Original Article

Sealing of pulp chamber dentin in endodontics: Influence of bond strategy and time-point application

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Abstract

Context: Seal the dentin of the pulp chamber during endodontic treatment to avoid interfering with the restorative treatment performed afterward.

Aims: The aim was to evaluate the effect of three adhesive systems applied in different bonding strategies (etch-and-rinse, self-etch, and universal adhesive) and time-point application (immediately after the cavity access preparation or after endodontic obturation) on the hybrid layer formation and dentinal penetrability.

Materials and Methods: Forty-eight sound molars were randomly distributed into six groups (n = 10) according to the adhesive system used: Forty-eight sound molars were randomly distributed into six groups (n = 10) according to the adhesive system used and the time-point application: Adper Scotchbond Multi-purpose (AS), Clearfil SE (CF) and Scotchbond Universal (SU) in strategy of immediate endodontic sealing (IES) or delayed endodontic sealing (DES). In IES-AS, IES-CF, and IES-SU groups, dentin sealing was performed immediately after the cavity access, while in DES-AS, DES-CF, and DES-SU, after root canal obturation. The specimens were sectioned in the long axis, in a buccal-lingual direction, and the dentinal penetrability of the adhesive systems was evaluated using confocal microscopy images. Hybrid layer formation was analyzed by scanning electron microscopy images.

Statistical Analysis Used: Dentinal penetrability data were analyzed with the ANOVA test and the Kruskal–Wallis test was performed for hybrid layer data ($\alpha = 0.05$).

Results: IES-CF showed the lowest dentinal penetrability (P < 0.05), while the other protocols were similar to each other (P > 0.05). No significant differences were found between groups regarding the hybrid layer formation (P > 0.05). Immediate and DES protocols do not influence the hybrid layer formation, regardless of the bond strategy used.

Conclusions: Sealing the pulp chamber dentin before endodontic treatment can improve the bond strength of the final restoration but the formation of the hybrid layer was not influenced by the bond strategy.

Keywords: Adhesive system; endodontic irrigation; endodontic treatment; immediate dentin sealing; sodium hypochlorite

INTRODUCTION

Residues from sodium hypochlorite (NaOCl) solution and

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endodontic sealer on the pulp chamber dentin may negatively affect the bonding between the adhesive system and dentin.^[1-6] Moreover, an inadequate and unstable hybrid layer and the absence of effective sealing in the final restoration can jeopardize the success of the endodontic treatment.^[7] Thus, it has been suggested to apply the adhesive system before endodontic obturation to reduce the persistence of residues.

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An endodontic sealing protocol (ES) can mitigate the adverse effects of these residues on the adhesive interface, increasing the clinical longevity of esthetic restorations.^[7] This protocol involves applying an adhesive system before the endodontic treatment ensuring the formation of a hybrid layer within the pulp chamber. This layer protects the dentin matrix and maintains a bond strength of the restoration after the completion of the endodontic treatment.^[7]

ES can be performed before chemical-mechanical preparation of the root canal (immediate endodontic sealing [IES]), preventing the deleterious effects of NaOCl on the polymerization of resin-based composites.^[8,9] Alternatively, it can be performed before or after endodontic obturation (delayed endodontic sealing [DES]) to prevent residue from the endodontic sealer and/or provisional restorative materials affect the adhesive interface.^[1,2] Previous studies have indicated that singlet oxygen is released during the degradation of NaOCl solution when irrigation is performed, which can negatively affect the polymerization of resin-based composites, mainly when using etch-and-rinse adhesive systems.^[8,9] In this context, IES protocol can counteract the deleterious effects of endodontic substances on the adhesive interface by applying the adhesive system to the dentin of the pulp chamber before the chemical-mechanical preparation of the root canal.

