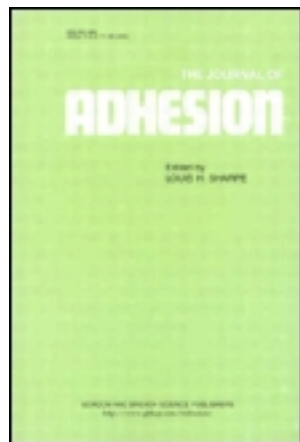


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## Influence of Different Solvents on the Morphology of the Hybrid Layer Using Two-Step Etch-and-Rinse Adhesives

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*To evaluate if different etch-and-rinse adhesive solvents influenced the hybrid layer's morphology.*

*Four one-bottle etch-and-rinse adhesive systems containing different solvents—Group A: Scotchbond 1XT<sup>TM</sup>– 3M ESPE, Group B: XP– Bond<sup>TM</sup>– Dentsply, Group C: Prime&Bond NT<sup>®</sup>– Dentsply, and Group D: One Coat Bond<sup>®</sup>– Coltène Whaledent—were applied onto 32 dentin discs which were thermocycled, prepared, and examined using field-emission scanning electron microscopy. Micrographs were scanned and the data were processed using Statistical Package for Social Sciences. The mean value and standard deviation were calculated and the Anova Multivariant Test was used.*

*The hybrid layer thickness average found was 3.23 μm (±0.53) in Group A, 3.13 μm (±0.73) in Group B, 2.53 μm (±0.50) in Group C, and 1.84 μm (±0.27) in Group D. Prime&Bond NT<sup>®</sup> presented a more inconsistent hybrid layer.*

*The solvent seems to play a significant role in hybrid layer structure and thickness.*

**KEYWORDS** *Etch-and-rinse dental adhesives; Hybrid layer; Solvents*

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## INTRODUCTION

Hybridization is a key phenomenon in bonding composite-resin restorations to dentin, and results from a molecular-level interaction between the resin and the demineralized collagen fibrils network. This interaction varies from a 3- to a 5- $\mu\text{m}$  [1] depth in etch-and-rinse systems; however, in self-etching systems, demineralization occurs at a shorter depth (0.5–1  $\mu\text{m}$  [2–4] or 0.5–1.5  $\mu\text{m}$  [5,6]).

The role of hybrid layer thickness in dentin bonding has often been questioned. Its existence is crucial and requires that the collagen fibrils network does not collapse when drying the dentin after acid-etching rinsing. However, in spite of the hybrid layer's role, its thickness is not considered important in the adhesion properties [7].

The main functions of the hybrid layer are adhesion, copolymerization with the composites, as well as simultaneously acting as a protective layer and preventing microorganisms and toxins from getting to the pulp through the dentin [8]. Hybridized dentin reduces the risk of microleakage, the incidence of secondary caries, and postoperative sensitivity [9,10].

Clinical longevity of the hybrid layer seems to involve both physical and chemical factors. Physical factors such as the occlusal chewing forces and the repetitive expansion and contraction stresses due to temperature changes within the oral cavity have been shown to affect the interface stability [11–13]. Saliva, food, beverages, and bacterial byproducts further challenge the tooth/biomaterials interface, resulting in various patterns of degradation and unprotected collagen fibrils, elution of resin monomers (probably due to sub-optimal polymerization), and degradation of resin components [14,11].

The etch-and-rinse adhesive systems contain a *primer*, whose function is to be the adhesion promoter, assuring the efficient wetness of the exposed collagen fibrils (through the hydrophilic end) and copolymerizing with hydrophobic adhesive resin (through the hydrophobic end). The solvents that can be used in these systems are water (inorganic solvent) or others with a high volatility level (whether the solvent readily forms a vapour), such as ethanol and acetone (organic solvents). There can also be adhesive systems that use water and ethanol as solvents. In order to achieve an adequate hybrid layer, it is important that the dentin is clinically wet (moist), due to the fact that the sensitive collagen fibrils network can collapse from excessive drying, preventing the interdiffusion of monomers inside it [15]. With the wet adhesion techniques, the channels between the demineralized dentin and the collagen fibrils are filled with water, solvent, conditioner, and/or oral fluids [16].

