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# Etch-and-Rinse and Self-Etch Adhesives Behavior on Dentin

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Additional information is available at the end of the chapter

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## Abstract

Hybridization is a key phenomenon in bonding resin-based composite restorations to dentin, and results from a molecular-level interaction between the resin and the demineralized collagen fibrils network. Nanoleakage occurs when small molecules or ions infiltrate into the hybrid layer. Our work aims to evaluate if the type of solvent and adhesive system influences the morphology of the hybrid layer and the occurrence of nanoleakage within it. Human molar teeth were distributed into six groups corresponding to adhesives with different solvents (Scotchbond™ 1XT, XP Bond™, Prime&Bond® NT, One Coat Bond®, AdheSE® and Xeno® V). Dentin disks (specimens) were cut from those teeth. The corresponding adhesive systems and a microhybrid composite (Synergy® D6) were applied onto them. The specimens were thermocycled, fixed, cut, polished, decalcified, dried, and, for nanoleakage evaluation, immersed into a tracer solution. The morphology and nanoleakage analyses were performed with a high-resolution scanning electron microscope (field-emission scanning electron microscope – FESEM), and the results were statistically processed. AdheSE® achieved the overall best performance. The type of adhesive, the number of steps, and the solvent seem to play a significant role in hybrid layer morphology and nanoleakage within it. The hybrid layer water absorption can determine the adhesion longevity.

**Keywords:** etch-and-rinse adhesives, self-etch adhesives, solvents, hybrid layer, nanoleakage

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## 1. Introduction

Most adhesive systems were designed to promote dentin bonding through the interaction of hydrophilic monomers with the collagen fibrils exposed on the dentin surface after etching.

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The effectiveness of current dentin adhesives is believed to depend on the leakage of monomers with high hydrophilic affinity into the network of collagen fibers in the etched dentin structure. This entanglement of monomers with collagen fibrils and scarce residual hydroxyapatite crystals forms a hybrid tissue known as resin-dentin interdiffusion zone or hybrid layer [1].

### 1.1. Hybrid layer

According to Nakabayashi, Kojima, and Masuhara (1982), the hybrid layer is the zone of contact between the restorative material and the solid dentin [2]. Nakabayashi's team was the first to demonstrate that resins could infiltrate the etched dentin to form a new structure composed of resin matrix reinforced by collagen fibers and named this biocomposite "hybrid layer" [3]. In the dentin, the bonding tension of adhesive systems depends mostly on the hybrid layer and then on the resin tags in dentin tubules, and, finally, on the chemical bond [4].

Hybridization is the key phenomenon in the bonding of resin-based composite restorations to the dentin. It results from the molecular interaction between the resin and the demineralized network of collagen fibers, which occurs at a depth of 3–5  $\mu\text{m}$  [5]. This depth decreases to 1–8  $\mu\text{m}$  [4] with etch-and-rinse systems and to 0.5–1  $\mu\text{m}$  with self-etch systems [6–8].

When resin interacts with the network of collagen fibers, a micromechanical bonding to the dentin occurs through the penetration of polymerizable resin monomers into the exposed collagen network, the open tubules, and their lateral branches—the canaliculi, thus originating, respectively, the hybrid layer or interdiffusion zone, the resin tags, and the resin microtags. The resin penetration through the dentin's tubular/canalicular system is important to the mechanical retention as it may increase the adhesion to the whole dentin [9].

Although the hybrid layer thickness is not important in adhesion, its existence is essential and depends on the collagen fibers not collapsing while the dentin is drying after etching [9]. Under ideal conditions, the formation of the hybrid layer should be the greatest bonding mechanism in the superficial dentin while the resin tags are the major contributors for bonding forces in the deep dentin. On the other hand, since the intertubular dentin is limited due to the enlargement and approximation of dentinal tubules, the hybrid layer contributes little to adhesion [10]. The microtags in the canaliculi provide greater retention and better sealing, and also hybridization, which in this case is named "canalicular hybridization" [11].

The main functions of the hybrid layer are bonding, copolymerization with resin-based composites, and simultaneously acting as a protective layer by preventing microorganisms and toxins from reaching the pulp through the dentin [12]. The hybridized dentin reduces the risk of microleakage, the incidence of secondary caries, and postoperative sensitivity [10].

The hybrid layer's role on dentin bonding has constantly been questioned. After etching, resin monomers do not thoroughly diffuse through the collagen network to the depth of the etched dentin. This incomplete resin leakage into the collagen network may produce a porous layer of collagen that is not protected by hydroxyapatite or impregnated with resin, which will lead to nanoleakage of the hybrid layer. This exposed collagen is thus subjected to hydrolysis and degradation, resulting in increased microleakage and failure with time [13]. Recent studies on

micropermeability showed that the hybrid layer can absorb and release water, acting as a semipermeable or even permeable membrane [14, 15]. Before the resin's penetration, part of the organic phase of the dentin matrix composed of type I collagen, proteoglycans, and other noncollagen proteins may not be impregnated with the adhesive, which causes posterior water absorption and may lead to the degradation of bonding forces by nanoleakage.

## 1.2. Nanoleakage

In 1995, Sano described the nanoleakage phenomenon as an important indicator for evaluating a material's sealing ability [16]. While in microleakage cracks may vary between 20 and 50  $\mu\text{m}$ , in nanoleakage, they have approximately 0.02  $\mu\text{m}$  [17]. Nanoleakage consists in the hybrid layer's permeabilization to small ions or molecules, even when there is no detectable development of interface failures [18, 19]. According to Prati et al., and other authors who studied nanoleakage [16, 18, 20], this phenomenon is believed to result from several mechanisms combined, including the incomplete leakage of monomers in the demineralized collagen matrix, the presence of hydrophilic monomers, and the insufficient removal of solvent or water that is trapped in the hybrid layer. It may also originate from the contraction of restorative material during polymerization or from the elution of residual adhesive monomers [3].

With etch-and-rinse adhesive systems, the demineralized dentin area becomes a weak point for adhesion due to the hydrolysis of type I collagen fibers and proteoglycans located there as they should be completely impregnated in adhesive to ensure a good protection throughout time [19, 21]. Therefore, the incomplete infiltration of resinous monomers in the demineralized dentin matrix should be the main reason for decreased adhesive forces. The poor resin leakage facilitates permeability to external fluids (as well as to oral and bacterial enzymes) and to endogenous proteolytic enzymes that are slowly released from the etched dentin after adhesion and impair the performance of the attached interface [19, 22].

