

# Hybridization quality and bond strength of adhesive systems according to interaction with dentin

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## ABSTRACT

**Objective:** To evaluate the hybridization quality and bond strength of adhesives to dentin. **Materials and Methods:** Ten human molars were ground to expose the dentin and then sectioned in four tooth-quarters. They were randomly divided into 5 groups according to the adhesive used: Two single-step self-etch adhesives – Adper Prompt (ADP) and Xeno III (XE), two two-step self-etching primer systems – Clearfil SE Bond (SE) and Adhe SE (ADSE), and one one-step etch-and-rinse system – Adper Single Bond (SB). Resin composite (Filtek Z250) crown buildups were made on the bonded surfaces and incrementally light-cured for 20 s. The restored tooth-quarters were stored in water at 37°C for 24 h and then sectioned into beams (0.8 mm<sup>2</sup> in cross-section). Maximal microtensile bond strength ( $\mu$ -TBS) was recorded (0.5 mm/min in crosshead speed). The results were submitted to one-way ANOVA and Tukey's test ( $\alpha = 0.05$ ). Thirty additional teeth were used to investigate the hybridization quality by SEM using silver methenamine or ammoniacal silver nitrate dyes. **Results:** SE reached significantly higher  $\mu$ -TBS ( $P < 0.05$ ); no significance was found between ADSE and XE ( $P > 0.05$ ), and between SB and ADP ( $P > 0.05$ ); ADSE and XE were significantly higher than SB and ADP ( $P < 0.05$ ). The bonding interface of SB showed the most intense silver uptake. SE and ADSE showed more favorable hybridization quality than that observed for ADP and XE. **Conclusions:** The bond strength and hybridization quality were affected by the interaction form of the adhesives with dentin. The hybridization quality was essential to improve the immediate  $\mu$ -TBS to dentin.

**Key words:** Adhesive systems, ammoniacal silver nitrate, bond strength, hybrid layer, silver methenamine

## INTRODUCTION

The adhesion of contemporary bonding systems is essentially micromechanical in nature. It is based upon the infiltration and *in situ* polymerization of a synthetic resin within the network collagen fibrils exposed by an acidic action, creating a new structure called the "hybrid

layer."<sup>[1,2]</sup> This structure seals the dentin, prevents post-operative sensitivity and secondary caries, and may act as an elastic buffer that compensates the tensions generated by the polymerization shrinkage of the restorative composite.<sup>[2]</sup> In order to achieve such conditions, the adhesives systems can interact with the dentin by etch-and-rinse or self-etch approaches.<sup>[1,3,4]</sup>

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The etch-and-rinse approach is considered a time-consuming and technique-sensitive bonding strategy since separate etching and rinsing steps are required. Multi-bottle etch-and-rinse adhesives involve three steps: Acid etching (mostly 30-40% phosphoric acid) followed by priming and the application of a fluid resin. The most popular two-step or "1-bottle" systems combine the primer and adhesive resin into one application. Despite the conventional three-step etch-and-rinse adhesives still perform most favorably and are most reliable in the long term, the two-step systems usually achieve acceptable adhesion to dentin.<sup>[1,4]</sup> However, the higher degree of hydrophilicity of the two-step etch-and-rinse adhesives makes them behave as permeable membranes after polymerization.<sup>[5,6]</sup> Moreover, a decreasing concentration gradient of resin monomer diffusion within the acid-etched dentin naturally occurs as a consequence of the etch-and-rinse approach, creating a porous zone especially at the bottom of the hybrid layer with increased nanoleakage expression.<sup>[4]</sup> This condition may cause post-operative sensitivity and accelerate the resin elution from hydrolytically unstable polymeric hydrogels within the hybrid layers.<sup>[7]</sup> As a consequence, the collagen fibrils remain unprotected and vulnerable to degradation by endogenous metalloproteinases, undermining the long-term durability of the restorations.<sup>[8,9]</sup>

In the case of self-etch adhesives, the non-rinsing acidic monomers are able to simultaneously etch and prime the dental tissues. Therefore, they attend the demand for simpler, more-user-friendly and less-technique-sensitive adhesives. However, nanoporosities and slight areas with exposed collagen fibrils without resin encapsulation can still be observed even with the reduced exposition of the collagen matrix network by the self-etch approach.<sup>[10-12]</sup> Such imperfections are impregnated by the silver nitrate in nanoleakage investigations, revealing areas of increased permeability within the polymerized resin matrix. According to Tay *et al.*,<sup>[10]</sup> this condition is promoted by the remaining water used as an ionizing medium for the self-etch adhesives, creating regions of incomplete polymerization and/or hydrogel formation.

The self-etch systems can hybridize the dental hard tissues through one or two operatory steps. In part depending on pH, the self-etch adhesives may be classified according to the interaction depth at dentin into ultra-mild (pH > 2.5 - "nano-interaction"), mild (pH ≈ 2 - interaction depth about 1 μm),

intermediate strong (pH 1-2, interaction depth between 1 and 2 μm), and strong (pH ≤ 1 - interaction of several micrometers' depth).<sup>[4]</sup> The latter are able to produce an interfacial ultra-morphology resembling that typically produced by etch-and-rinse adhesives.<sup>[4]</sup> Nevertheless, several *in vitro* studies have shown that despite the reasonable bonding potential of the strong self-etch adhesives,<sup>[13,14]</sup> reduced bond strength<sup>[15,16]</sup> and increased interfacial nanoleakage are recorded in comparison to the multi-step adhesives, especially regarding the strong simplified adhesives rich in HEMA.<sup>[10,17]</sup> In fact, these less-favorable *in vitro* results were confirmed by the inferior clinical performance of the strong one-step self-etch adhesives.<sup>[18,19]</sup>

The adhesive-dentin bond strength can be affected by a number of factors such as the dentin region used for bonding, the composition of the adhesive system and the application mode, polymerization characteristics (especially regarding energy dose and conversion degree), and variables related with the method used to evaluate the bond strength. With regards to the hybridization quality, revealed by the nanoleakage investigation, it is associated in most studies with the degradation phenomena and bonding durability, but little is known about its influence on the immediate bond strength to dentin. Therefore, the purpose of this study was to investigate the structural integrity of the hybrid layers and peripheral areas of two single-step self-etch adhesives, two two-step self-etching primer systems, and one one-step etch-and-rinse adhesive using silver methenamine or ammoniacal silver nitrate dyes for scanning electron microscopy (SEM) nanoleakage investigation, as well as evaluate the resin-dentin bond strength. The hypotheses tested were that (i) the interaction form of the adhesives to dentin affects hybridization quality and bond strength, and that (ii) the hybridization quality cannot be related with the immediate bond strength.

