Original Research
Endodontic Therapy

Effect of the time-point of acid etching on the persistence of sealer residues after using different dental cleaning protocols

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Abstract: Endodontic sealer residues remaining within the pulp chamber dentin after root canal obturation and cleaning with various solvents may compromise the appearance and the durability of dental restorations. Acid etching is routinely performed prior to application of dentine adhesive systems, but is effect on residual sealer material and the optimal time-point for performing etching, are unknown. Here, we evaluated the effect of acid etching on the dentin surface when performed either immediately or 7 days after removal of the endodontic sealer with two solvents, i.e., 95% ethanol or xylol. Forty crowns fragments from bovine incisors were impregnated with sealer and divided into 4 groups (n = 10 each), according to the dentin cleaning protocol and to the acid etching time-point: G1, 95.0% ethanol and immediate acid etching; G2, xylol and immediate acid etching; G3, 95.0% ethanol and acid etching after 7 days; and G4, xylol and acid etching after 7 days. Scanning electron microscopy (SEM) images (2000 ×) were obtained from each specimen and the number of open dentinal tubules counted and compared. Another 40 fragments were similarly prepared, and SEM images were obtained (500 ×) to score and compare the persistence of sealer residues on the dentin. G4 showed the most open dentinal tubules and the least epoxy resin-based sealer residues on the dentin surface ($p < 0.05$). The least epoxy resin-based sealer residues was obtained when acid etching, using 37% phosphoric acid, was performed after 7 days after cleaning the dentin with xylol.

Keywords: Endodontics; Dentin; Microscopy, Electtron, Scanning.

Introduction

The presence of endodontic sealer residues within the pulp chamber dentin after root canal obturation may cause dental crown discoloration and/or may negatively affect the function of adhesives, compromising the durability of dental restorations.[1,2,3,4]

Epoxy resin-based sealers are most commonly used in contemporary endodontics and have satisfactory physicochemical and biological properties.[5,6] Nevertheless, the persistence of residues from these sealers on the dentin surface significantly reduces the strength of bonding of self-etching adhesive systems to pulp chamber dentin.[4] Various substances

have been recommended to remove such residues from the dentin, such as ethanol, acetone, isopropyl alcohol, and amyl acetate.[7,8] However, even after application of these substances, endodontic sealer residues are still present on the dentin surface.[7,9,10,11]

Xylol is an organic solvent that has the capacity to solubilize endodontic sealers, including epoxy resin-based sealer.[12,13] It is routinely used in endodontic retreatment techniques, including manual and rotatory approaches, for removing obturation material from the root canal.[14,15] However, its efficacy for cleaning dentin impregnated with endodontic sealer is not known.

On the other hand, 37% phosphoric acid, which is routinely used for acid etching prior to applying dentin adhesive systems, is a promising final irrigant for removing the smear layer from root dentin.[16,17] Nonetheless, it is not known if this procedure favors the removal of endodontic sealer residues that remain after cleaning the dentin with chemical substances in endodontically treated teeth. Moreover, these sealers require a long time to achieve final setting, and it is not clear at which time-point it is optimal to perform acid etching.[18,19]

Therefore, the aim of this study was to evaluate the effect of acid etching on the dentin surface, performed either immediately or 7 days after removal of the endodontic sealer with solvents.

Methodology

This study was approved by the Animal Ethical Committee of Araraquara Dental School – Unesp, SP, Brazil (protocol 11/2014).

Forty freshly extracted bovine incisors were selected for this study. The roots were sectioned at the cement-enamel junction using a diamond disc (Brasseler, Savannah, USA) and discarded. The crowns were longitudinally sectioned using a diamond disc at low speed (Isomet; Buehler Ltd., Lake Bluff, USA) under constant irrigation in the mesial-distal direction, and a fragment (50 mm × 50 mm) was obtained from the buccal surface of each dental crown (Figure 1A). Subsequently, the fragments were individually immersed in 10 mL of 2.5% sodium hypochlorite for 15 min, then immersed in 10 mL of 17% EDTA (Biodinâmica Ind. Com, Ibiporã, Brazil) for 3 min, and dried using absorbent paper.

An epoxy resin-based sealer (AH Plus; Dentsply De Trey, Konstanz, Germany) was mixed in a 1:1 ratio of paste A and B, according to the manufacturer´s instructions. The mixture was spread over the dentin surface using a microbrush (Microbrush Int., Grafton, WI, USA) until a visible sealer layer could be observed (Figure 1B and 1C). The material was left undisturbed on the dentin surface of each specimen for 15 min.[20]

Then, the specimens were divided into 4 groups (n = 10 each), according to the dentin cleaning protocol and acid etching time. G1 (E-I-AE) was cleaned with 95.0% ethanol and immediately acid etched; G2 (X-I-AE) was cleaned with xylol and immediately acid etched; G3 (E-D-AE) was cleaned with 95.0% ethanol and acid etched 7 days later, and G4 (X-D-AE) was cleaned with xylol and acid etched 7 days later.