Even though self-etch adhesive systems condition and simultaneously leak into the underlying substrate, a discrepancy between the demineralization and resin infiltration depths may occur [23, 24]. Several references prove that nanoleakage in interfaces formed by self-etch adhesives is not exclusively caused by the incomplete leakage of resin in the demineralized dentin. Other reported causes are areas within the adhesive layer from where water is incompletely removed, thus resulting in areas of improper polymerization and/or hydrogel formation, and hydrophilic areas of acidic monomers that are more prone to water caption [23]. Sometimes, in both etch-and-rinse and self-etch adhesive systems, channels filled with water form in the adhesive layer—water trees, and these are manifested by water bubbles in the resin-dentin interface. This phenomenon may contribute directly to resin degradation due to the extraction of nonpolymerized monomers or small oligomers with time [25].

Besides occurring in the hybrid layer, nanoleakage may also occur in the adhesive layer [16, 26]. Moreover, water absorption by hydrophilic resin monomers in the hybrid and adhesive layers may contribute to the degradation of bonding forces over time [27] since the permeable hybrid layer seems to be highly susceptible to slow water hydrolysis.

Ammoniacal silver nitrate ( $\text{AgNO}_3$ ) has been used to identify regions filled with water, with leakage failure, and with the deterioration of hydrophilic polymers in the hybrid and adhesive layers [25]. Silver nitrate is an excellent marker as silver ions are very small and have high solubility, thus allowing the preparation of highly concentrated solutions [28]. The flow of silver ions increases in water trees.

### 1.3. Objectives

This work was aimed to evaluate if the type of solvent and adhesive system influence the morphology of the hybrid layer and the occurrence of nanoleakage within it, based on high-resolution electronic microscopy (FESEM) analysis.

### 1.4. Null hypothesis

The type of solvent and the adhesive system used in two-step etch-and-rinse adhesive systems do not influence the hybrid layer morphology, quality, and thickness, as well as the occurrence of nanoleakage within it.

## 2. Material and methods

### 2.1. Sample preparation

After appraisal and approval by an Ethics Committee, 78 caries-free human molars ( $N = 78$ ), extracted for periodontal or orthodontic reasons, were included in this study. These teeth had been disinfected in 0.5% chloramine and had been stored for up to 6 months in distilled water (according to ISO/TS 11405, 2003). They were cross-cut into 1 mm-thick dentin disks—specimens—using a slow-speed diamond saw (Accuton 2-Struers, Copenhagen, Denmark). A standardized smear layer was created on the occlusal surface of each specimen using 600-grit silicon carbide (SiC) paper (for 1 min under cooling with distilled water).

### 2.2. Restorative procedures

Specimens were randomly allocated into the following six adhesive/solvent groups (**Table 1**):

- Group A: Adper™ Scotchbond™ 1XT (3 M ESPE, Seefeld, Germany)—ethanol and water;
- Group B: XP Bond™ (Dentsply, Konstanz, Germany)—tert-butanol;
- Group C: Prime&Bond® NT (Dentsply, Konstanz, Germany)—acetone;
- Group D: One Coat Bond® (Coltène Whaledent, Altstätten, Switzerland)—solvent free, 5% water;
- Group E: AdheSE® (Ivoclar Vivadent, Schaan, Liechtenstein)—water;
- Group F: Xeno® V (Dentsply, Konstanz, Germany)—water/tert-butanol.

Two layers of each adhesive system were applied on the dentin disks, following the manufacturers' instructions, and then light cured for 20 s (BluePhase, Ivoclar Vivadent, Schaan, Liechtenstein). After adhesive application, a hybrid composite resin (Synergy® D6, Coltène Whaledent, Altstätten, Switzerland) was applied and light cured in two increments of 2 mm. Light curing was performed at 1200 mW/cm<sup>2</sup> (BluePhase, Ivoclar Vivadent, Schaan, Liechtenstein).

Adhesives	Composition
Adper™ Scotchbond™ 1XT (3M-ESPE)* Lot number: 5 FL	Bis-GMA, HEMA. Dimethacrylates polyacenoic copolymer. 5 nm diameter 10% of weight silica spherical particles. Solvents: ethanol and water
XP Bond™ (Dentsply) Lot number: 0609000250	Carboxylic acid modified dimethacrylate (TCB resin); phosphoric acid modified acrylate resin (PENTA); urethane dimethacrylate (UDMA); triethyleneglycol dimethacrylate (TEGDMA); 2-hydroxy ethylmethacrylate (HEMA); butylened benzenediol [stabilizer]; ethyl-4-dimethylamir obenzoate; camphorquinone; functionalized amorphous silica. Solvent: tert-butanol
Prime & Bond™ NT (Dentsply) Lot number: 0508000096	Di- and trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate), photoinitiators, stabilizers, nanofillers—amorphous silicon dioxide cetylamine hydrofluoride. Solvent: acetone
One Coat Bond™ (Coltene Whaledent) Lot number: 0090783	HEMA, UDMA, HPMA, hidroxypropylmethacrylate, glycerol, methacrylates, polyalkenoate methacrylized, amorphous silica Solvent: 5% water
AdheSE™ (Ivoclar Vivadent, Schaan – Liechtenstein) Batch number: Primer (1)-M02841 Bonding Agent (2): L56767	AdheSE primer: dimethacrylate, phosphoric acid acrylate, initiators and stabilizers in an aqueous solution AdheSE bond: HEMA, dimethacrylate, silicon dioxide, initiators, and stabilizers. Solvent: water
Xeno™ V (Dentsply, Konstanz – Germany) Batch number: 1002000450	Bifunctional acrylate, acidic acrylate functionalized phosphoric acid ester, acrylic acid, water, tertiary butanol, initiator, stabilizer. Solvents: water and tert-butanol
Bis-GMA = bisphenol A glycidyl methacrylate; HEMA = hydroxyethylmethacrylate; UDMA = Urethanedimethacrylate; HPMA = hydroxypropylmethacrylate.	
*Adper™ Scotchbond™ 1XT (Europe) is the same as Adper™ Single Bond <i>Plus</i> (USA) and Adper™ Single Bond 2 (Latin America, Gulf countries, and Pacific region including countries such as Australia, New Zealand, and Hong-Kong).	

**Table 1.** Composition and batch numbers of the adhesives used in the study.

### 2.3. Assessment of the hybrid layer morphology

All 48 specimens were stored at 37°C with 100% humidity for 24 h (Hemmet, Schwabach, Germany) and thermal-cycled (500 cycles) in distilled-water baths at 5 and 55°C (Aralab, mod

200E, Cascais, Portugal) with a dwell time of 20 s. After storage under the same conditions for another 24 h, specimens were fixed in glutaraldehyde and rinsed. Then, they were cross-cut in half, creating 96 restored hemidisks ( $n = 16$ ). The hemidisks were polished with a sequence of sandpapers (320, 500, 1000, and 1500) and diamond pastes with 3, 1, and  $\frac{1}{4}$   $\mu\text{m}$  (Kemet® diamond spray, Kapellen, Belgium) using polishing cloths (DP-NAP, Struers, Copenhagen, Denmark). Immediately after, the hybrid layers were revealed by denaturation and decalcification of the specimens. Finally, the specimens were dehydrated in ethanol and hexamethyldisilazane (HMDS) [20, 29].