## MATERIALS AND METHODS

Forty caries-free, human third molars were collected according to the local Institutional Review Board (# 073/2007) with the informed consent of the donors. The teeth were disinfected in 1% thymol, stored in distilled water, and used within one month following extraction. The roots of the teeth were severed along the cement-enamel junction using a diamond-impregnated disk (Extec, Enfield, CT, USA) under water lubrication in a specific cutter machine (Isomet 1000, Buehler, Lake Bluff, IL, USA).

After that, the occlusal enamel of the teeth was ground using a wet #180-grit silicon carbide (SiC) paper in order to expose a flat dentin surface.

Five adhesive systems were used: The single-step self-etch adhesives – Adper Prompt (ADP) and Xeno III (XE), the two-step self-etching primer systems – Clearfil SE Bond (SE) and Adhe SE (ADSE), and the single-step etch-and-rinse adhesive – Adper Single Bond (SB). The brands, components, pH values, and application protocols of the adhesives evaluated are listed in Table 1.

### Microtensile bond testing

Ten teeth were used. Each tooth received two longitudinal sections, perpendicular to each other, using a diamond disk (KG Sorensen, Barueri, SP, Brazil) mounted in a slow-speed handpiece. These sections produced four tooth-quarters that were randomly distributed in five groups with 8 tooth-quarters each according the adhesive evaluated. Prior to bonding procedures, the exposed dentin

surfaces were wet-polished with #600-grit SiC paper under running water for 60 s to create a standard smear layer. The adhesives were applied on the prepared dentin surfaces and light-cured using a quartz-tungsten-halogen curing unit (XL 3000-3M ESPE, St. Paul, MN, USA) with a power density of 600 mW/cm<sup>2</sup>. After that, three 2-mm-thick increments of a resin composite (Filtek™ Z250, 3M ESPE) were built up on the bonded dentin surface of each tooth-quarter and individually light-cured for 20 s, constituting a crown of 6.0 mm in height. The restored tooth-quarters were stored in distilled water at 37°C for 24 h. After this period of storage, they were longitudinally sectioned in both the “x” and “y” directions across the bonded interface with a diamond-impregnated disk (Extec, Enfield) under water cooling at 300 rpm in a specific cutter machine (Isomet 1000, Buehler) to obtain 3-4 sticks of approximately 0.8 mm<sup>2</sup> in cross-sectional area for each restored tooth-quarter (25 sticks per group). The sticks were individually fixed to a custom-made testing jig (Geraldeli’s device)<sup>[20]</sup> with a cyanoacrylate glue (Model Repair II Blue, Dentsply-Sakin, Japan)

**Table 1: Materials, brands, components, pH values, and application protocols of the investigated adhesives**

Materials	Brand	Components	pH	Application protocol
Single-step, etch-and-rinse adhesive	Adper single bond (3M ESPE, St. Paul, MN, USA)	Scotchbond Etchant: 37% H3PO3	0.6	a (15s); b (15s); c; d; e; j (20s)
		Adhesive: Bis-GMA, HEMA, dimethacrylates, methacrylated polyalkenoic acid, copolymer, initiators, water and ethanol	4.7	
Two-step, self-etch adhesives	Clearfil SE Bond (Kuraray, Osaka, Japão)	Primer: Water, MDP, HEMA, hydrophilic dimethacrylates, camphoroquinone.	1.9	f (20s); e; g; j (10s)
		Adhesive: MDP, Bis-GMA, HEMA, camphoroquinone hydrophobic dimethacrylate, N/N-diethanol p-toluidine bond, colloidal silica	2.8	
	Adhe SE Ivoclar (Vivadent, Schaan, Liechtenstein)	Primer: Dimethacrylate, phosphonic acid acrylate, initiators, stabilizers, water Adhesive: HEMA, Bis-GMA, GDMA, silicon dioxide, initiators, stabilizers	1.7 7	f (20s); e; g; j (10s)
One-step, self-etch adhesives	Adper Prompt 3M (ESPE, St. Paul, MN, USA)	Prompt A: Methacrylated phosphoric esters, Bis-GMA, initiators based on camphoroquinone, stabilizers Prompt B: Water, HEMA, polyalkenoic acid, stabilizers	0.8 (mixed)	h; i (rub with medium-force for 15s); e; j (10s)
	Xeno III (Dentsply De Trey, Konstanz, Germany)	Liquid A: HEMA, purified water, ethanol, UDMA resin, BHT, highly dispersed silicon dioxide Liquid B: Phosphoric acid modified polymethacrylate resin, mono fluoro phosphazene modified methacrylate resin, UDMA resin, BHT, camphoroquinone, EDMAB	1.0 (mixed)	h; i (leave for 20s); e (2s); j (10s)

Bis-GMA indicates bisphenol-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-10-methacryloyloxydecyl dihydrogen phosphate, GDMA: Glycidyl dimethacrylate, UDMA: Urethane dimethacrylate, BHT: Butylated hydroxytoluene, EDMAB: Ethyl 4-dimethyl amino benzoate, Application techniques - a: Acid etching; b: Rinsing; c: Dry with kimwipe; d: Application of two coats of adhesive, e: Gentle air dry, f: Application of two coats of the primer; g: Application of one coat of the adhesive, h: Mixture equal amounts of the solutions A and B with for 5 seconds, i: Application of the resulting acid solution, j: Light-cure

and subjected to tensile load using a universal testing machine (Instron 4411, Instron Corporation, Canton, MA, USA) at a crosshead speed of 0.5 mm/min until failure. No premature failure was observed.