For each specimen, the dentin surface was wiped using a cotton pellet saturated with 95% ethanol (Rinse-N-Dry; Vista Dental, Racine, WI, USA) or xylol (Quimidrol, Joinville, SC, Brasil), until the surface appeared visibly clean (Figure 1D). Acid etching was then performed immediately, or 7 days after dentin surface cleaning, by applying 37% phosphoric acid for 15 sec; then specimens were rinsed with distilled water for 10 sec, and dried with absorbent paper.

The specimens were subsequently allowed to dry at room temperature for 7 days and thereafter were dehydrated inside a closed chamber containing silica gel, for 24 h. Subsequently, the specimens were mounted onto metal stubs, sputter coated with gold (single cycle; 120 sec) under vacuum inside a metallizing chamber (MED 010, Balzers Union, Balzers, Liechtenstein), and were then examined by scanning electron microscopy (SEM) with a JEOL 6060 (JEOL Ltd., Tokyo, JPN) operated at 15 kV. Four different images were obtained from each specimen and the most representative image was selected at 500 × magnification. The number of open dentinal tubules presenting no residue was counted using the Photoshop CS5 program. The data were analyzed using ANOVA and Tukey's test.

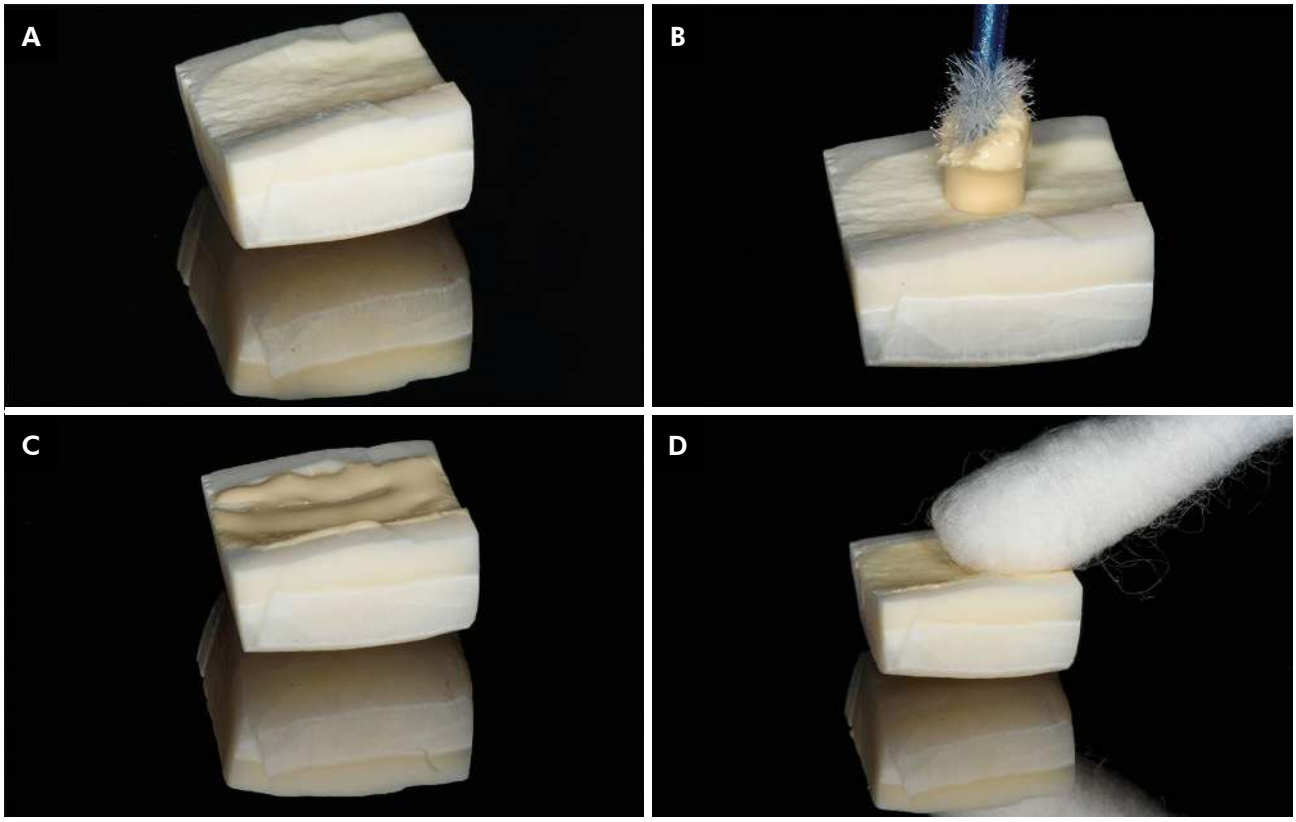

Figure 1. (A) specimen preparation; (B) the epoxy resin-based sealer application; (C) endodontic sealer applied to the dentin; (D) dentin surface cleaning.