#### 2.4. Nanoleakage assessment

A total of 30 specimens were placed in a greenhouse (Hemmet, Schwabach, Germany) at 37°C and 100% humidity. Afterward, they were vertically cut with a microtome (Accuton 2, Struers, Copenhagen, Denmark) into 0.9 mm thick slabs, originating 60 restored hemidisks (slabs)—10 specimens per group ( $n = 10$ ). Each slab was then coated with two layers of nail polish, except for 1 mm around the adhesive interface. Specimens were allowed to rehydrate in distilled water for 20 min before being immersed in 50 wt% aqueous ammoniacal silver nitrate (pH 9.5) for 24 h. Specimens were then immersed in a photodeveloper solution for 8 h (Intra Periomat, Dental Durr, Bietigheim, Bissingen, Germany) under a fluorescent light (Phillips, Amsterdam, Holland) to reduce the silver or diamine silver ions into metallic silver grains [27]—and were washed in water for 1 min.

Fixation was performed by immersion in a solution of 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.4) for 12 h at 4°C. Specimens were then rinsed three times with 20 ml of 0.2 M buffered sodium cacodylate (pH 7.4) for 1 h, followed by distilled water for 1 min. Surface polishing was achieved using decreasing abrasive grits (600, 800, and 1200) of silicon carbide paper, and diamond spray (Kemet diamond spray) of 2  $\mu\text{m}$  and 1  $\mu\text{m}$  on polishing cloths (DP-NAP, Struers, Copenhagen, Denmark), followed by ultrasound bath in 100% ethanol for 10 min, and demineralization in 0.5% silica-free phosphoric acid for 1 min to remove surface debris. Afterward, the specimens were dehydrated in ascending concentrations of ethanol in water (25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min, and 100% for 60 min) and were dried by immersion in hexamethyldisilazane for 10 min [29].

#### 2.5. Specimens preparation for the FESEM observation

Specimens were placed in electron microscopy stubs and sputter-coated with Au-Pd (JEOL Fine Coat Ion Sputter JFC-1100, Tokyo, Japan). They were observed in field-emission scanning electron microscope (FESEM) (JEOL JSM 6301 F, Tokyo, Japan) using secondary electrons (SE) and an accelerating voltage of 10 kV for the morphology specimens, and backscattered electrons (BE) and an accelerating voltage of 15 kV for the nanoleakage specimens. Silver was detected using the EDS Microanalysis System (Oxford Inca Energy 350®, Oxford Instruments, Oxfordshire, UK).

## 2.6. Morphology assessment

The dentin-resin interdiffusion zone of the 16 hemidisks from each group was observed under FESEM (JEOL JSM 6301 F, Tokyo, Japan) at 10 kV, using SE. Electronic microphotographs were then taken at different magnifications and scanned with the EDS Microanalysis System (Oxford Inca Energy 350®, Oxford Instruments, Oxfordshire, UK).

For the analysis of the hybrid layer morphology, in order to standardize the measurements, six images were taken with different magnifications (right, center, and left at 400×, 500×, 800×, and 1500×) per specimen. The hybrid layer thickness was measured in the 1500× images using the “offline” mode of the software (Oxford Inca Energy 350®, Oxford Instruments, Oxfordshire, UK). The hybrid layer thickness was observed in the three images at 1500×, per specimen, and was averaged in order to obtain a single value to represent each tooth.

## 2.7. Nanoleakage assessment

For the analysis of nanoleakage within the hybrid layer, 10 hemidisks from each group were observed under FESEM. Six images were taken with different magnifications (right, center, and left at 400× and 2000×; 1000× was used for water-tree observation) per specimen. The total length of the hybrid layer and the extension of the ammoniacal silver nitrate impregnation were measured in the 400× images using the “offline” mode of the software (Oxford Inca Energy 350®, Oxford Instruments, Oxfordshire, UK). The nanoleakage length within the hybrid layer observed in the three images at 400×, per specimen, was averaged in order to obtain a single value to represent each tooth.

## 2.8. Statistical analysis

Scanning electron microscopy observations were registered in an Excel file and were analyzed using the IBM SPSS version 19 software. To detect and localize statistically significant differences in the studied variables from the six groups of adhesives, the Kolmogorov–Smirnov, Levene’s, and one-way or Brown–Forsythe ANOVA tests were used. Then, multiple comparisons (and correlations) were performed with the Tamhane posthoc and Bonferroni tests for morphology and nanoleakage in the hybrid layer per 305 μm, respectively. In the comparative analyses between the six studied adhesives, 5 and 10% significance levels were applied, with Bonferroni correction, and only differences with  $P < 0.0033$  and  $P < 0.0066$ , respectively, were considered statistically significant.

# 3. Results

## 3.1. Morphology assessment

The final number of specimens used for the statistical analysis was the same as the initial number ( $N = 96$ ) as no specimen was lost during the experimental procedure, even though some in the Xeno® V Group presented fractures.

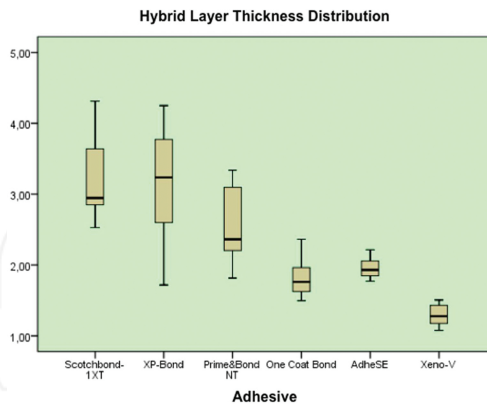


The hybrid layer thickness values obtained with the different adhesive systems were the following (**Table 2** and **Figure 1**):

- Scotchbond™ 1XT, 3.23  $\mu\text{m} \pm 0.53 \mu\text{m}$ ;
- XP Bond™, 3.13  $\mu\text{m} \pm 0.73 \mu\text{m}$ ;
- Prime&Bond® NT, 2.53  $\mu\text{m} \pm 0.50 \mu\text{m}$ ;
- One Coat Bond®, 1.84  $\mu\text{m} \pm 0.27 \mu\text{m}$ ;
- AdheSE®, 1.96  $\mu\text{m} \pm 0.14 \mu\text{m}$ ;
- Xeno® V, 1.29  $\mu\text{m} \pm 0.14 \mu\text{m}$ .