Materials used during endodontic obturation can lead to tooth discoloration and promote marginal microleakage at the adhesive interface, even after cleansing using ethanol or xylol,^[1,2,10-12] being another drawback of endodontic treatment. Thus, applying the adhesive interface before root canal obturation (DES) can prevent dentin from the impregnation of these residues.^[7]

A previous study recommended the use of a two-step self-etching adhesive system (Clearfil SE [CF]) for immediate sealing endodontics.^[7] This adhesive system functions through a chemical interaction between functional monomers/hydroxyapatite and slight hybridization to the organic matrix of dentin.^[13,14] "All-in-one" self-etching adhesive systems (Universal Adhesives) were launched to simplify the operative procedures and present a bonding mechanism similar to two-step self-etching adhesives. The acidic monomers partially dissolve hydroxyapatite, demineralizing the dentin, and creating a favorable surface for adhesive infiltration into the smear layer. Consequently, it results in a hybrid layer composed of smear layer residues and incorporated minerals.^[15-17]

Despite the options for immediate or DES, the optimal timing for application and the choice of adhesive system remain uncertain.^[13,14] Therefore, this study aimed to evaluate the adhesive interface between pulp chamber dentin and the adhesive system, after immediate (IES) or delayed (DES) ES, using a two-step self-etching adhesive (CF) or a universal adhesive (Scotchbond Universal [SU]), compared to three-step etch-and-rinse adhesive system (Adper Scotchbond Multipurpose [AS]) on the dentinal penetrability and hybrid layer formation. The null hypotheses tested were: (I) there is no difference between ES protocols and (II) there is no difference between adhesive systems, regarding dentinal penetrability and hybrid layer formation.

MATERIALS AND METHODS

Specimens' preparation

This study received proper approval from the Research Ethics Committee of the local School of Dentistry, (CAAE: 68355317.0.0000.5416). Forty-eight human mandibular molars with sound dental crowns and similar anatomical dimensions at the enamel-dentin junction, (measuring 6.0 ± 0.5 mm in the buccolingual direction and 8.0 ± 0.5 mm in the mesiodistal direction) were selected. The molars had two or more root canals, as confirmed by radiographic examination. The sample size (n = 10) was determined based on previous research studies.^[1,2,7]

Cavity preparation

A mesio-occlusal cavity was carried out with its proximal margins placed 1 mm below the cementoenamel junction. The cavities were prepared according to a previous study^[7] in the following dimensions: 4 mm wide in buccolingual direction, 4 mm deep occlusal, and 2 mm wide in proximal mesiodistal direction. The cavity walls presented approximately 15° occlusal divergence.

Cavity preparations were performed using a #3070 diamond-coated bur (KG Sorensen, Sao Paulo, SP, BR), and finished with 3203F diamond-coated bur (KG Sorensen, Sao Paulo, SP, BR) under constant water-cooling. Then, the pulp chamber roof was removed using a diamond bur (Endo Access Bur; Dentsply-Sirona, Ballaigues, Switzerland) under water-cooling.

Experimental groups

The specimens were randomly allocated into six groups (n = 10), according to the time-point of the ES and type of adhesive system. The experimental groups investigated are described below:

IES + AS Multi-Puporse (3M ESPE, St. Paul, MN, USA): Dentin was etched with 37% phosphoric acid (Condac 37; FGM, Joinville, SC, BR) for 20 s, and rinsed with water jets. After that, dentin was gently dried with absorbent paper points. A three-step etch-and-rinse adhesive system (AS, 3M ESPE, St. Paul, MN, USA) was applied over the pulp chamber dentin and light-cured (Valo; Ultradent, South Jordan, UT, USA) for 10 s with an irradiance of 1000 mW/cm²; IES-CF (Kuraray, Osaka, JPN): The primer was actively applied for 20 s over the pulp chamber dentin, and then the self-etch adhesive was immediately applied and light-cured (Valo; Ultradent, South Jordan, UT, USA) for 10 s with an irradiance of 1000 mW/cm²;

IES + SU (3M ESPE, St. Paul, MN, USA): An "all-in-one" adhesive system was applied to dentin, and light-cured (Valo; Ultradent, South Jordan, UT, USA) for 10 s with an irradiance of 1000 mW/cm²;

DES + AS, DES-CF (DES + CF), and DES + SU: In all these groups, the ES protocol was performed as previously described for each type of adhesive system but after the root canal obturation, and pulp chamber dentin was cleansed with 95% ethanol (Synth, São Paulo, SP, BR).