#### Failure mode classification

After the microtensile bond testing, the dentin and composite sides of the failed specimens were sputter coated (40 mA for 120 s) with gold/palladium (SCD 050; Balzers, Schaan, Leichtenstein) and then examined through SEM (JSM 5600LV; JEOL, Tokyo, Japan) with  $\times 85$  magnification, operating in secondary electron mode and accelerating voltage of 15 kV. Failure modes were classified into one of the following categories:<sup>[21]</sup> type I: cohesive failure in the hybrid layer; type II: cohesive failure in the resin composite; type III: cohesive failure in the dentin; and type IV: cohesive failure in the adhesive layer and resin composite; type V: cohesive failure in the adhesive resin and hybrid layer; or type VI: cohesive failure in the adhesive resin and dentin. In cases of uncertainty, examination under higher magnifications (650-5000 $\times$ ) was performed in order to confirm the nature of the failure. Statistical differences between the mean bond strength of the five bonding systems tested were submitted to one-way ANOVA and Tukey's HSD post-hoc test, at a pre-set alpha of 0.05. The statistical unit was sticks, not teeth.

#### Scanning electron microscopy for hybridization quality evaluation

Thirty teeth were randomly divided into five groups according to the adhesive evaluated. Two discs of 1 mm ( $\pm 0.5$ ) in thickness were prepared from each tooth by means of two parallel sections of the remaining coronary portion. The same adhesive system was applied to the adjacent dentin surfaces. The discs were then laminated into disc-pairs using a thin layer ( $\pm 1.0$  mm in thickness) of the flowable composite FilteK Flow (3M ESPE), similar to the sandwich technique first described by Inokoshi *et al.*<sup>[22]</sup> The dentin-resin-dentin sandwiches were light-cured (XL 3000-3M ESPE) for 160 s in four different directions. After that, each sandwich was sectioned perpendicularly to the resin-dentin bonding interface, producing 12 half-sandwiches by group that were immediately immersed in 2.5% glutaraldehyde in 0.1M sodium phosphate buffer at 7.2 pH, for 24 h at 37°C. The half-sandwiches were further subdivided into three groups according to the specific specimens' preparation for the following types of SEM (JSM 5600LV; JEOL) analysis: resin-dentin ultramorphological analysis (control), hybridization quality investigation using silver methenamine, or ammoniacal silver nitrate dyes.

For the ultramorphological analysis of the resin-dentin bonding interfaces, common procedures were employed to specimen preparation including demineralization in 50% phosphoric acid for 5 s, deproteinization by immersion in 10% NaOCl for 15 min, dehydration in ascending ethanol series (20%, 30%, 40%, 50%, 70%, 90%, and 100% for at least 20 min per step), and immersion in hexamethyldisilazane (Electron Microscope Sciences, Fort Washington, PN, USA) for 10 min.<sup>[23,24]</sup> After chemical dehydration, the processed bonding interfaces were mounted in aluminum stubs, sputter coated (40 mA for 120 s) with gold/palladium (SCD 050; Balzers) and observed under a SEM (JSM 5600LV; JEOL), operating in secondary electron mode, working distance 20 mm and accelerating voltage of 20 kV.

For both silver methenamine and ammoniacal silver nitrate dyes uptake, the half-sandwiches were coated with two layers of nail varnish applied to within 1 mm of the bonded interfaces. Ammoniacal silver nitrate was prepared according to the protocol first described by Tay *et al.*<sup>[17]</sup> The specimens were placed in the ammoniacal silver nitrate in darkness for 24 h, rinsed thoroughly in distilled water, and immersed in photo developing solution for eight hours under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface. Silver methenamine was prepared according to the protocol described by de Goes and Montes.<sup>[25]</sup> The specimens were immersed in the freshly prepared solution and left for 90 min at 60°C in a pre-heated oven. After this period, the specimens were rinsed in distilled water for 3 min. Specimens were then transferred to a 0.2% gold chlorine bath for 30 s, rinsed in distilled water for 1 min, placed in a 3% sodium thiosulfate bath for 3 min, and finally rinsed profusely in tap water. The specimens were then stained.

After that, the specimens were wet-polished with #600-grit SiC paper to remove the nail varnish. They were then placed inside an acrylic ring attached to double-sided adhesive tape and embedded in epoxy resin (Buehler Ltd, Lake Bluff, IL, USA). After the epoxy resin set, the specimens were lightly finished with #1000-grit and #2000-grit SiC papers under water and polished with 6-, 3-, 1-, and 0.25- $\mu$ m-grit diamond pastes (Buehler Ltd, USA). The specimens were ultrasonically cleaned with distilled water and air-dried at room temperature for 24 h. The resin-dentin interfaces were mounted in aluminum stubs, submitted to carbon evaporation (SCD 050; Balzers), and analyzed using a SEM (JSM 5600LV; JEOL), operating in back-scattering electron mode,

working distance 20 mm and accelerating voltage of 20 kV. Representative SEM micrographs were taken at  $\times 2000$  magnification.

## RESULTS

### Bond strength results and failure analysis

The microtensile bond strength ( $\mu$ -TBS) results of the adhesives evaluated are presented in Table 2. Clearfil SE Bond (SE) reached significantly higher bond strength than that of obtained by the others adhesives ( $P < 0.05$ ). No significance was found when the bond strength of Adhe SE (ADSE) was compared to that of produced by Xeno III (XE) ( $P > 0.05$ ); as well, the results of Adper Single Bond (SB) and Adper Prompt (ADP) did not differ significantly ( $P > 0.05$ ).