Once the solvent is the monomer carrier it seems that it has a great importance in the formation of the hybrid layer and in the adhesion phenomenon. Currently there is a lack of information relating to the type of solvent present in the adhesive system and its influence in the formation,

morphology, and quality of the hybrid layer as well as its durability with time. It is known that the solvents are the carriers for the monomers into the dentin, so we wanted to know if the variation of those carriers in the two-step etch-and-rinse adhesives could influence the morphology and thickness (even knowing that the hybrid layer's thickness is not as important as its quality) as well as the quality of the hybrid layer (absence of empty spaces beneath it). We did not use bond strength evaluation because the main aim of this study was only to determine the hybrid layer's morphology as well as a quality analysis and its relationship with the solvents present in the etch-and-rinse adhesives.

By analyzing electron micrographs, this work aimed to evaluate the structure, thickness, and quality of the hybrid layer after artificial aging using thermocycling. In this study, we investigated morphology differences originated by different types of solvents present in the adhesives applied according to the manufacturer's instructions and not the application techniques.

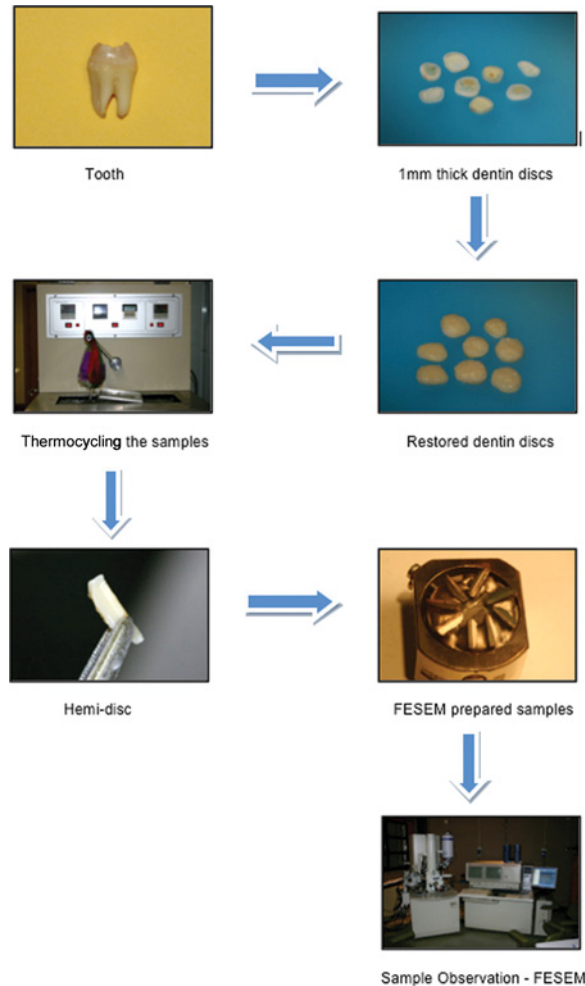
The tested hypothesis was that the type of solvent used in a two-step etch-and-rinse adhesive system can influence the thickness and quality of the hybrid layer.

## MATERIALS AND METHODS

We conducted an experimental comparative *in vitro* study to evaluate and quantify the structure and thickness of the hybrid layer by comparing two-step etch-and-rinse adhesives with different solvents. For that purpose, we used field-emission scanning electron microscopy (FESEM) to analyze the prepared samples.

The 32 caries-free molars extracted for periodontal or orthodontic reasons yielded the same amount of 1-mm thick dentin discs—Scheme 1—(one sample from each tooth - 32 samples) by cross-cutting the tooth with a slow-speed diamond disc (Accuton-Struers, Bollerup, Denmark). A standardized *smear layer* was created with a 600-grit silicone carbide (SiC) paper on the occlusal dentin surface (after removing the occlusal one-third of the tooth). We then divided the dentin discs into four different adhesive/solvent groups ( $n = 8$ )—Group A: Scotchbond 1XT™ – 3M ESPE, Seefeld, Germany (ethanol/water); Group B: XP – Bond™ – Dentsply, Konstanz, Germany (tertiary-butanol); Group C: Prime&Bond NT® – Dentsply, Konstanz, Germany (acetone); and Group D: One Coat Bond® – Coltène Whaledent, Altstätten, Switzerland (solvent free –5% water) Table 1.

We applied 37% phosphoric acid (Ivoclar Vivadent®) following the manufacturers' instructions for the etching times of all the adhesives (15 seconds), and we washed the discs with air/water and dried or rinsed according to the adhesive manufacturers' directions. After that, we applied the two-step etch-and-rinse adhesives following again the manufacturers' instructions and



**SCHEME 1** Research protocol steps. (Color figure available online.)

light-cured them for 20 seconds with a BluePhase<sup>®</sup> (Ivoclar Vivadent, Schaan, Liechtenstein) light emission diode (LED) curing light at a 1200-mW/cm<sup>2</sup> intensity. As we were not evaluating different ways of applying the adhesives, they were applied according to the manufacturer's instructions. The aim of this study was to investigate differences in different types of solvents present in the adhesives and not with the technique of application (as stated previously).

