the sealer, filling techniques, and different levels of residual moisture in the root canal[5]. Clinicians should be aware of these characteristics and try to establish a protocol that can promote greater percentage and maximum depth of sealer penetration into dentinal tubules[6].

Final irrigation of root canals with different chemical solutions has been proposed[5,7-9] to improve sealer penetration into dentinal tubules. For many years, drying the dentine walls prior to filling procedures has been carried out with vacuum cleaner tips and absorbent paper points[2,3,7]. Ethanol solution is a dehydrating agent[10] that has been tested to improve dentine wettability and to increase sealer penetration along the root canal walls[8]. Some authors suggest there is a higher compatibility between ethanol-saturated dentine and hydrophobic resin monomers, preventing collagen shrinkage and allowing for higher impregnation[11], infiltrating hydrophobic monomers into the demineralised dentine collagen matrix[12-14]. The use of 70% or 100% ethanol increased dentine wettability and improved the interaction between resin-based endodontic sealer and dentine root canal walls[15]. These findings suggest that different ethanol concentrations could enhance sealer penetration into dentinal tubules and corroborate the better endodontic treatment outcomes.

Hence, the aim of this study was to evaluate the effect of ethanol-conditioned dentine on endodontic sealer penetration into dentinal tubules by confocal laser scanning microscopy (CLSM).

Materials and Methods

Specimen preparation

Forty recently extracted human maxillary anterior teeth were selected. The teeth were cleaned with an ultrasonic scaler and washed with saline solution. Preoperative mesiodistal and buccolingual radiographs were taken to ensure the presence of a single root canal. The teeth were stored in 0.2% thymol solution at 4 °C. The crowns were removed using a 0.3-mm saw microtome (Isomet 1000; Buehler, Lake Bluff, IL, USA) to standardise root length at 18 mm. The canals were accessed and the working length established. All canals were instrumented with the crown-down technique using rotary nickel-titanium K3 files (SybronEndo, Glendora, CA, USA) according to the "Procedure pack" guidelines for instrumentation. The canals were prepared up to apical size 25/06. After each instrument change, the canals were irrigated with 5 mL of saline solution and filled with 2% chlorhexidine gel, an auxiliary chemical substance (Endogel, Essencial Farma – Itapetininga, SP, Brazil). Apical patency was maintained by passing # 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) through the apical foramen between files. The canals were then irri-

gated with 3 mL of 17% ethylenediaminetetracetic acid for 3 min, followed by a final rinse with 5 mL of saline solution. All irrigation solutions and auxiliary chemical substances were introduced into the root canal using a 5-mL disposable plastic syringe (Ultradent Products Inc, South Jordan, UT, USA) and a 20 x 0.55 mm (24 G) needle (Becton Dickinson (BD) – Curitiba, Brazil) inserted 2 mm short of the working length.

The samples were randomly divided into four groups (n=10) according to the drying methods: G1 – wet: the root canals were dried for only 5 s using a capillary tip 0.014 attached to the vacuum adapter (Ultradent Products Inc, South Jordan, UT, USA); G2 – paper points: the root canals were dried with a capillary tip 0.014 attached to the vacuum adapter dried for 5 seconds, followed by the use of two absorbent paper points (Endo Points Industrial Amazônica, Manacapuru, AM, Brazil); G3 – 70% ethanol: the canals were filled with 70% ethanol using a 5-mL disposable plastic syringe (Ultradent Products Inc, South Jordan, UT, USA) with 20 x 0.55 mm (24 G) needle inserted 2 mm short of the working length. After 1 min, the ethanol solution was removed with a 0.014 capillary tip attached to the vacuum adapter dried for 5 s then with the use of two paper points; and G4 – 100% ethanol: the same procedure applied in G3 was performed using 100% ethanol. The canals were then obturated with AH Plus (Dentsply – Konstanz, Germany) and Autofit gutta-percha cones (Analytic Endodontics, Orange, CA, USA) using Schilder's technique. The sealer was labelled with 0.1% fluorescent rhodamine B (Sigma-Aldrich, St. Louis, MO, USA) for CLSM[9,15,16]. In endodontics, CLSM is used to determine the degree of adaptation and penetration of the root canal filling into dentine walls and into dentinal tubules, respectively[17]. Rhodamine must be incorporated with cement at a ratio of 0.1%. It did not interfere with the physicochemical properties of endodontic sealers[13,16]. Autofit gutta-percha cones were coated with sealer and placed 2 mm short of the working length. Radiographs were exposed from facial and proximal surfaces to make sure no voids were present. The access cavities were sealed with intermediate restorative material (Coltosol, Vigodent / Coltene – RJ, Brazil). The teeth were stored in an incubator at 37 °C and 100% relative humidity for 24 h to allow sealers to set.