Hybrid layer thickness		
Adhesives	Mean ( $\mu\text{m}$ )	Standard deviation ( $\mu\text{m}$ )
Scotchbond™ 1XT	3.2287	0.53253
XP Bond™	3.1335	0.7315
Prime & Bond® NT	2.5346	2.3617
One Coat Bond®	1.84	0.2744
AdheSE®	1.964	0.13571
Xeno® V	1.291	0.14187

**Table 2.** Mean hybrid layer thickness and standard deviation of the different adhesive systems.



**Figure 1.** Mean hybrid layer thickness and standard deviation of the different adhesive systems.

Scotchbond™ 1XT showed a greater mean thickness than One Coat Bond®, AdheSE®, and Xeno® V (1.26–1.94  $\mu\text{m}$ ,  $P < 0.001$ ). XP Bond™ also showed a greater mean thickness than One Coat Bond®, AdheSE®, and Xeno® V (1.17–1.84  $\mu\text{m}$ ,  $P < 0.001$ ). Prime&Bond® NT showed a hybrid layer thicker than One Coat Bond® (0.69  $\mu\text{m}$ ;  $P < 0.002$ ) and Xeno® V

(1.24  $\mu\text{m}$ ;  $P < 0.001$ ). One Coat Bond<sup>®</sup> had a hybrid layer thicker than Xeno<sup>®</sup> V (0.55  $\mu\text{m}$ ;  $P < 0.001$ ). AdheSe<sup>®</sup> had a greater mean hybrid layer thickness than Xeno<sup>®</sup> V (0.67  $\mu\text{m}$ ;  $P < 0.001$ ). Finally, Xeno<sup>®</sup> V showed the lowest values of all, in a statistically significant way (Figure 2).

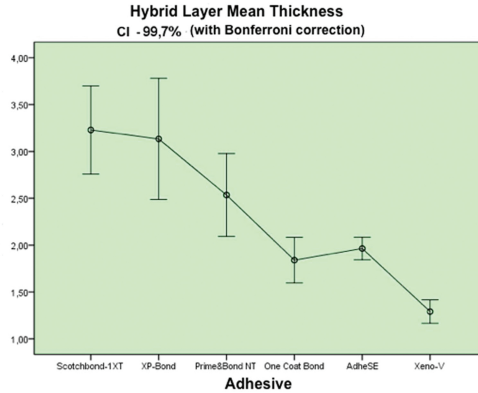


Figure 2. Comparison of the mean hybrid layer thicknesses, with Bonferroni correction, between the six adhesive groups.

### 3.1.1. FESEM images (morphology)

#### 3.1.1.1. Scotchbond<sup>™</sup> 1XT

Figure 3A and B shows the adhesive layer (AL), the hybrid layer (HL), the resin tags (T), and dentin (D) with different magnifications (800 $\times$  and 1500 $\times$ ). In both figures, the hybrid layer of Scotchbond<sup>™</sup> 1XT presents good quality.

#### 3.1.1.2. XP Bond<sup>™</sup>

Figure 4A and B shows the adhesive layer (AL), the hybrid layer (HL), and, beneath that, the resin tags (T) at 500 $\times$  and 1500 $\times$  magnifications. In both figures, the hybrid layer of XP Bond<sup>™</sup> also presents good quality.

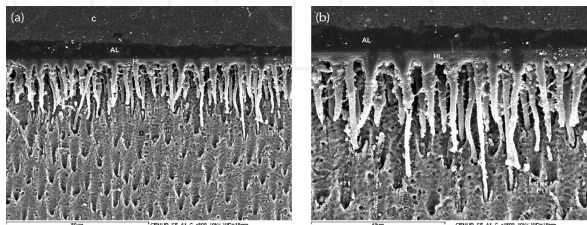


Figure 3. A and B.

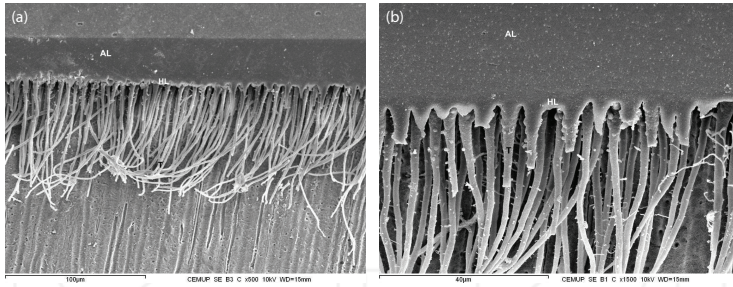


Figure 4. A and B.

### 3.1.1.3. Prime&Bond® NT

Figure 5A and B shows the adhesive layer (AL) and a space in the underlying hybrid layer (HL) marked with arrows that represent a failure in the resin leakage. These figures also show resin tags (T). In this case (Prime&Bond® NT), the hybrid layer is impaired, which may imply greater nanoleakage.

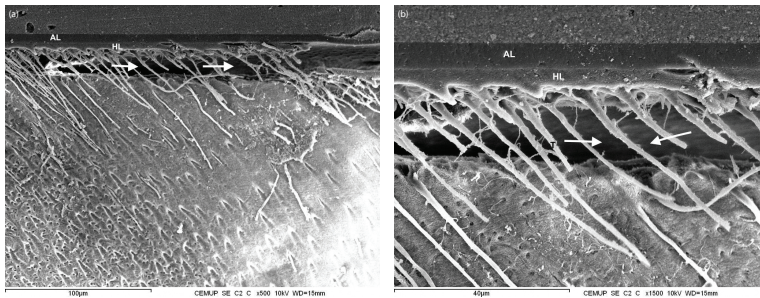


Figure 5. A and B.

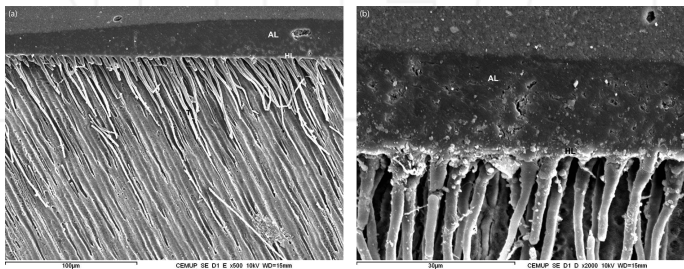


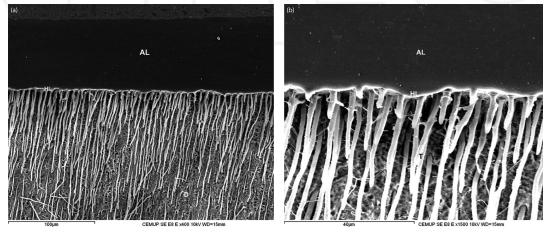
Figure 6. A and B.

#### 3.1.1.4. One Coat Bond®

**Figure 6A and B** shows the adhesive layer (AL), the hybrid layer (HL) and the resin tags (T) different magnifications (500× and 2000×). In both figures, the hybrid layer of One Coat Bond® presents good quality.