We then applied a 2-mm microhybrid composite-resin layer (Artemis<sup>®</sup> – Shade A3 – Ivoclar Vivadent, Schaan – Liechtenstein) and light-cured it for about 40 seconds.

The samples were stored at 37°C in a chamber with 100% humidity for 24 hours (Hemmet, Schwabach, Germany), and then thermocycled

**TABLE 1** Composition of Adhesives

| Adhesives  | Composition   |
|--|---|
| <i>Scotchbond 1XT<sup>TM</sup></i><br>(3M-ESPE)<br>Lot number: 5 FL          | Bis-GMA, HEMA, dimethacrylates, polyalkenoic copolymer, 5-nm diameter 10% by weight of silica spherical particles<br>Solvents: ethanol and water  |
| <i>XP – Bond (Dentsply)</i><br>Lot number: 0609000250                        | Carboxylic acid modified dimethacrylate (TCB resin), Phosphoric acid modified acrylate resin (PENTA), Urethane dimethacrylate (UDMA), Triethyleneglycol dimethacrylate (TEGDMA), 2-hydroxyethylmethacrylate (HEMA), Butylated benzenediol (stabilizer), Ethyl-4-dimethylaminobenzoate, Camphorquinone, Functionalized amorphous silica<br>Solvent: tertiary-butanol |
| <i>Prime&amp;Bond NT<sup>®</sup></i><br>(Dentsply)<br>Lot number: 0508000096 | Di- and trimethacrylate resins, PENTA (dipentaerythritol penta acrylate monophosphate)<br>Photoinitiators, Stabilizers, Nanofillers - Amorphous silicon dioxide cetylamine hydrofluoride<br>Solvent: acetone  |
| <i>One Coat Bond<sup>®</sup></i> (Coltène Whaledent)<br>Lot number: 0090783  | HEMA, UDMA, HPMA, hidroxypropylmethacrylate, glycerol, methacrylates, polyalkenoate methacrylized, amorphous silica<br>5% water   |

Bis-GMA = bisphenol A glycidyl methacrylate; HEMA = hydroxyethylmethacrylate.

TCB = butane-1,2,3,4-tetracarboxylic acid, di-2-hydroxyethylmethacrylate ester.

PENTA = dipentaerythritolpentaacrylate monophosphate; UDMA = urethanedimethacrylate.

TEGDMA = triethyleneglycoldimethacrylate; HPMA = hydroxypropylmethacrylate.

(500 cycles) in distilled water baths at 5° and 55°C (Aralab, mod 200E, Cascais, Portugal) for alternate periods of 20 seconds. 500 cycles were chosen because we followed the ISO/TS 11405 standard (2003) that indicates that a thermocycling regimen comprised of 500 cycles in water between 5 and 55°C is an appropriate artificial aging test. The dwell time varies from author to author and can reach from 10 [17] to 120 seconds [18]. Twenty seconds were chosen because in the ISO/TS 11405 (2003) indicates that “the exposure to each bath should be at least 20 seconds” and it is the protocol followed by our Department in the Faculty of Dentistry, University of Porto based on ISO and several authors.

A literature review [19] concluded that 10,000 cycles corresponds approximately to 1 year of *in vivo* functioning. We did not find specific information about the correspondence of 500 cycles of thermocycling time of wear *in vivo*, but based on the evidence we believe that is approximately 0.05 years.

After storage under the same conditions for 24 additional hours, the samples were fixed with 3% glutaraldehyde in a buffered solution of 0.2 M sodium cacodylate (pH = 7.2) for 24 hours, at 4°C, and then rinsed.

The discs were then cross-cut in half, creating 64 restored hemi-discs — Scheme 1 (specimens are standardized because all the cuts were made perpendicular - as close to the center of the specimens as possible - to the

restored dentin discs to originate the hemi-discs following the protocol of such important authors as Perdigão, Tay, and Schneider). The hemi-discs were polished with a sequence of sandpapers (320, 500, 1000, and 1500) and diamond paste (3, 1, and  $\frac{1}{4}$   $\mu\text{m}$ ) (Kemet<sup>®</sup>-diamond spray, Kemet, Kapellen, Belgium). Immediately after that, the samples were decalcified. Sodium hypochlorite 10% was used to remove the organic phase (unprotected collagen) after decalcification of the specimens. Then, the samples were dehydrated in ethanol and hexamethyldisilazane (HMDS) for critical point drying [20,21].