Rhodamine B was added to the prime/adhesive at a concentration of 0.04 mg to 0.4 mL of liquid in all protocols for confocal laser scanning microscopy (CLSM) analysis.^[18] All adhesive systems were handled according to the manufacturer's recommendations.

Chemical-mechanical preparation and endodontic obturation

In the IES protocols, the root canal openings were filled with a gutta-percha point immediately after the operative procedure and before the chemical-mechanical preparation of the root canal. In the DES protocols, the operative procedures were performed after root canal obturation.

After the #10 K-file glide path (Dentsply Maillefer, Ballaigues, Switzerland) was achieved, the real working length was established 1 mm below the real root length. Mechanical preparation was performed using rotary instruments (ProTaper; Dentsply Maillefer, Ballaigues, Switzerland) to F2 instrument with irrigation of 5 mL of 2.5% NaOCl at each instrument change. Each root canal was irrigated with 17% ethylenediaminetetraacetic acid (EDTA) for 3 min, and 5 mL of distilled water according to a previous study.^[19]

Root canals were dried with absorbent paper points, and obturated with epoxy resin-based sealer (AH Plus; Dentsply Konstanz, GER), and F2 gutta-percha master cone (ProTaper; Dentsply, Petrópolis, RJ, BR). Gutta-percha excesses were removed using heated instruments at the level of the cementum-enamel junction. Afterward, pulp chamber dentin was cleansed with 95% ethanol (Synth, São Paulo, SP, BR).

Cavity restoration

A thin layer (~ 0.5 mm) of flowable resin composite (Filtek Z350 XT Flow; 3M, Dubai, UAE) was applied over the dentin sealing surface, and light-cured (Valo; Ultradent, South Jordan, UT, USA) for 40 s, in both dentin sealing protocols.

After the completion of endodontic obturation, the surface of the flowable resin composite was etched with 37% phosphoric acid for 10 s and then restored with a bulk-fill resin composite (Opus Bulk-Fill; FGM, Joinville, SC, BR).

Then, the dental crown was cross-sectioned 2 mm below the cementoenamel junction and centrally sectioned in a buccolingual direction. Two slices were obtained (mesial and distal with 2.0 ± 0.1 mm thick). A mesial slice was used to evaluate the dentinal penetrability, and a distal one to analyze the hybrid layer formation.

Dentin penetrability evaluation

Dentinal penetrability was measured (in micrometers) at the buccal interface of the mesial slice using Confocal laser scanning microscopy (CLSM) (Leica, Mannheim, Germany) at $\times 100$. Ten measurements were performed on each specimen (1 µm between each one) from the pulp chamber floor onto the occlusal face of a dental crown. Analyzes were carried out with Image J Software (National Institutes of Health, USA). The arithmetic mean of dentin penetrability value for each specimen.

Hybrid layer formation

The distal slices were polished with #600 and #1200 silicon carbide sandpapers (Wurth, Cotia, SP, BR), and immersed in distilled water in an ultrasonic bath for 5 min. After drying, the slices were sequentially immersed in 18% hydrochloric acid solution (30 s), deionized water (5 min), 5% NaOCl (10 min), and deionized water (10 min). Then, the specimens were stored at 37° C for 24 h.^[19,20]

One impression of each slice was obtained using additional silicone (Express XT; 3M ESPE, Sumaré, SP, BR), and then epoxy resin replicates (Epofix; Struers Inc., Cleveland, OH, USA) were obtained. The replicates were submitted to the metallization process and analyzed with scanning electron microscopy (JSM 6400; Jeol Co., Tokyo, JPN). Images of the buccal surface of the cervical region replicas were evaluated at ×500.