**Table 2: Bond strength (means $\pm$ standard deviations) of the adhesives evaluated**

Adhesive systems	Bond strength (MPa)
Clearfil SE bond	51.48 ( $\pm 15.24$ ) a
Adhe SE	37.08 ( $\pm 12.55$ ) b
Xeno III	36.24 ( $\pm 11.95$ ) b
Adper single bond	26.20 ( $\pm 4.39$ ) c
Adper prompt	24.92 ( $\pm 6.17$ ) c

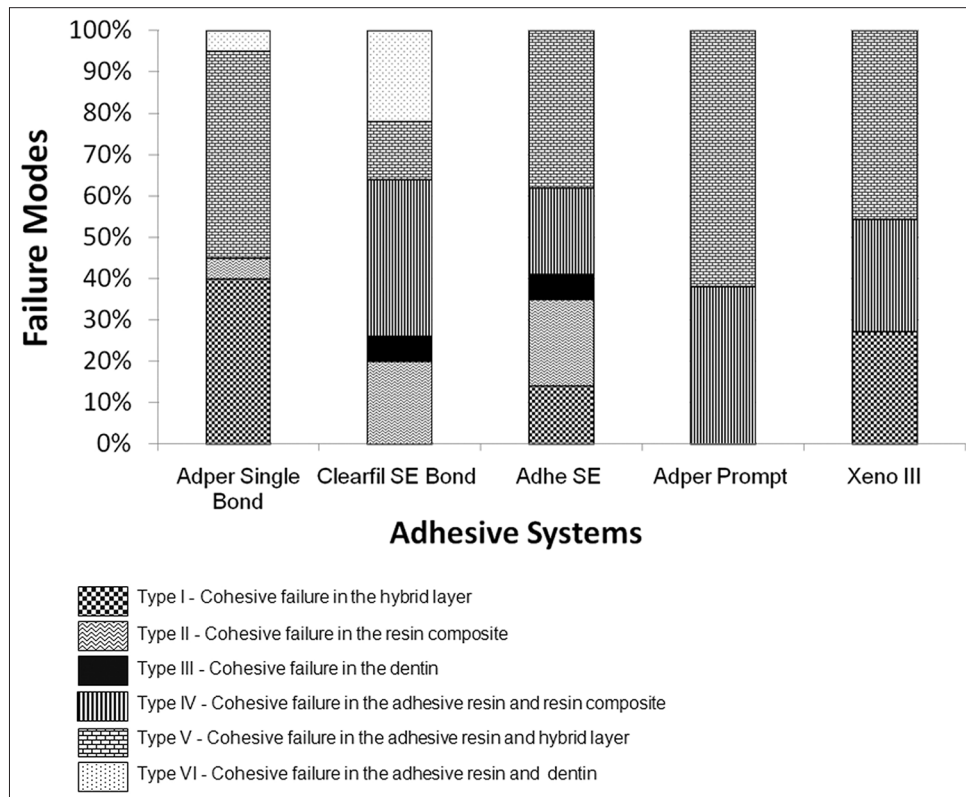
Means indicated by different letters are significantly different at  $P < 0.05$ ,  $N = 25$

The bond strength of ADSE and XE were significantly higher than those produced by SB and ADP ( $P < .05$ ).

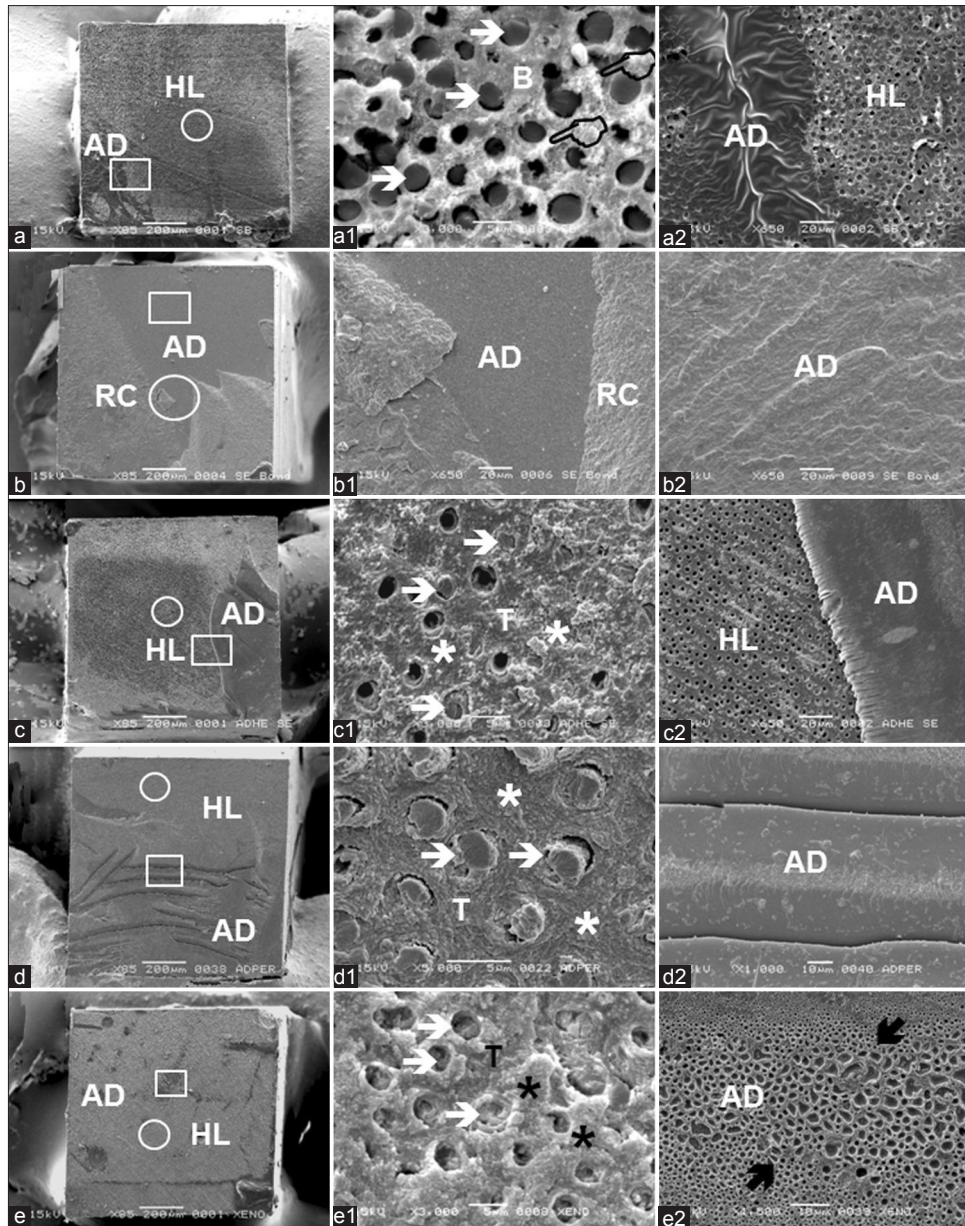
The distribution of the failure pattern (%) as analyzed by SEM can be observed in Figure 1. Representative SEM images of the predominant failure mode for all of the adhesives evaluated are in Figure 2. The type V failure mode was predominant for all of the adhesives except SE, which showed higher incidence of the type IV failure mode. The one-step self-etch adhesives XE and ADP did not exhibit the type II failure mode. The type III failure mode was observed only for the two-step self-etching primers SE and ADSE. As well, the type VI failure mode was seen only for SB and SE. The type IV failure mode was not found for the etch-and-rinse SB.