Each group now comprised of 16 hemi-discs that were prepared to be observed under the field-emission scanning electron microscope (FESEM, Jeol JsM 6301F, Jeol, Tokyo, Japan at 10–15 kV, focused on the dentin-resin interdiffusion zone (Scheme 1). Electron micrographs were then taken with 800x and 1500x magnification and scanned with the EDS microanalysis system (Oxford Inca Energy 350<sup>®</sup> – Oxford Instruments, Oxfordshire, United Kingdom). Six microphotographs were taken in each sample (hemi-disc): two in the left-side (one with 800x magnification and another with 1500x), two in the centre (one with 800x magnification and another with 1500x), and two in the right-side (one with 800x magnification and another with 1500x).

All the micrographs were evaluated according to a subjective analysis of the hybrid layer structure and an objective evaluation of its thickness, independently measured at three different points (one in the left-side, one in the centre, and one in the right-side of the hemi-discs) in the sample using the “offline” mode of the Inca Energy 350 software installed in a personal computer. Only one measurement per image was taken using a micrometric ruler. So, six images were taken by hemi-disc as explained above but in each image taken we only could measure the hybrid layer at one point because the offline mode of the Inca Energy 350 software only permits one measurement per image.

Note: We extracted one dentin disc per tooth, then we applied the adhesive and composite. The restored discs were cut into two parts (hemi-discs). So, eight samples became 16 per group. In each hemi-disc we took six FESEM photographs corresponding to 96 images per group).

The electron microscopy scan observations were recorded in an Excel<sup>®</sup> file, and the data were processed using statistical package for social sciences (SPSS), version 11.5. Descriptive statistics were used to analyze the measurements of the studied hybrid layers. For each hemi-disc we considered a mean value resulting for the three evaluation points.

The mean value (and standard deviation) of each sample from each group was then compared with the ones of other groups using the Anova multivariant test – general linear model (GLM). A pairwise comparison was made between measurements of the hybrid layer's average thickness.

## RESULTS

## Descriptive Analysis

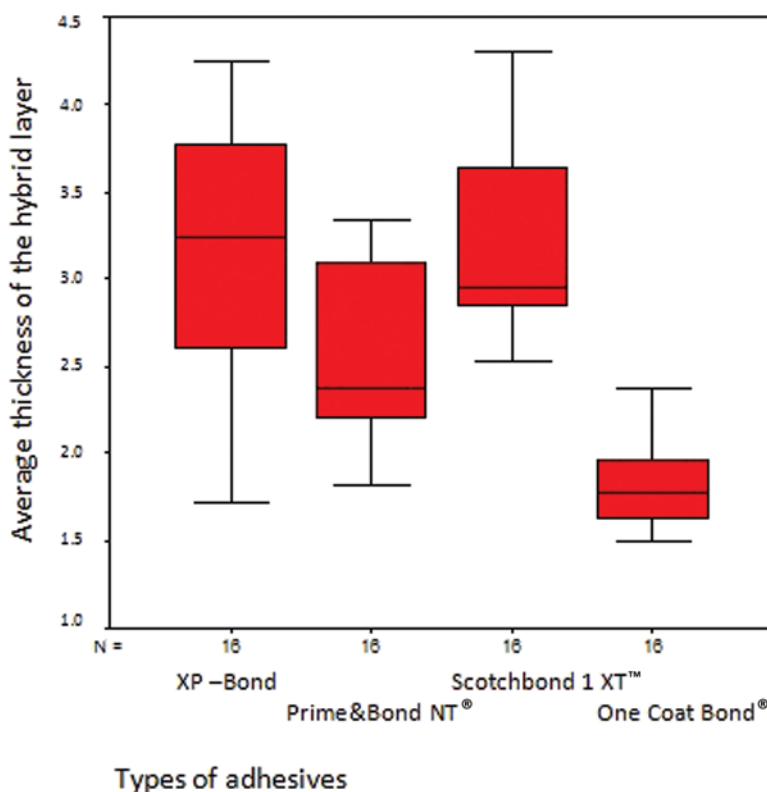
After measuring the 16 samples in each group, at three different points, we found a hybrid layer average thickness of  $3.23\ \mu\text{m}$  ( $\pm 0.53$ ) in Group A,  $3.13\ \mu\text{m}$  ( $\pm 0.73$ ) in Group B,  $2.53\ \mu\text{m}$  ( $\pm 0.50$ ) in Group C, and  $1.84\ \mu\text{m}$  ( $\pm 0.27$ ) in Group D (Chart 1).