The hybrid layer formation in the adhesive interface after SEM analysis was classified in the following parameters according to a previous study:^[21] "0" represented the continuity of the hybrid layer in all specimens, i.e. an absence of fissures in all thirds; "1" represented continuity in two thirds; "2" represented continuity in at least one-third; and "3" indicated that all thirds presented a discontinuity in the hybrid layer or fissures.

Statistical analysis

The normality of the data was verified by Shapiro–Wilk

test (P > 0.05). Dentinal penetrability data were submitted to ANOVA and Tukey tests. Hybrid layer formation data were submitted to the Kruskal–Wallis test. A significance level of 5% was adopted for all tests. The software IBM SPSS Statistics version 22 (IBM SPSS, New York, NY, USA) was used in all tests.

RESULTS

Dentin penetrability evaluation

Table 1 shows the arithmetic mean and standard deviation (in micrometers) of dentinal penetrability according to the dentin sealing protocols. Figure 1 shows the dentinal penetrability of the adhesive system according to the time-point application and type of adhesive system.

The IES-CF group showed the smallest extent of dentin penetration (P < 0.05). The other protocols presented similar results (P > 0.05), regardless of the time-point of sealing (immediate or delayed).

Hybrid layer formation

No difference was observed among dentin sealing protocols regarding the hybrid layer formation (P > 0.05). Table 2 shows the median, maximum, and minimum values of the scores attributed to the hybrid layer formation, according to the dentin sealing protocol. Figure 2 shows the continuity pattern of the hybrid layer formation at the adhesive interface, according to the dentin sealing protocol, time-point application, and type of adhesive system.

DISCUSSION

ES has been shown to enhance the longevity of aesthetic restorations after endodontic treatment and also decreases marginal infiltration between between adhesive system and dentin.^[13] In our study, we found that hybrid layer formation was similar in all sealing protocols; however, the dentinal penetrability was lowest in the case of IES using CF Therefore, our null hypotheses were rejected.

The analysis of internal marginal adaptation, hybrid layer formation using scanning electron microscopy, and dentinal penetrability with CLSM are considered reliable methods for evaluating the adhesive interface. In our study, we used confocal laser microscopy to evaluate the adhesive system penetrability into the pulp chamber dentin to compare it with the SEM evaluation.^[9,10,21]

Table 1: Mean and standard deviation (μm) of dentinal penetrability of the adhesive systems according to the endodontic sealing protocols

Group	Mean±SD		
IES-AS	14.30 ^b ±3.08		
IES-CF	$7.99^{a} \pm 1.58$		
IES-SU	13.37 ^b ±1.34		
DES-AS	16.38 ^b ±3.43		
DES-CF	12.52 ^b ±1.12		
DES-SU	15.36 ^b ±3.80		

^{a,b}Different letters show a statistically significant difference (P<0.05). IES: Immediate endodontic sealing, DES: Delayed endodontic sealing, AS: Adper Scotchbond Multi-purpose, CF: Clearfil SE bond, SU: Scotchbond universal, SD: Standard deviation



Figure 1: Dentinal penetrability of the adhesive system according to the endodontic sealing protocols. (a), Immediate sealing with Adper Scotchbond Multi-purpose; (b) immediate sealing with Clearfil SE Bond; (c) immediate sealing with Scotchbond Universal; (d) delayed sealing with Adper Scotchbond Multi-purpose; (e) delayed sealing with Clearfil SE Bond; (f) delayed sealing with Scotchbond Universal



Figure 2: Adhesive interface according to the endodontic sealing protocols. (a), Immediate sealing with Adper Scotchbond Multi-purpose; (b) immediate sealing with Clearfil SE Bond; (c) immediate sealing with Scotchbond Universal; (d) delayed sealing with Adper Scotchbond Multi-purpose; (e) delayed sealing with Clearfil SE Bond; (f) delayed sealing with Scotchbond Universal Universal