### Hybridization quality

Representative SEM micrographs of the bonding interface of the adhesives studied are depicted in Figures 3-5. The hybrid layer of SB was about 5–6  $\mu\text{m}$  in thickness with funnel-shaped resin tags distributed along the interface [Figure 3a]. When ammoniacal silver nitrate was used, specimens bonded with SB showed the most intense pattern of silver nitrate uptake, in which the whole extension of the hybrid layer seemed to be impregnated [Figure 3c]. In



**Figure 1:** Distribution of the failure pattern (%) of the adhesives evaluated



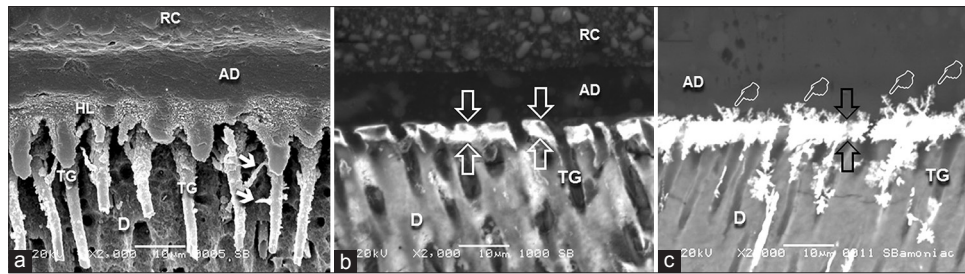
**Figure 2:** Representative SEM images of the dentin side of fractured specimens bonded with the adhesives evaluated. (a - a2) Specimens bonded with Adper Single Bond (SB); (b - b2) Specimens bonded with Clearfil SE Bond (SE); (c - c2) Specimens bonded with Adhese SE (ADSE); (d - d2) Specimens bonded with Adper Prompt (ADP); (e - e2) Specimens bonded with Xeno III (XE). (a - e) Low-power magnification of whole area of the fractured specimens. (a1- e1) Higher magnification of the circled area. (a2- e2) Higher magnification of the area limited by a rectangle. (B) Bottom of the hybrid layer; (T) Top of the hybrid layer; (AD) Adhesive resin; (HL) Hybrid layer; (RC) Resin composite; (white arrows) dentinal tubules filled by resin tags; (asterisk) intertubular dentin covered by the adhesive; (pointer) exposed collagen fibrils; (between black arrows) blistering microstructures

addition, tree-like silver deposits called “water trees” protruding from the adhesive interface toward the adhesive layer could also be observed. When silver methenamine was used, the nanoleakage expression was predominately observed at the bottom of the hybrid later [Figure 3b].

The self-etch systems SE, ADSE, and XE exhibited a shallow interaction with the dentin that was characterized by thin hybrid layers and cylindrical

resin tags [Figures 4a, 4d, and 5d]. A thicker hybrid layer about 3–4  $\mu\text{m}$  thick with funnel-shaped resin tags was observed for ADP [Figure 5a], which was comparable to that produced by SB.

Silver penetration for both two-step self-etch systems SE and ADSE, using ammoniacal silver nitrate, was practically restricted to the hybridization zone [Figure 4c and f]. It was concentrated mainly in the region between the non-demineralized dentin and



**Figure 3:** Representative SEM images of the resin-dentin interfaces bonded with Adper Single Bond. (a) Secondary electron image of unstained specimen (control). An authentic hybrid layer with 5–6- $\mu\text{m}$ -thick was observed. Funnel-shaped resin tags (TG) with lateral branches (white arrow) were noted. (b) Back-scattering image of silver-methenamine-stained specimen. The silver nitrate uptake was observed at the bottom of the hybrid layer (between white arrows). (c) Back-scattering image of ammoniacal-silver-nitrate-stained specimen. Intense silver penetration occurred throughout the hybrid layer (between black arrows). Water trees were observed in the adhesive layer (pointer). (RC) Resin composite; (AD) Adhesive layer; (HL) Hybrid layer; (D) Dentine

the hybrid layer, showing also some extensions for the adhesive layer. An intermittent silver deposition along the bonding interface was observed for SE [Figure 4c], while a more continuous nanoleakage pattern occurred for ADSE [Figure 4f]. The amount of silver deposits for both SE and ADSE was evidently smaller when compared to the deposits observed for SB, ADP, and XE [Figures 3c, 5c, and 5f]. The analysis of the hybrid layer formed by SE, after the action of the silver methenamine, exhibited silver clusters outlining the bottom of the hybrid layer to form a spotted nanoleakage pattern [Figure 4b]. In the case of ADSE, the silver deposits are closer to each other to form a nanoleakage pattern with a diffuse aspect [Figure 4e].