#### 3.1.1.5. AdheSE®

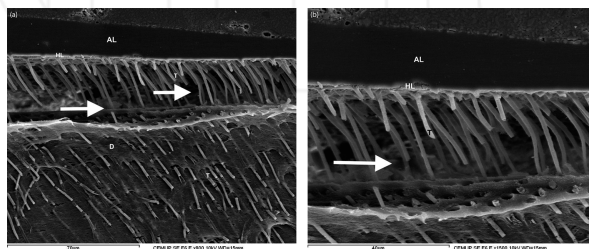
**Figure 7A and B** shows the adhesive layer (AL), the hybrid layer (HL), and, beneath that, the resin tags (T). **Figure 7B** shows the adhesive layer (AL), the hybrid layer (HL), and the underlying resin tags (T) with greater magnification. In both figures, the hybrid layer of AdheSE® presents good quality.



**Figure 7.** A and B.

#### 3.1.1.6. Xeno® V

**Figure 8A and B** shows the adhesive layer (AL) and a space in the underlying hybrid layer (HL) marked with arrows that represent a failure in the resin leakage. That space corresponds to the area where the collagen fibers that were not involved by adhesive resin were located before the specimens' preparation. Those collagen fibers were degraded by either the sodium hypochlorite/hydrochloride acid during preparation or some hydrolysis during thermocycling. These figures also show the dentin (D) and the resin tags (T) of which some are fractured. In this case (Xeno® V), the hybrid layer is impaired, which may imply greater nanoleakage.



**Figure 8.** A and B.

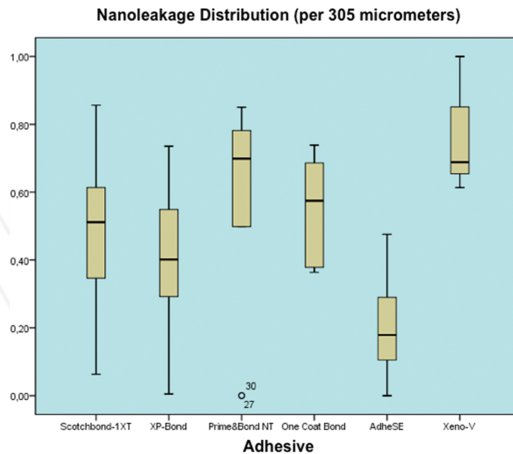
### 3.2. Nanoleakage assessment

The final number of specimens used for the statistical analysis was the same as the initial number ( $N = 60$ ) as no specimen was lost during the experimental procedure, even though some presented fractures, again in the Xeno® V group.

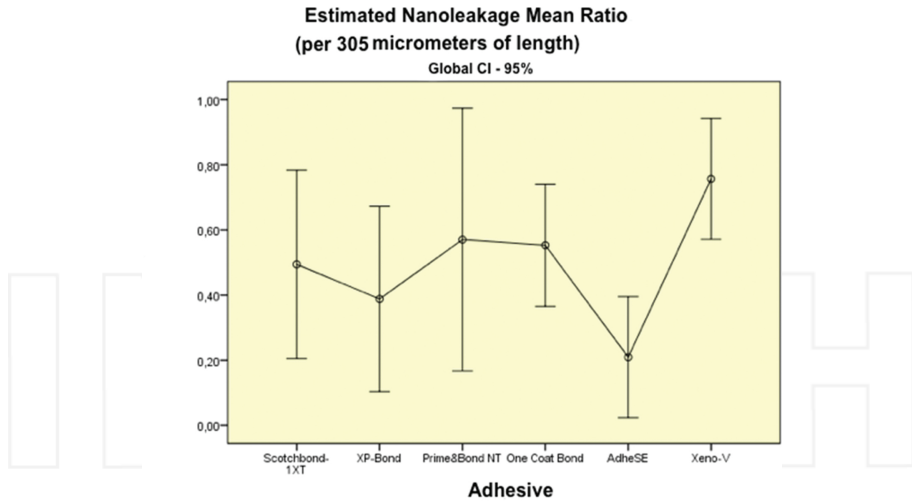
Nanoleakage percentage in the hybrid layer per 305 $\mu\text{m}$		
Adhesives	Mean (%)	Standard deviation (%)
Scotchbond™ 1XT	49.43	22.73
XP Bond™	38.81	22.36
Prime&Bond® NT	57.03	31.68
One Coat Bond®	55.29	14.74
AdheSE®	20.94	14.63
Xeno® V	75.68	14.58

**Table 3.** Mean percentages and standard deviations of the nanoleakage observed in the hybrid layer (per 305  $\mu\text{m}$ ) of the different adhesive systems used in this study.

Scotchbond™ 1XT showed a nanoleakage of  $49.43 \pm 22.73\%$ , XP Bond™ of  $38.81 \pm 22.36\%$ , Prime&Bond® NT of  $57.03 \pm 31.68\%$ , One Coat Bond® of  $55.29 \pm 14.74\%$ , AdheSE® of  $20.94 \pm 14.63\%$ , and Xeno® V of  $75.68 \pm 14.58\%$  (**Table 3** and **Figure 9**). Prime&Bond® NT had two outliers, one of which was severe (**Figure 9**). Even so, these outliers were not enough to impede the application of parametrical analysis methods, and their exclusion did not help to detect statistical significances.



**Figure 9.** Distribution of the nanoleakage percentage in the hybrid layer (per 305  $\mu\text{m}$ ) by the six adhesive system groups (with outliers).



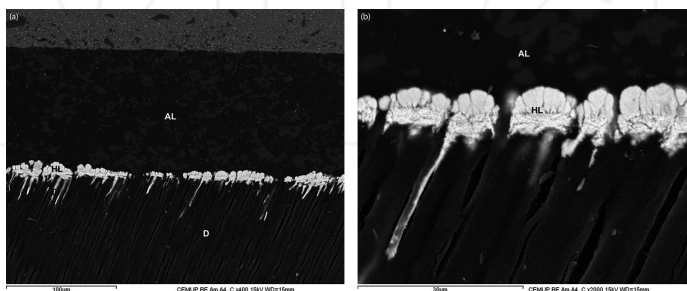
**Figure 10.** Comparison of the estimated mean nanoleakages for each adhesive system group (with Bonferroni correction).

After outliers' elimination, more analyses were conducted. For a 95% confidence, AdheSE® showed in average less 54.74% nanoleakage than Xeno® V ( $P < 0.001$ ); no statistically significant differences were found in the remaining groups (**Figure 10**).

### 3.2.1. FESEM images (nanoleakage)

#### 3.2.1.1. Scotchbond™ 1XT

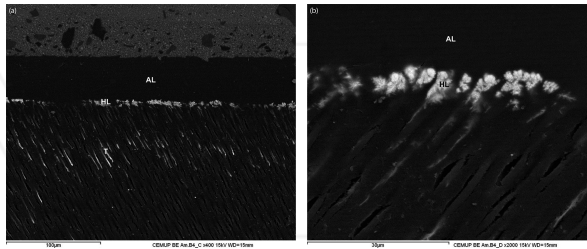
**Figure 11A** and **B** illustrates the results of Scotchbond™ 1XT and show the adhesive layer (AL), the underlying hybrid layer (HL), the resin tags (T) and dentin (D) at 400× and 2000× magnifications. The hybrid layer reveals water absorption as it is impregnated with the ammoniacal silver nitrate (lighter shade).