Additionally, 40 additional dentin fragments were obtained from different bovine dental crowns and were submitted to a similar protocol as described above. However, these specimens were mounted onto metal stubs and were directly examined using SEM (PsemAspex Express; FEI Company, Eindhoven, Netherlands), operating at 20 kV, to avoid cracks occurring in the specimens during the dehydration and metallization processes.

After the SEM images were obtained, the persistence of endodontic sealer residues on each specimen was assessed by assigning scores ranging from 1 to 4, as described by Kuga et al.[7] Score 1: the impregnated surface showed persistence of sealer in less than 25% of the total image area. Score 2: the impregnated surface showed persistence of sealer ranging from 25% to 50% of the total image area. Score 3: the impregnated surface showed persistence of sealer ranging from 50% to 75 % of the total image area. Score 4: the impregnated surface showed persistence of sealer in more than 75% of the total image area. The data obtained were analyzed using the Kruskal-Wallis and Dunn tests.

For all analyses, $p < 0.05$ was considered significant.

Results

The cleaning protocol employing xylol and acid etching performed after 7 days yielded a larger number of open dentinal tubules than did immediate acid etching, regardless of whether xylol or 95% ethanol was used for cleaning (both $p < 0.05$), or etching 7 days after cleaning with 95% ethanol ($p < 0.05$). No significant differences were found among other groups ($p > 0.05$). Table 1 shows the mean and standard deviation of the incidence of open dentinal tubules for all cleaning protocols and etching times. Representative images of the open dentinal tubules are shown in Figure 2.

Similar differences were observed in relation to the persistence of residues on the dentin

surface. Table 2 shows the median, minimum, and maximum values, and first and third quartiles of the scores representing the amount of residue present on the dentin surface after employing the various cleaning protocols and etching times. Figure 3 (A-D) shows representative images of the dentin surface after applying the different dentinal cleaning protocols.

Discussion

In the present study, we observed an interaction between the time-point of performing acid etching, using 37% phosphoric acid, and the chemical solution used to remove sealer residues from the dentin surface. Cleaning the dentin surface with xylol and performing acid etching after 7 days resulted in more open dentinal tubules and less epoxy-based sealer residues (AH Plus) on the dentin surface.

The methodology used to evaluate the cleaning effects of chemical substances on dentin impregnated with endodontic sealer has previously been published.[7,20,21] Ethanol is routinely recommended as a chemical solution for removal of sealers from the pulp chamber; however, ethanol cannot completely remove the sealer residues from dentin.[7]

Although xylol is recommended as a solvent for epoxy resin-based sealer, it also does not completely remove sealer residues from the dentin surface.[7,12] Due to the inefficiency of these chemical solutions, we considered it necessary to determine whether acid etching could synergistically facilitate removal of the endodontic sealer residues.

Performing acid etching immediately after the cleaning protocol using xylol resulted in greater persistence of residues than when using delayed etching. Since the time required for final setting of epoxy resin-based sealers is relatively long, the mechanical action performed during the acid etching and subsequent irrigation with distilled water to remove the phosphoric acid may have favored dispersion of sealer residues on the dentin surface, as shown in Figure 3B.[18,19] In contrast, the final setting of the epoxy resin-based sealer had been achieved by 7 days.[19,21]

The concept that "like dissolves like" (polar solvents are better at dissolving polar substances), and vice versa explains the higher incidence of remaining sealer residues for the cleaning protocol employing ethanol.[9] Kuga et al.[7] have observed, through SEM, that the 95% ethanol and isopropyl alcohol are ineffective in cleaning dentin impregnated with AH Plus sealer. In the present study, the