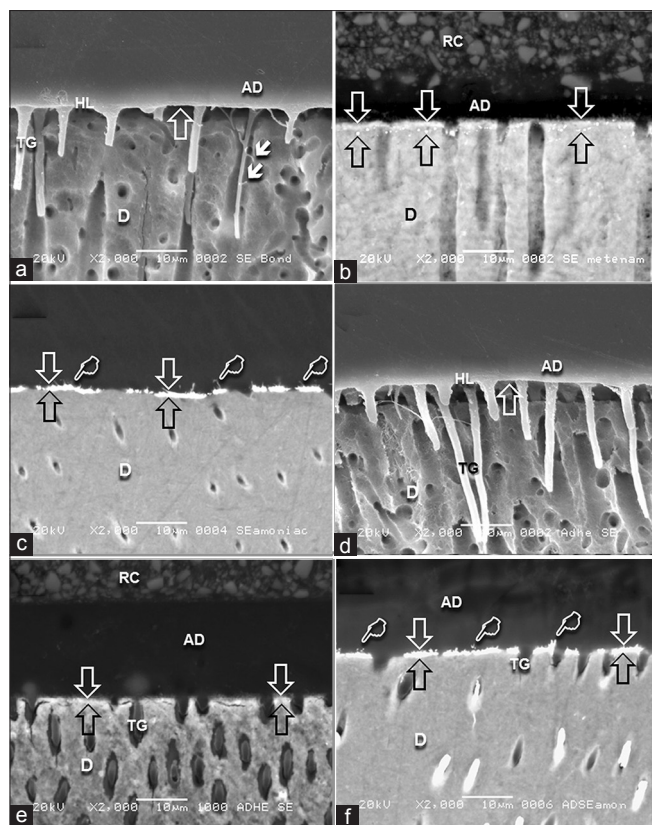
The ammoniacal silver nitrate produced intense silver deposition in the bonding interface for both one-step self-etch adhesives XE and ADP [Figures 5c and f]. The nanoleakage uptake was predominantly at the hybridization zone for XE, but clusters of silver deposits were also observed at the interaction zone between the adhesive and resin composite. The specimens bonded with ADP showed a complex silver nitrate uptake at the hybrid layer with water trees in a similar way to that observed for the etch-and-rinse SB. When submitted to silver methenamine, both ADP and XE exhibited spotted nanoleakage patterns [Figure 5b and e] similar to that produced by SE at the bottom of the hybrid layer [Figure 4b].

## DISCUSSION

The hybridization quality is key to achieving reliable bond strength and hermetic seal of the dentin surface.<sup>[2]</sup> The results of the present study showed that each adhesive system resulted in different bond strength and degree of nanoleakage, which corroborated the results of previous studies.<sup>[17,26,27]</sup>

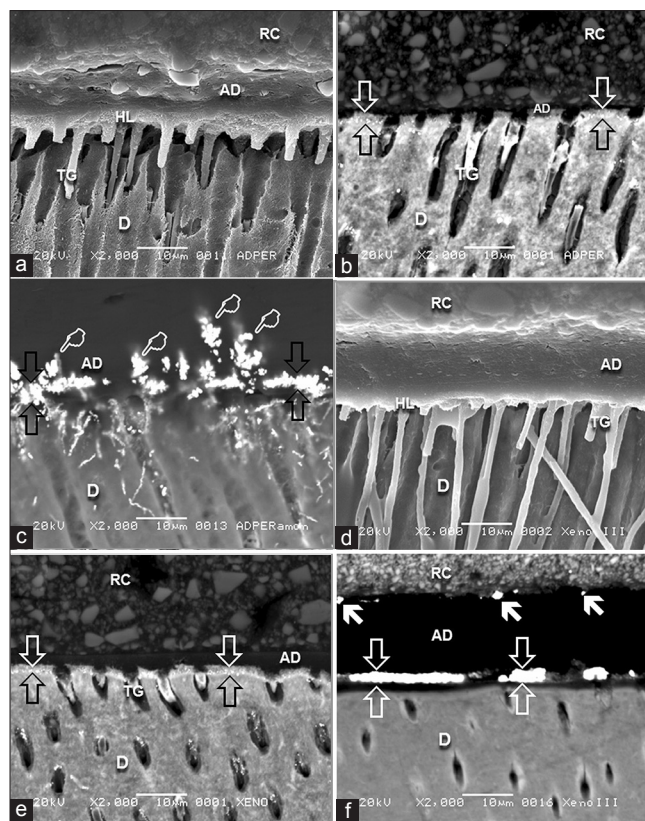
It was also demonstrated that the interaction form of the adhesives systems to dentin importantly affected the hybridization quality and bond strength; hypothesis (i) was accepted.

Sano *et al.*<sup>[28]</sup> first described the hybridization quality investigation to explore the sealing ability of the dentin adhesives using a silver nitrate tracer. However, the remnant dentin apatites and amorphous calcium phosphates, which are re-precipitated in the bonded interfaces of self-etching adhesives, may be dissolved when immersed in such a mildly acidic solution of silver nitrate (pH 4.2),<sup>[29]</sup> producing artifactual microporosities, and then false-positive results.<sup>[17]</sup> Therefore, a basic version of this solution, the ammoniacal silver nitrate (pH 9.5), was used to eliminate unwanted acidity of the conventional silver nitrate tracer solution. Besides ammoniacal silver nitrate, the silver methenamine dye (pH 8.1) was used in the present study to complement the investigation of the hybridization quality of the adhesives to dentin. This silver dye was claimed to offer reduced risks of super-estimating the nanoleakage expression in a similar way to ammoniacal silver nitrate.<sup>[25]</sup> The silver methenamine was basically designed by Gromori<sup>[30]</sup> for histologic visualization of carbohydrate derivatives in animal tissues. Many years later, the silver methenamine was applied to dental hard tissues for visualization of hypo-mineralized areas in human teeth.<sup>[30,31]</sup> These studies concluded that the silver methenamine can stain collagen of hypo- and unmineralized areas of the dentin and should be employed to demonstrate abnormal patterns of mineralization. The studies of Perdigão *et al.*<sup>[32]</sup> and de Goes and Montes (2004)<sup>[25]</sup> confirmed this information and demonstrated that the silver methenamine is a useful method for nanoleakage investigation, marking the exposed collagen fibrils within the hybrid layer.