**Figure 11.** A and B.

### 3.2.1.2. XP Bond™

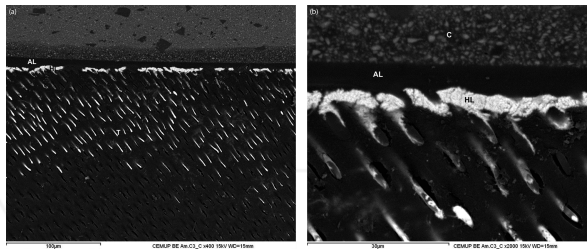
**Figure 12A and B** illustrates the results of XP Bond™ and show the adhesive layer (AL), the underlying hybrid layer (HL), and the resin tags (T) at 400× and 2000× magnifications. Figures reveal that the hybrid layer also absorbed some water as it is impregnated with the ammoniacal silver nitrate (lighter shade).



**Figure 12.** A and B.

### 3.2.1.3. Prime&Bond® NT

**Figure 13A and B** illustrates the results of Prime&Bond® NT and show the adhesive layer (AL), the underlying hybrid layer (HL), and the resin tags (T) at 400× and 2000× magnifications. It is also possible to identify the resin-based composite (C). The hybrid layer reveals water absorption as it is impregnated with the ammoniacal silver nitrate (lighter shade).



**Figure 13.** A and B.

### 3.2.1.4. One Coat Bond®

**Figure 14A–C** illustrates the results of One Coat Bond® and show the adhesive layer (AL), the underlying hybrid layer (HL), and the resin tags (T) at 400× and 2000× magnifications. The hybrid layer reveals water absorption as it is impregnated with the ammoniacal silver nitrate (lighter shade). In **Figure 14A**, the phenomenon of water trees (WT) formation is clearly visible. It is represented by a branched pattern and reveals nanoleakage in the adhesive layer because the hybrid layer acted as a permeable membrane, thus allowing water to flow through it.

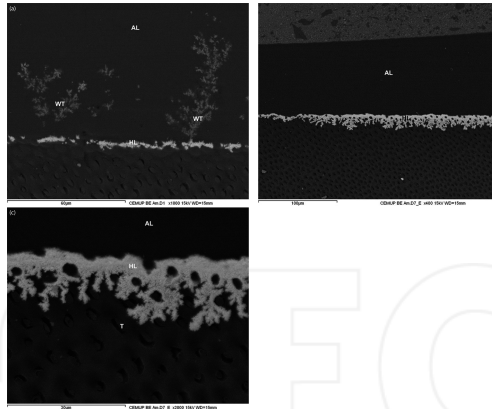


Figure 14. A, B, and C.

### 3.2.1.5. AdheSE®

**Figure 15A** and **B** illustrates the results of AdheSe® and show the adhesive layer (AL) and the underlying hybrid layer (HL) at 400× and 2000× magnifications. The hybrid layer has absorbed practically no water, and thus the ammoniacal silver nitrate solution was practically not impregnated with this adhesive.

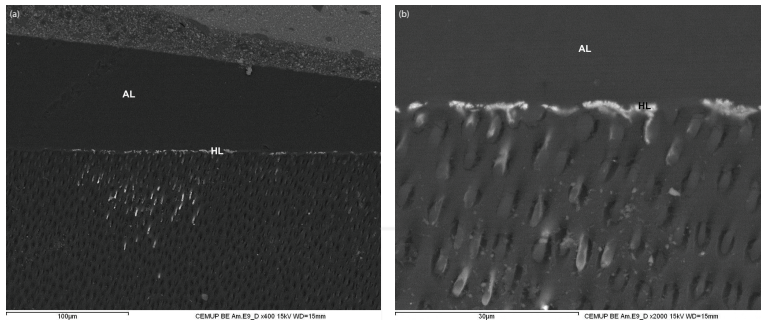


Figure 15. A and B.

### 3.2.1.6. Xeno® V

**Figure 16A** and **B** illustrates the results of Xeno® V and show the adhesive layer (AL), the underlying hybrid layer (HL), and the resin tags (T) at 400× and 2000× magnifications. The dentin (D) is also identified. The hybrid layer is almost completely impregnated with ammoniacal silver nitrate. The specimens suffered fractures, and this is clearly visible in all figures. Fracture of the resin tags (T) is also visible.



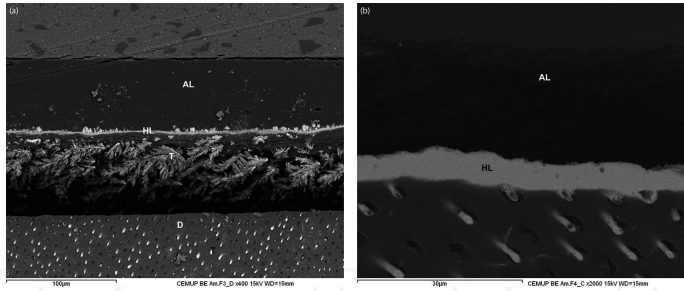


Figure 16. A and B.

## 4. Discussion

### 4.1. Hybrid layer morphology

In this study, we evaluated the hybrid layer quality, which may be very important for assessing the bonding process performance and predicting the adhesives durability in the long term in the oral environment. However, that evaluation is somewhat subjective and sometimes may be difficult to perform.

Milia and Santini reported that the identification of a high-quality hybrid layer with strong and stable adhesion is associated with a homogeneous area where monomers leak completely and fill the collagen matrix [30]. When the whole demineralized dentin (with exposed collagen fibers) is protected, i.e., surrounded by adhesive, a high-quality hybrid layer is achieved. When this does not occur, as in the case of etch-and-rinse adhesives, there is a discrepancy between the demineralization and the resin penetration, resulting in a “space” underneath the hybrid layer, which is clear in the electron microscopy images of the Prime&Bond® NT (**Figure 5A and B**). Moreover, according to some studies, regardless of the number of application steps, acetone-based etch-and-rinse adhesives have shown a performance worse than the water/ethanol-based ones. The main reason for their compromised clinical results in the long term is the high technical sensitivity of acetone-based adhesives [31, 32].