Table 2: Median, minimum, and maximum values; first (1Q) and third (3Q) quartiles of scores attributed to the hybrid layer formation according to the endodontic sealing protocols

Group	IES-AS	IES-CF	IES-SU	DES-AS	DES-CF	DES-SU
Median	0	1	0.5	0	0	0.5
Minimum-	0-3	0-3	0-1	0-1	0-1	0-3
maximum						
1Q-3Q	0-2	0-2	0-1	0-0.25	0-1	0-1.25

No significant difference was found among the protocols (*P*>0.05) IES: Immediate endodontic sealing, DES: Delayed endodontic sealing, AS: Adper Scotchbond Multi-purpose, CF: Clearfil SE bond, SU: Scotchbond universal

While a previous study^[7] demonstrated improved internal adaptation when IES was performed using CF, our study found that this same sealing approach exhibited the lowest dentinal penetrability among all the protocols. This adhesive system contains a higher percentage of hydrophobic molecules (Bis-GMA), and a small percentage of hydrophilic molecules (e.g., 2-hydroxyethyl methacrylate (HEMA)). The reduced dentinal penetrability observed in IES-CF may be attributed to the incorporation of rhodamine B into the fluid resin (bond) of CF.^[15] This is significant because the adhesion mechanism of self-etching adhesive systems involves interaction with the smear layer, followed by chemical bonding with the hydroxyapatite from dentin. Thus, any factor affecting adhesion and/or its chemical composition can directly impact the hybrid layer formation.^[21]

Another possible reason for the lower dentinal penetrability in IES-CF may be related to the initial composition of the smear layer formed immediately after endodontic access. This composition might differ from the smear layer formed after the completion of endodontic treatment, especially with the presence of sealer residues (AH Plus) on the dentin surface.^[1,2,11,13] These differences could cause the penetrability of the two-step self-etching adhesive system (CF) to behave differently under varying conditions and techniques.

Dentin sealing protocols using SU exhibited dentinal penetrability similar to the etch-and-rinse adhesive protocol. "All-in-one" adhesive does not contain a hydrophobic fluid resin in its composition. Thus, its acidic monomers are dissolved in water and organic solvent, and after their ionization, they promote dentin demineralization, allowing a greater infiltration in the smear layer, and resulting in higher dentinal penetrability.^[16,17]

The hybrid layer formation was evaluated by scanning electron microscopy using scores attributed to the characteristics.^[21] Similar formation image's was observed among all dentin sealing protocols. During the chemical-mechanical preparation, a 2.5% NaOCI solution was used for endodontic irrigation. However, the final irrigation was performed with 17% EDTA and distilled water. It is worth noting that the concentration of NaOCI does not significantly affect the adhesive interface when self-etching adhesive systems are employed.^[3,4] In addition, solutions with lower concentrations of hypochlorous acid and the final irrigation protocol likely helped minimize its adverse effects.^[5] Therefore, the immediate dentin sealing protocol is recommended when higher concentrations of NaOCI solution are utilized or when it is the sole irrigant for the root canals.^[6,7]

Our findings corroborate with a previous study,^[7] as both studies showed favorable results regarding the dentinal penetrability of the adhesive systems. This suggests that all ES strategies can be considered viable options. In

this context, the use of simplified adhesive systems with easy handling is a time-saving alternative, considering the clinical time already required for performing the endodontic treatment.

To assess the long-term performance and justify the clinical applicability of these ES protocols, further studies with artificial aging are warranted. Moreover, it is valuable to evaluate different types of adhesive systems associated with various irrigating solutions and endodontic sealers. This broader investigation can help to predict the effectiveness of the ES protocols under various clinical conditions.

Within the limitation of this study, some conclusions can be drawn:

- The time-point application of ES (immediate or delayed) did not interfere with the formation of a hybrid layer, regardless of the type of adhesive system employed
- Regarding dentinal penetrability, the IES using a two-step self-etch adhesive system (CF) yielded lower results.

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Conflicts of interest

There are no conflicts of interest.

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