**Figure 4:** Representative SEM images of the resin-dentin interfaces bonded with Clearfil SE Bond (SE) (a-c) and Adhese SE (ADSE) (d-f). Secondary electron images of unstained specimens (control) bonded with SE (a) and ADSE (d), showing the interaction zone (arrow) with thin hybrid layers (HL) and cylindrical resin tags (TG). Back-scattering images of stained specimens (SE – b and c; ADSE – e and f) showing the hybridization quality. When silver methenamine was used, a spotted nanoleakage pattern was observed for SE (b), which was characterized by silver clusters outlining the bottom of the hybrid layer (between arrows). ADSE (e) exhibited a diffuse nanoleakage pattern at the bottom of the hybrid layer (between arrows). The ammoniacal silver nitrate resulted in a clearer silver impregnation along the bonding interfaces. An intermittent nanoleakage pattern was produced for SE (c) (between arrows), while it was more continuous for ADSE (f) (between arrows). Both SE and ADSE exhibited some extensions of silver deposits toward the adhesive layer (pointer). (RC) Resin composite; (AD) Adhesive layer; (D) Dentine

The results of the immediate bond strength were related with the hybridization quality of the adhesives evaluated. According to this analysis, the less-favorable hybridization patterns, considering the intensity, distribution, and morphological aspect of the silver uptake were associated with lower bond strength to dentin; hypothesis (ii) was rejected. One may argue that no quantitative assessment of the nanoleakage is a limitation of the study. However, it is important to consider that the results of the present study demonstrated that the hybridization quality have relevant influence in the immediate bond strength to dentin, while the amount of silver uptake was supposed to be more properly associated with the long-term bonding durability.



**Figure 5:** Representative SEM images of the resin-dentin interfaces bonded with Adper Prompt (ADP) (a-c) and Xeno III (XE) (d-f). Secondary electron images of unstained specimens (control) showing a hybrid layer about 3-4 µm thick for ADP (a) with funnel-shaped resin tags (TG); XE (d) exhibited a thin hybrid layer with cylindrical TGs. When silver methenamine was used, both ADP (b) and XE (e) exhibited spotted nanoleakage patterns at the bottom of the hybrid layer. Ammoniacal silver nitrate yielded intense silver observed in the bonding interfaces for both ADP (c) and XE (f). Clusters of silver deposits were observed at the interaction area between the adhesive and resin composite for XE (pointer). Specimens bonded with ADP showed water trees in the adhesive layer (pointer). (RC) Resin composite; (D) Dentine

For a better understanding of the results of the present study, it is of paramount importance to take into account the composition of the adhesives. One common characteristic among them is the presence of water as an organic solvent [Table 1], which plays an important role in both etch-and-rinse and self-etch approaches. Regarding the etch-and-rinse adhesives, water is responsible for carrying resinous monomers into dentin after the etching step, and contributes to support the collagen fibrils expanded according to the wet-bonding technique.<sup>[4]</sup> However, the solvent cannot be completely removed after the application of the adhesive, especially from water-based adhesives (for instance, SB – water/ethanol solvent) that show minor solvent evaporation in comparison with ethanol-based or acetone-based ones.<sup>[33]</sup> As a consequence, the solvent remains in the bonding interface and may disturb the polymerization reaction of the resin



systems,<sup>[34,35]</sup> which is extremely deleterious for the adhesion.<sup>[3,4]</sup> This condition could have importantly influenced the intense silver uptake observed for SB [Figure 3b and c], and also the ultimate bond strength to dentin, which was significantly lower than that produced by the self-etch systems SE, ADSE, and XE.

Another aspect that may importantly have affected the hybridization quality was the adhesion approach of the adhesive systems to the dentin. The etch-and-rinse approach used the phosphoric acid to etch the dentin prior the resin monomers infiltration, creating a demineralization zone of 5 to 8  $\mu\text{m}$  in thickness. However, this deep and complex etching pattern was not completely filled by the resin monomers of the adhesive, especially at the bottom of the hybrid layer, where the collagen fibrils remain exposed.<sup>[4]</sup> Using methenamine silver solution, the presence of silver impregnated predominantly at the bottom of the hybrid layer for the specimens bonded with SB was evident [Figure 3b]; this characterizes the discrepancy between the depths of demineralization and resin infiltration. When bonding interfaces of SB [Figure 3b] and ADP [Figure 5b] are compared, the discrepant zone for SB is evidently thicker. In spite of the similarity in terms of aggressiveness between ADP and SB, which results in similar hybrid layers regarding the morphologic aspect and thickness [Figures 3a and 5a], the discrepant zone of ADP is naturally minimized due to the self-etch approach [Figure 5b]. Such a condition can be considered a goal of this adhesion strategy once it contributes to reduce the nanoleakage at the bottom of the hybrid layer, which represents a more susceptible area for degradation by the action of the endogenous metalloproteinases.<sup>[8,9]</sup>

Despite the minimization of the discrepant zone due to the self-etch approach, ADP showed an intense silver uptake after ammoniacal silver nitrate staining [Figure 5c], and the bond strength was not significantly different from that obtained by SB. In general, the most simple-to-use one-step self-etch adhesives are composed of intricate mixes of hydrophilic and hydrophobic monomers, requiring relatively high amounts of water to make possible this complex chemical combination.<sup>[36]</sup> Due to the deleterious effects of water upon adhesion,<sup>[3,4]</sup> this category of strong self-etch adhesives so far should be considered 'compromise' materials,<sup>[3]</sup> which have been documented to possess several shortcomings.<sup>[15,37]</sup> As a consequence, the immediate bond strength of ADP

was reduced, and the degree of the nanoleakage observed is at the same level of the etch-and-rinse SB.