The roots were sectioned perpendicularly (1 mm thick) with a slow-speed, water-cooled 0.3-mm saw microtome (Buehler Isomet- Lake Bluff, IL, USA). Four sections from each third were obtained at distances of 2, 6, and 10 mm from the apex. After sectioning, gutta-percha was gently removed by a probe, without touching in sealer or dentine areas, removed from the root canal and the sections were polished manually with silicone carbide abrasive papers. The specimens were mounted onto glass slides and examined with a Leica TCS-SPE confocal microscope (Leica, Mannheim, Germany) and Leica Microsystems software (LAS-AF).

CLSM analysis

Six images from each section were obtained (Fig 1A), merged, and exported to Image J software (Fig 1B). The image of the final section was evaluated by the depth of sealer penetration into dentinal tubules at four different points.

Percentage of root canal wall coverage

In each image of the sections, the circumference of the root canal wall was outlined and the perimeter was measured with the Image J software measuring tool (Fig 1C).

Then, the areas along the canal walls in which the sealer had penetrated into dentinal tubules were outlined and measured by the same method (Fig 1D). The outlined distances were divided by the circumference of the canal to calculate the percentage of each canal wall area covered by sealer in that section. The depth of sealer penetration did not matter in this evaluation.

Maximum depth of sealer penetration

The depth of sealer penetration into dentinal tubules was measured at four standardised points in each image (Fig 1E). They were 2 pairs of points, each one located on left and right, up and down sides of each root third slice, centralizing the sample. The canal wall was the starting point, and the final point of each pair marked the maximum depth of sealer penetration into dentinal tubules (Fig. 1F) which was measured on each slice of each third root section for each section, so an average was obtained and used.

Percentage of penetrated area

The limits of each section and root canal wall were outlined and the area was measured with the Image J software measuring tool (Fig 1G). Thus, it was possible to calculate only the dentine wall area, by subtracting the root canal space. Then, the areas along the dentine in which the sealer had penetrated into dentinal tubules were outlined and measured using the same method (Fig 1H). The total area into which the sealer had penetrated was divided by the dentine wall area to calculate the percentage of penetrated area.