In single-step self-etch systems, such as the Xeno® V, the same phenomenon appears in the FESEM images (**Figure 8A and B**). In this situation, the cause may lie on the phase separation and poor resin penetration due to an early evaporation of the solvent, or on a poor polymerization of the adhesive due to great quantities of water being absorbed by the hybrid layer of these adhesives [15], which might have lead to adhesive fracture during polymerization. If the solvent does not completely evaporate before light cure, failures may occur, weakening the hybrid layer and causing an early failure of the restoration [33]. Thus, the hybrid layer has poor quality. According to the literature, the good quality of a hybrid layer is essential for adhesion, even more than its thickness. That is why self-etch adhesives often provide greater forces of adhesion to the dentin than etch-and-rinse ones, despite the latter having a larger

thickness (up to 5  $\mu\text{m}$  versus 0.5–1.5  $\mu\text{m}$ ) [34]. Although these thickness values vary in different studies, they are coherent with the ones obtained in our study. This situation is related to the fact that self-etch adhesives show less discrepancy between dentin demineralization and resin penetration.

Therefore, there is no correlation between the hybrid layer thickness and bonding forces, which suggests that hybrid layer quality is more important than its thickness for the success of bonding forces [35]. The values obtained in this study for the hybrid layer thickness of One Coat Bond® are coherent with the ones obtained in the study by Breschi et al., which varied between 1.4 and 2.1  $\mu\text{m}$  [1]. Xeno® V showed systematically lower values of mean hybrid layer thickness.

For adhesion to be effective, as well as durable, it is essential that the formed hybrid layers do not have defects. Adhesives with poor-quality hybrid layers have short durability in comparison with the ones that form high-quality hybrid layers, which are more qualitatively filled by resin [36]. In poor-quality hybrid layers, collagen fibers or fibrils that are not enveloped by resin will originate a porous interface—nanoleakage, and are thus more prone to hydrolysis (as are all the proteins) and degradation with time; these are areas of adhesion failure [18, 19, 36, 37]. Again, this study's results are coherent with previous studies, as, in the case of Xeno® V, a poor-quality hybrid layer lead to a high percentage of nanoleakage with ammoniacal silver nitrate (75.68%). This situation also occurred with Prime&Bond® NT, showing the highest nanoleakage percentage (57.03%) of all the tested etch-and-rinse systems (and the second highest of all the six groups) (Table 3 and Figure 9).

Collagen fibrils exposed in the resin-dentin interfaces may be digested by matrix metalloproteinases (MMPs) of the host [38]. According to this study, Prime&Bond® NT and Xeno® V may cause bonding problems in the long term due to failures in the filling of nanospaces that were detected in the FESEM images. Therefore, it is possible to extrapolate that the in vivo behavior of these adhesives may involve problems in the mean/long term, considering the precautions that the extrapolation of in vitro results implies. All other adhesive groups achieved an acceptable qualitative formation of the hybrid layer.

#### 4.2. Nanoleakage within the hybrid layer

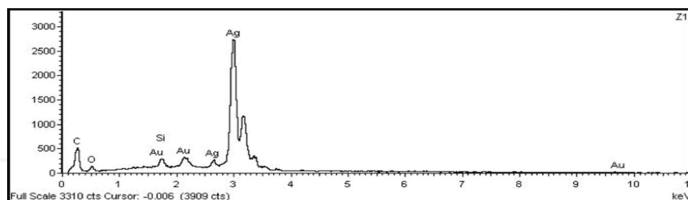
Nanoleakage is thought to start in the interface between the adhesive layer and the hybrid layer, which is the weakest point in the interior of the dentin-restoration contact area [39, 40] or in the interior of a hybrid layer that has not been perfectly penetrated by adhesive resin [28, 41].

The percentage of nanoleakage with ammoniacal silver nitrate per 305  $\mu\text{m}$  length of the hybrid layer was evaluated in every FESEM microphotograph. It is interesting that the specimens prepared with the two-step etch-and-rinse adhesive systems (Prime&Bond® NT and One Coat Bond®) and with the single-step self-etch adhesive system (Xeno® V) originated a greater rate of penetration of the respective hybrid layers with silver ions (per 305  $\mu\text{m}$ ) when compared with the other adhesive systems used in this study (Table 3 and Figure 9). These results may be explained by several factors related specifically to the composition of the adhesive systems.

In fact, several factors influence the development of nanoleakage, such as the type of solvent (water versus acetone), the individual chemical components of the adhesive system (ex: HEMA, PENTA, and Bis-GMA), and the different molecular weights of its components, which vary from 130 Da in HEMA to 513 Da in Bis-GMA, influencing the adhesive's viscosity. Other additives, such as glutaraldehyde, and the application method (dry bonding versus wet bonding) may also influence nanoleakage. With etch-and-rinse adhesive systems, dry bonding increases nanoleakage due to the collapse of collagen fibers, which interferes with the penetration of resin [4].

The reticular mode of the nanoleakage pattern, especially the silver deposits (**Figure 17**), which were perpendicular to the hybrid layer surface, corresponds to the morphologic manifestation of water trees [27]. This well-known phenomenon is clearly identified in the FESEM microphotographs (**Figure 14A**) of the One Coat Bond® and Scotchbond™ 1XT groups (although it appears in a much inferior proportion in the latter).

Given that simplified or two-step etch-and-rinse adhesive systems (such as Scotchbond™ 1XT, XP Bond™, Prime&Bond® NT, and One Coat Bond®) incorporate the primer and adhesive components in a single solution, they originate suboptimal or imperfect hybridization due to having poor ability to penetrate the demineralized dentin substrate. Moreover, their hydrophilic nature makes them more prone to water absorption and, consequently, more susceptible to the effects of hydrolytic degradation. These adhesives have a high percentage of hydrophilic monomers, thus having great permeability after polymerization and facilitating the occurrence of areas filled with water within the hybrid layer [15]. Usually, phosphoric etch adhesives (etch-and-rinse adhesives) tend to cause more nanoleakage than the two-step self-etch ones, as has been shown in some studies [16]; this fact is coherent with our study's results.



**Figure 17.** Hybrid layer spectra analysis reveals the presence of silver (Ag).

Furthermore, the solvents in these two-step etch-and-rinse adhesive systems also have an increased difficulty to evaporate, which increases their risk of becoming trapped more often in the adhesive layer after polymerization [6, 42]. This situation may have occurred especially with Scotchbond™ 1XT and One Coat Bond® as water is a cosolvent in the first one and the solvent in the second one. Water has (at 20°C) less volatility and vapor pressure (17.5 mmHg) comparing with organic solvents such as ethanol (43.9 mmHg) or acetone (184 mmHg), and, thus, is more difficult to evaporate, which may have led to the nanoleakage percentages observed in these adhesives. Between both adhesives with water as a solvent, One Coat Bond® had greater nanoleakage than Scotchbond™ 1XT, and this may be explained by the fact that

water is a poor solvent for organic components (such as monomers), which are usually more hydrophobic. Also, adding a secondary solvent, such as ethanol or acetone, helps to overcome this limitation [43]. This fact may explain the better performance of Scotchbond™ 1XT, in terms of nanoleakage percentage, compared with One Coat Bond®, as well as the worst solvent evaporation with the latter, and consequent greater water flow represented by the ammoniacal silver nitrate (**Table 3** and **Figures 11A** and **B**, **14A-C**). Moreover, the high boiling temperature and low vapor pressure of water make this solvent hard to remove from adhesive solutions after these have been applied to the dental substrate. Furthermore, Pashley et al. demonstrated that some monomers, such as HEMA (found in both Scotchbond™ 1XT and One Coat Bond®) lower, even more, the water vapor pressure, which may make difficult the removal of the remaining water [43, 44]. These facts also help explain the greater tendency of One Coat Bond® to form water trees compared with all the other adhesives (**Figure 14A-C**), which may negatively influence the bonding of One Coat Bond® in the long term (despite the good results obtained after 500 thermal cycles).