The analysis of the failure mode distribution [Figure 1] shows predominance of type V failure mode (cohesive in the adhesive resin and hybrid layer) for both SB [Figures 2a, 2a', and 2a''] and ADP [Figures 2d, 2d', and 2d''], suggesting that these regions are less resistant against mechanical efforts. Regarding the etch-and-rinse SB, the authors speculated that there was a tendency for failure to initiate at the severely demineralized dentin not infiltrated by the resin monomers (discrepant zone), propagating then within the adhesive resin. On the other hand, the predominance of type V failure mode for ADP may be explained based on the presence of water blisters and poly-HEMA hydrogels into both hybrid and adhesive layers.<sup>[6,10,38]</sup> After the evaporation of the water, in which the monomers are mutually soluble, the chemical equilibrium of the adhesive is broken, resulting in above mentioned microstructures that capture the water,<sup>[15,39,40]</sup> and are responsible to create a complex pattern of silver deposition [Figure 5b and c]. A similar situation that results in intense silver uptake was also observed with the one-step self-etch XE [Figure 5e and f]. Especially for XE, the blistering microstructures could be identified in the SEM analysis of the failure pattern [Figure 2e2]. The sorption of water by these microstructures causes the internal plasticization of the resinous matrix, reducing the intensity of the chemical bond between the polymer chains. Thus, the absorbed moisture acts as a plasticizer agent, lowering the glass transition temperature of cured resin, and then its physical properties<sup>[41]</sup> and bond strength of the one-step self-etches adhesives to dentin.<sup>[42]</sup>

The self-etch adhesives are in general composed of acidic resin monomers, hydrophilic monomers (HEMA), and functional dimethacrylates. The presence of water is also crucial for this category of bonding systems. In this case, water ionizes the acidic monomers, providing the ability to etch and infiltrate the tooth substrates simultaneously.<sup>[4]</sup> The presence of low molecular weight and hydrophilic resin monomers such as 2-hydroxyethyl methacrylate (HEMA) is necessary, acting as a kind of solvent that enhances the penetration capability of dentinal substrates and can be polymerized with the others resin monomers. So, this category of resin monomers works as an auxiliary of the acidic monomers not readily soluble in water, attending in the demineralization and polymerization

processes.<sup>[1,43]</sup> Other bi- or multifunctional monomers are included in the formulation of the adhesives to increase the resistance of the adhesive via cross-links between polymer chains. Despite the similar basic composition, the self-etch adhesives exhibit particularities in their chemical components and water content. Consequently, the adhesives show particular interaction and bond strength with the dentin, as observed in the present study.

Unlike the one-step self-etch adhesives (AD and XE), the two-step self-etch systems have lower acidities and degrees of hydrophilicity, which minimize the possibility of creating water blisters.<sup>[3,15]</sup> Such characteristics determined the particular interaction of SE with dentin [Figure 4a-c], and are thought to significantly contribute to the higher magnitude of bond strength when compared with those produced by the other adhesives evaluated [Table 2]. The superior hybridization quality of SE was considered decisive for the low incidence of cohesive fractures in the hybrid layer (type I), suggesting that the hybridization zone is mechanically resistant against tensile tensions. Another important aspect to be considered is the chemical bonding between the monomer 10-MDP, present in the composition of SE [Table 1], and the hydroxyapatite-crystals that remain around the collagen fibrils due to the ultra-mild demineralization.<sup>[1,3,44]</sup> The resultant two-fold micro-mechanical and chemical bonding mechanisms of SE help explain the better results regarding the hybridization quality and bond strength.

Besides the more-favorable degree of hydrophilicity and pH of the two-step self-etch adhesives SE and ADSE in comparison with the simplified ones, the improved hybridization quality SE and ADSE was also importantly influenced by the application of a hydrophobic adhesive layer.<sup>[45]</sup> Adhesive systems that have the hydrophobic monomers stored in a separate bottle tend to produce a more-resistant barrier for water permeability, and thus a more-effective sealing of the dentin.<sup>[12,46]</sup> In spite of the similar characteristics of the two self-etch systems evaluated, they showed different behaviors in this study. ADSE provided significantly lower bond strength values than those obtained for SE and predominance of cohesive failure in the adhesive resin and in the hybrid layer (type V) [Figure 2c, c1 and c2]. The authors of the present study speculated that a larger amount of water is required for ionizing the acidic monomers of the more-aggressive ADSE, giving the adhesive a

higher degree of hydrophilicity. As a result, ADSE exhibited a more-intense and continuous silver uptake after ammoniacal silver staining [Figure 4f] than that observed for SE [Figure 4c], which is thought to have negatively influenced the bond strength and failure pattern distribution.

The same magnitude of bond strength between the two-step ADSE and the one-step XE self-etch adhesives may be attributed to its similar hybridization quality [Figures 4e, 4f, 5e, and 5f] and also upon the aggressiveness, which in part depends upon the pH of the adhesives. ADSE and XE are considered mild and intermediate strong adhesives, respectively, for which the interaction depth with the dentin is about 1-2  $\mu\text{m}$ .<sup>[3]</sup> Comparing the one-step self-etch adhesives ADP and XE, the significant difference in the bond strength observed between them was influenced not only by the difference in aggressiveness and hybridization quality to dentin, but it may also have been affected by the presence of inorganic fillers in the composition of XE [Table 1]. The inorganic phase contributes to form a thicker adhesive layer over the hybrid layer that is better polymerized due to the proportionally lower oxygen inhibition.<sup>[47]</sup> The failure pattern distributions are consistent with this interpretation, since XE showed lower incidence of cohesive failure involving the adhesive resin when compared to that observed in ADP.

The results of the present study demonstrated that the simplified and more-hydrophilic systems SB, ADP, and XE underperform when compared to the two-step self-etch adhesives SE and ADSE, which show less silver uptake and higher bond strength. Therefore, the hybridization was key to providing good dentin bonding, corroborating the results of a previous study.<sup>[48]</sup> Especially regarding the one-step self-etch adhesives, represented in the present study of ADP and XE, the severely compromised bonding to dentin apparently pushed them today toward the more-promising mild self-etch adhesives.<sup>[3]</sup> In an effort to improve the performance of the one-step self-etch adhesives, a new generation of "single-component" self-etch adhesives with higher pH was recently introduced. In general, the increase in pH leads to a reduction in the degree of hydrophilicity, since less water would be required for the ionization of acidic monomers. Therefore, further studies are necessary to investigate the performance of this recent concept for the one-step self-etch adhesives.

## CONCLUSIONS

Within the limits of the present study, the following can be concluded:

- The adhesive systems evaluated showed particular hybridization quality and bond strength, which depended upon not only the actual category of the adhesives, but also on the composition of the adhesives and the interaction to dentin.
- The hybridization quality is related with the immediate  $\mu$ -TBS to dentin, and is essential to improve the bonding effectiveness.

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