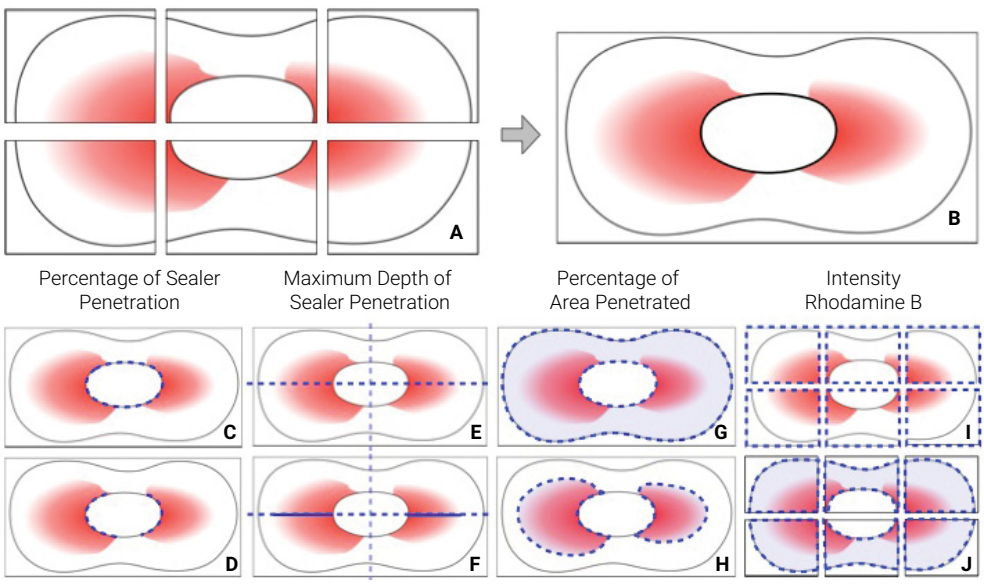

Figure 1. Design evaluation parameters of each radicular section. (A) Captured images in CLSM; (B) Merged image at Image J software; (C) Root canal wall perimeter; (D) Root canal wall perimeter covered by sealer; (E) Four standardized points on each image; (F) Maximum depth sealer penetration; (G) Area of each section; (H) Area penetrated with sealer; (I) Intensity values of rhodamine B and (J) Dentin area outlined and rhodamine B intensity.

Rhodamine B intensity in dentinal tubules

In each section of the images, the dentin area was outlined and rhodamine B intensity (Fig. 1I-J) was determined. Then, the intensity of rhodamine B in each section was summed, according to each root third, and the overall value was calculated.

Statistical analysis

Statistical analysis was performed with SAS software. Data normality evaluation was performed by Shapiro-Wilk test and one-way ANOVA and Tukey's tests were carried out to compare each treatment at a specific root canal third. The level of significance was set at $p < 0.05$.

Results

Sealer penetration occurred on each side of the root canal wall, resulting in statistically similar perimeter percentages in all groups and at all root canal thirds (Fig 2A).

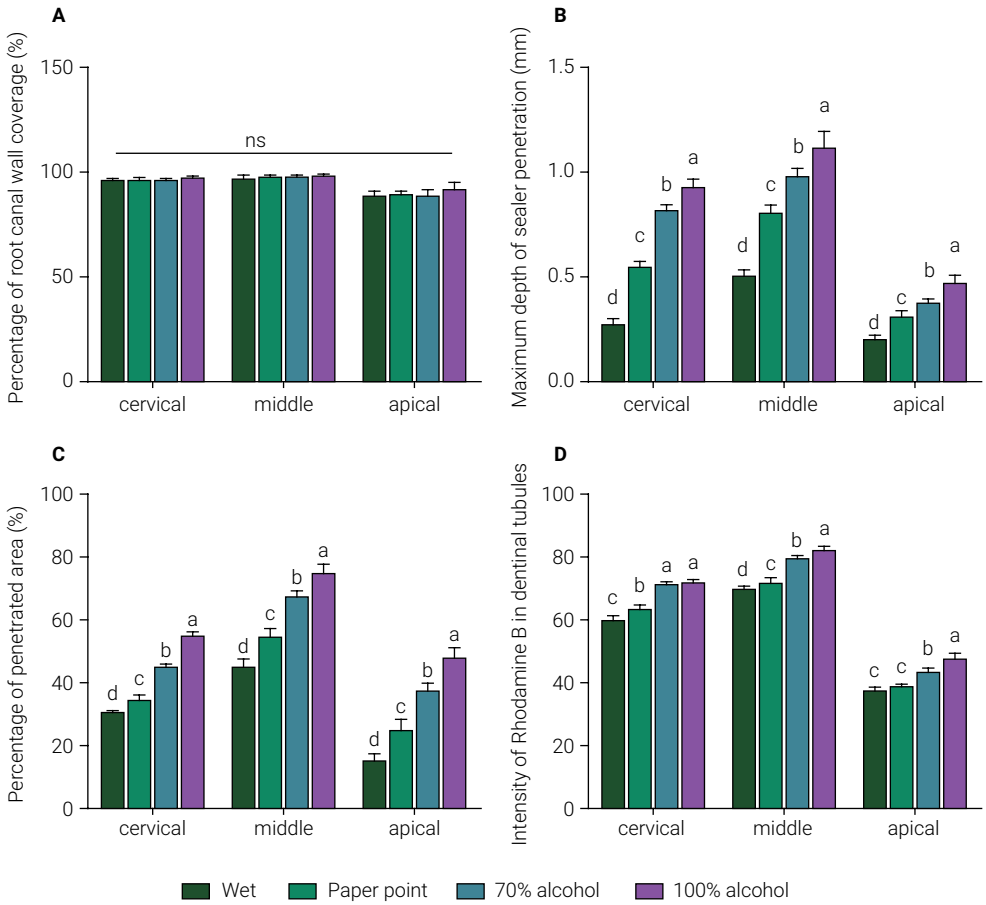

Figure 2. Representative graphics of evaluation parameters. A. Percentage of root canal wall covered; B. Maximum depth of sealer penetration; C. Percentage of penetrated area; D. Intensity of Rhodamine B in dentinal tubules.