The slightly higher nanoleakage percentage of the Prome&Bond-NT® is likely to be associated more with the difficult penetration of monomers probably due to the fast evaporation of acetone (which has high vapor pressure and high volatility) or to the inability of rehydration of the collapsed collagen fibers [43]. In this study, we used dentin disks, which may present some level of dehydration of the dentin and absence of positive pulpal pressure, influencing more the performance of an adhesive such as Prime&Bond® NT in the dentin. Many etch-and-rinse adhesive systems usually contain acetone to facilitate water removal [43, 45] as acetone takes the place of the water previously existent in the dentin, removing it after pursuing it. This fact would be optimal if a degree of humidity that allowed acetone to function that way were always present. However, that does not always occur and, especially, in samples with some degree of dehydration, acetone has some difficulty to act, i.e., transport monomers, correctly. Thus, discrepancies may occur due to the improper or incomplete resin filling of open intertubular dentin nanospaces (which vary in size between 15 and 20 nm, according to Fawzy et al.) [46] after etching, and result in nanoleakage (**Figure 13A** and **B**). The PENTA monomer in the Prime&Bond® NT may also have contributed somewhat to the increased nanoleakage because, according to Perdigao et al., this monomer tends to be more hydrophilic [47]. To contradict the propensity of adhesive forms to be more hydrophilic, in the past years, there has been a tendency to reduce the quantity of strongly hydrophilic monomers, such as HEMA, and replace them with UDMA or TEGDMA [43]. XP Bond™, being the most recently released etch-and-rinse adhesive of this study, already has UDMA and TEGDMA, thus compensating the HEMA and PENTA (more hydrophilic) also present in its composition.

Every adhesive evaluated in this study has HEMA in its composition, except for Prime&Bond® NT and Xeno® V. Therefore, the hydrophobic resin component inside the residual water is prone to divide itself into resin globules and water bubbles—the phase separation of resinous materials [48, 49]. In this process, after acetone or tert-butanol has started evaporating, the monomer/solvent balance is broken as water separates from the other components of the

adhesive. When the adhesive is light cured, these water bubbles get trapped in the adhesive layer, which may lead to an impaired adhesive effectiveness [6].

Regarding monomers, the AdheSE® also includes *bis*-acrylamide, which is a monomer more hydrolytically stable than the other hydrophilic monomers used in adhesive forms in the dentin [50]. This characteristic may explain why this adhesive had a much lower mean nanoleakage percentage in the hybrid layer ( $20.94 \pm 14.63\%$ ) compared with the others. AdheSE® also showed the second lowest standard deviation, and this provides reliability to the results.

In the AdheSE® group's sample, an hydrophobic resin was applied in a stage different than the primer's one, which originated a much lower nanoleakage percentage when compared with the other five groups, even with the elimination of the outliers of Prime&Bond® NT. The values are statistically significant when comparing AdheSE® with Prime&Bond® NT, One Coat Bond®, and Xeno® V (**Table 3** and **Figures 9** and **10**). Consequently, we can predict a great stability of this adhesive with time. Therefore, the existence of two stages (or two steps) in self-etch adhesives may contribute to a better sealing of the adhesive interface [51] and that effect may prevent the occurrence of water trees and promote adhesion durability [27].

A great penetration of the ammoniacal silver nitrate was observed in the hybrid layer of the Xeno® V group ( $75.68 \pm 14.58\%$ ) (**Table 3** and **Figures 9**, **16A** and **B**). This group's sample originated the highest mean nanoleakage percentage ( $75.68 \pm 14.58\%$ ) in comparison with the other groups, and this was statistically significant when comparing Xeno® V with XP Bond™ and AdheSE® after eliminating the outliers, or even with AdheSE® before outliers were eliminated (**Table 3** and **Figures 12A** and **B**, **15A** and **B**). These results are coherent with several current studies on single-step self-etch adhesive systems that demonstrated the presence of nanoleakage in the hybrid layer due to a poor penetration of resin into the collagen network. Hashimoto et al. found that nanoleakage in these adhesives could be seen in transmission electron microscopy (TEM) [52]. Thus, due to the greater water absorption by single-step self-etch adhesives, these may act as permeable membranes [15, 53]. To contradict that disadvantage, some authors advocate that applying an hydrophobic lining may be essential to improve the adhesion durability [54], as demonstrated by Sillas Duarte Jr. et al. (2009) in a study on etch-and-rinse and self-etch adhesives [55].

A high hydrolysis in the hybrid layer may lead to reduced bonding forces with time, and thus Prime&Bond® NT, One Coat Bond®, and Xeno® V may reveal problems in the long term. Even One Coat Bond® showed water trees, and this water flow through the adhesive layer leads to bonding forces degradation in the long term, which could not be evaluated in this study.

As demonstrated by some studies, a hybrid layer with a high nanoleakage percentage (such as in the case of Prime&Bond® NT and Xeno® V) leads to a greater decline of bonding forces with time due to unprotected collagen fibers present in the resin, which makes them easily degraded by metalloproteinases [19, 22]. Thus, it would be interesting to conduct a complementary study to evaluate the nanoleakage percentage in the hybrid layer of adhesive systems for a long time (at least one year).

Finally, clinically, adhesive systems should be hydrophilic during their application and, then, they should become hydrophobic to seal the restoration margins thoroughly for a significant amount of time [16].

## 5. Conclusions

The adhesive with the overall best performance in the dentin was the two-step self-etch system containing water as solvent (AdheSE®), having shown a good morphology with a high-quality hybrid layer and low nanoleakage percentages. These good results appear to be correlated with the application of a hydrophobic resin over the hydrophilic primer in a distinct step. According to our research and other scientific studies, besides other elements that compose the adhesives, the solvent appears to play a significant role in morphology and nanoleakage. The latter is always present in the adhesive interfaces produced by the current commercial adhesives available in the market. The type of adhesive and the number of steps also seems to be important to water absorption by the hybrid layer, which leads to the nanoleakage phenomenon and can determine the adhesion longevity. Thus, according to our research, the null hypothesis was rejected.

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