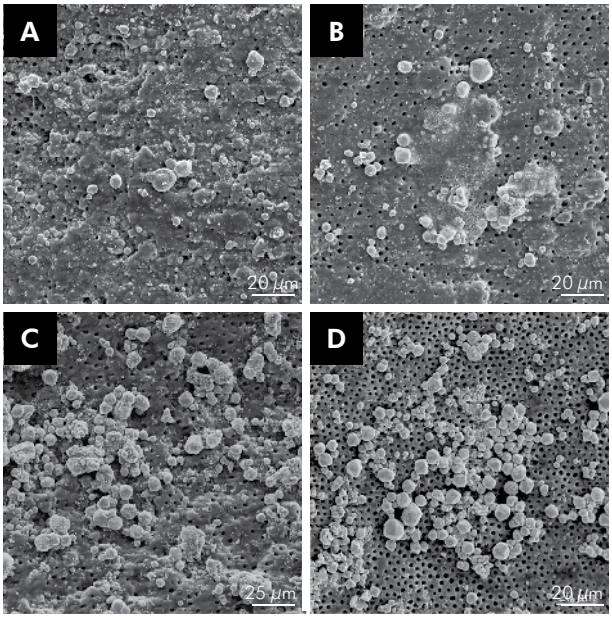

Figure 2. Representative image of the open dentinal tubules (A) G1 (E-I-AE), 95% ethanol and immediate acid etching; (B) G2 (X-I-AE), xylol and immediate acid etching; (C) G3 (E-D-AE), 95% ethanol and etching 7 days later; and (D) G4 (X-D-AE), xylol and acid etching after 7 days.

Table 1. Mean and standard deviation of the number of open dentinal tubules (per 14.4 mm^2), according to the cleaning solutions and acid etching time-point used.

Variable	G1 (E-I-AE)	G2 (X-I-AE)	G3 (E-D-AE)	G4 (X-D-AE)
Mean	250^b	264^b	315^b	912^a
SD	207.04	172.51	166.48	350.64

G1 (E-I-AE): 95% ethanol and immediate acid etching; G2 (X-I-AE): xylol and immediate acid etching; G3 (E-D-AE): 95% ethanol and acid etching after 7 days; G4 (X-D-AE): xylol and acid etching after 7 days. ^{a,b}Different letters indicate significant statistical difference ($p < 0.05$).

Table 2. Median, maximum and minimum values, and the first and third quartile of the scores attributed to the remaining epoxy resin-based sealer (AH Plus) residues, according to the cleaning chemical protocol and acid etching time-point (n = 10, each group).

Variable	G1 (E-I-AE)	G2 (X-I-AE)	G3 (E-D-AE)	G4 (X-D-AE)
Median	2[b]	2[b]	2[b]	1[a]
Minimum	2	2	1	1
Maximum	3	3	3	2
Q1	2	2	2	1
Q3	2.75	2	2	1

G1 (E-I-AE): 95% ethanol and immediate acid etching; G2 (X-I-AE): xylol and immediate acid etching; G3 (E-D-AE): 95% ethanol and acid etching after 7 days; G4 (X-D-AE): xylol and acid etching after 7 days. [a,b]Different letters indicate significant statistical difference ($p < 0.05$).

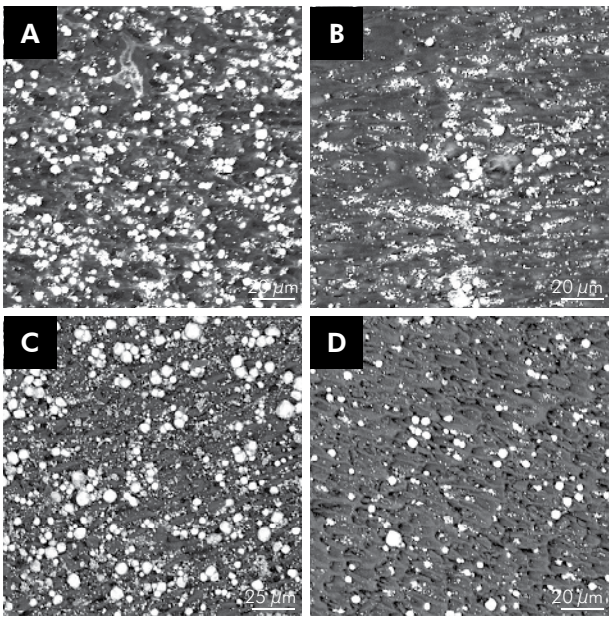

Figure 3. Representative image of residual persistence on dentin surface (A) G1 (E-I-AE), 95% ethanol and immediate acid etching; (B) G2 (X-I-AE), xylol and immediate acid etching; (C) G3 (E-D-AE), 95% ethanol and acid etching 7 days later; and (D) G4 (X-D-AE), xylol and acid etching 7 days later.

groups that used 95% ethanol presented endodontic sealer residues regardless the acid etching time. This concentration of ethanol contains water in its composition, while the epoxy resin is hydrophobic; thus, regardless of the etching time-point, this may have led to incomplete solubilization of sealer residues, and have contributed to the presence of endodontic sealer residues on the surface dentin.

As residual endodontic sealer may compromise the longevity of dental restorations,[22,23] further studies should evaluate which substances are effective for removal of endodontic sealer residues, without interfering with the hybrid layer formation after acid etching and application of a dentin adhesive system.

Conclusions

In the present study, the lowest amount of epoxy-based sealer residues remained when acid etching, using 37% phosphoric acid, was performed 7 days after dentin cleaning with xylol.

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