On the other hand, 100% ethanol-conditioned dentine promoted deeper sealer penetration (Fig 2B), higher percentage of penetrated area (Fig 2C), and higher rhodamine B intensity (Fig 2D), followed by the 70% ethanol, paper point, and wet groups ($p<0.05$). The only two exceptions were the non-significant differences in rhodamine B intensity ($p>0.05$) between the 100% and 70% ethanol groups (cervical third) and the absorbent paper point and wet groups (apical third). Figure 3 shows the representative patterns of sealer penetration into dentinal tubules in all experimental groups and at all root canal thirds.

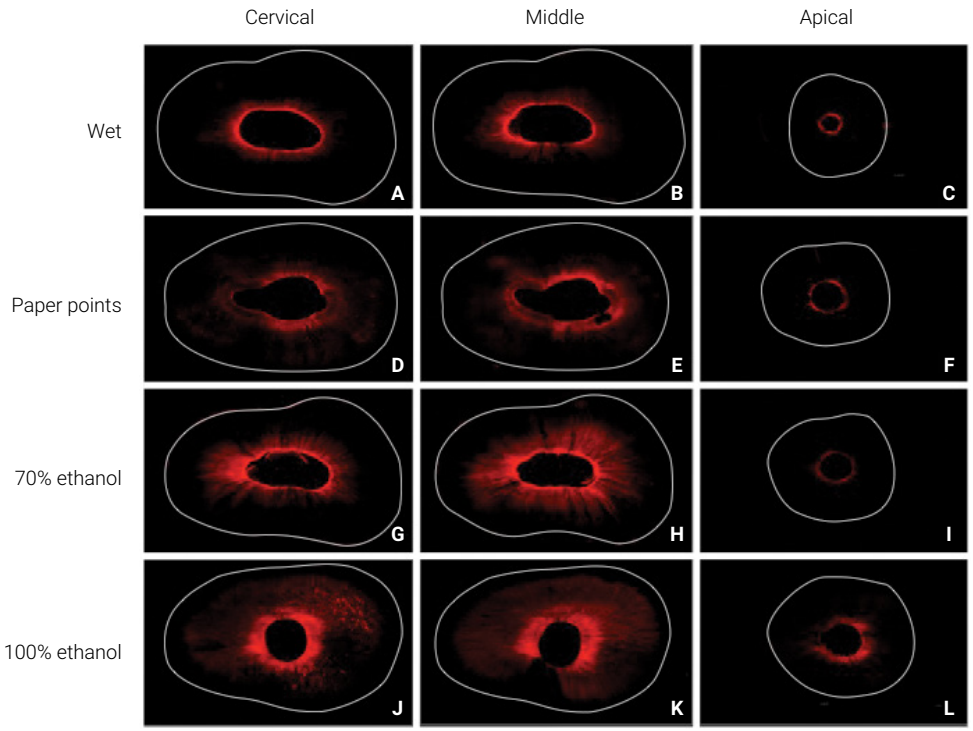

Figure 3. CLSM images according to drying methods and root canal thirds. A – C: wet group images showed lower penetration of sealer into dentinal tubules at all thirds; D – F: Paper points group images with more penetration of sealer into dentinal tubules, than wet groups; G – I: 70% ethanol group images showed better sealer penetration, when compared to wet and paper points group and J – L: 100% ethanol group images presented a greater amount of sealer penetrated into dentinal tubules, especially at middle third.

Discussion

Scanning electron microscopy (SEM) has been widely used to evaluate sealer penetration into dentinal tubules[3,7,18-20]. SEM micrographs allow observation of the dentinal tubules and accurate measurement of sealer penetration depth at high magnification[21]. Although this technique may be advantageous, some requirements need to be analysed such as artefacts during specimen preparation and inability to obtain a detailed overall view at low magnification[20]. Benefits of CLSM have been shown in the assessment of sealer penetration into dentinal tubules[15,17,21,22]. Background infor-

mation away from the focal plane enables the acquisition of images with fewer artefacts[11]. Another advantage of CLSM is the control over the depth of the field, which allows obtaining excellent images in different planes. In this study, CLSM images allowed detailed visualisation of sealer penetration into dentinal tubules and measurement of the percentage of sealer penetration area at each root canal third. Also, CLSM provided the rhodamine B intensity values in each dentine sample, which could suggest that higher rhodamine B intensity is related to greater sealer volume in dentinal tubules. The ethanol solutions used for the final rinse demonstrated that the root canal cervical third presented a significantly higher perimeter percentage and maximum depth of sealer penetration when compared to the middle and apical thirds[5,7,22]. However, the quantity, volume, and orientation of dentinal tubules at each root third are different[23]. Moreover, root dentine is not uniformly mineralised, and the density or number of dentinal tubules increases from the apical-coronal direction to the root surface[23]. Apical dentine is more frequently sclerosed and the tubules are irregular in number and cannot be observed in some areas[18,23]. Therefore, root canal thirds were not compared in this study because establishing comparisons among them could not be reliable and would not provide any important information about sealer penetration.

The root canal perimeter in which the endodontic sealer could penetrate into dentinal tubules has been analysed and differences were found after several final rinse regimens, sealer applications, or filling techniques[8,24-27]. Our results did not show any difference among groups when root canal wall coverages were compared. Despite the different evaluation techniques, the same root canal coverage results were achieved after a final rinse with 95% ethanol prior to obturation[25]. Although our previous study showed that ethanol could improve root canal surface wettability[24], an adequate sealer placement and/or a good filling technique could overcome any difference in surface substrates. A uniform sealer distribution can be obtained even when different sealer penetration into dentinal tubules is observed. This emphasises that sealer penetration into dentinal tubules would be better evaluated by other methods such as maximum depth of sealer penetration, percentage of sealer penetration area, or intensity of fluorescent dye using CLSM.

Penetration of sealers into dentinal tubules can form a physical barrier to prevent bacterial microleakage and recontamination of the root canal system[24], maintaining their bactericidal effect[24,28], which favours the healing of periapical lesions. Besides, the intensity of rhodamine B in dentinal tubules shows a higher sealer volume, as well as better filling and sealer performance, leading to periapical repair. These findings have been poorly discussed in previous studies and are probably more meaningful and more clinically relevant than those of other evaluations.

Final rinse with 95% ethyl ethanol presented significantly deeper sealer penetration than the rinse with NaOCl solution[8], as in this study 100% ethanol-conditioned dentine promoted deeper sealer penetration. Ethanol is generally considered a dehydrating medium[10], which removes the water from among the collagen fibrils[29] and dentinal tubules[30]. The reduction of root dentine wetness improved the wettability of the dentine surface in our previous study[24], which could let into more penetration and flow of sealer upon dentin, corroborating with this study and others[8,30]. This could also occur

on the inner surfaces of dentinal tubules, favouring diffusion of sealer into deeper regions, as founded. Therefore, the decreased wetness on the dentin wall or tubules may improve adhesion[31] or penetration of hydrophobic materials such as AH Plus.

Moreover, the ethanol solution was used to condition the root canal prior to filling[8] or bonding[14], instead of dehydrating the dentine using a technique known as 'ethanol wet-bonding'. Conditioning of the dentine promotes a surface with hydrophobic characteristics. An interaction with a hydrophobic sealer – such as "AH Plus" - would provide a low contact angle between sealer and surface, higher sealer penetration, greater mechanical interlock into the tubules, retention, sealing ability and, consequently, *in vivo* antibacterial effectiveness[6,32]. This technique combines the reduction of polarity of the collagen network with the low polarity of highly hydrophobic resins, since hydrophobic monomers can better infiltrate into the ethanol-saturated demineralised dentine. Therefore, as epoxy resin binds to collagen[14], especially in the demineralised dentine, it is suggested that in the presence of ethanol instead of water, some hydrophobic materials, such as AH Plus, have improved flow into dentinal tubules and infiltration into collagen interfibrillar spaces.

In conclusion, a 1-minute final rinse with 100% ethanol seems to be a good dentine conditioning method before root canal drying procedures, as it can improve sealer penetration into dentinal tubules, which clinically could led into entombing more bacteria and enhancing endodontic treatment outcomes.

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