When comparing the hybrid layer thickness among the four analyzed adhesives, Group D presented the lowest average ( $1.84\ \mu\text{m}$ ); however, it was also the one with the lowest value dispersion (variance;  $0.075\ \mu\text{m}^2$ ) (Chart 1).

In addition, Group D systematically exhibited lower hybrid layer thickness values, as can be observed in Chart 1.

## Statistical Analytical Analysis: Comparison of the Hybrid Layer Average Thickness

Table 2 illustrates a pairwise comparison between measurements of the hybrid layer's average thickness, showing some statistically significant



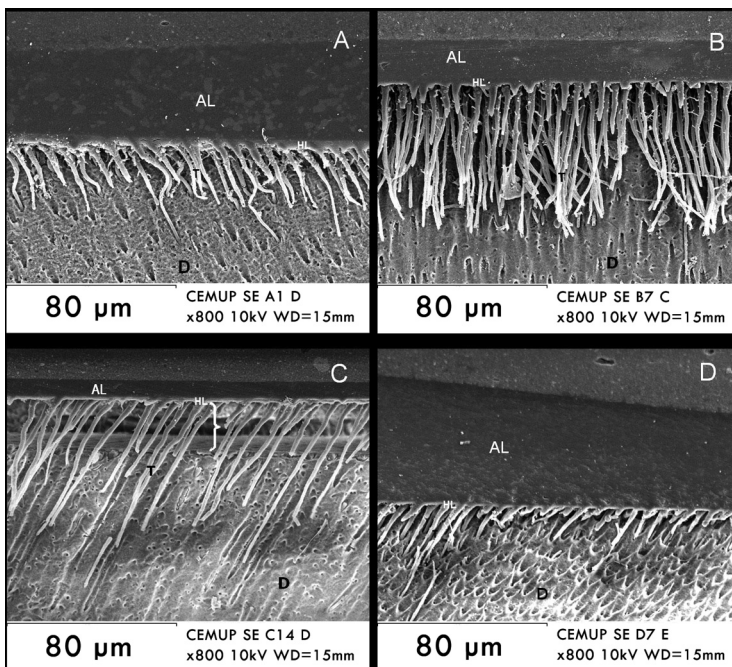
**Chart 1** Hybrid layer average thickness – Comparison of the four adhesives. (Color figure available online.)



**TABLE 2** Hybrid Layer Average Thickness - *Pairwise Comparisons*

| Dependent variable             | (I) Adhesive types | (J) Adhesive types | Mean difference (I-J) | Sig.(a)          |
|--------------------------------|--------------------|--------------------|-----------------------|------------------|
| Hybrid layer average thickness | XP – Bond          | XP – Bond™         |                       |                  |
|                                |                    | Prime&Bond NT®     | <b>.599(*)</b>        | <b>0.003</b>     |
|                                |                    | Scotchbond 1 XT™   | -0.095                | 0.62             |
|                                |                    | One Coat bond®     | <b>1.321(*)</b>       | <b>&lt;0.000</b> |
|                                | Prime&Bond NT      | XP – Bond™         | <b>-.599(*)</b>       | <b>0.003</b>     |
|                                |                    | Prime&Bond NT®     |                       |                  |
|                                |                    | Scotchbond 1 XT™   | <b>-.694(*)</b>       | <b>0.001</b>     |
|                                |                    | One Coat Bond®     | <b>.722(*)</b>        | <b>0.001</b>     |
|                                | Scotchbond 1 XT    | XP – Bond™         | 0.095                 | 0.62             |
|                                |                    | Prime&Bond NT®     | <b>.694(*)</b>        | <b>0.001</b>     |
|                                |                    | Scotchbond 1 XT™   |                       |                  |
|                                |                    | One Coat Bond®     | <b>1.416(*)</b>       | <b>&lt;0.000</b> |
|                                | One Coat Bond      | XP – Bond™         | <b>-1.321(*)</b>      | <b>&lt;0.000</b> |
|                                |                    | Prime&Bond NT®     | <b>-.722(*)</b>       | <b>0.001</b>     |
|                                |                    | Scotchbond 1 XT™   | <b>-1.416(*)</b>      | <b>&lt;0.000</b> |
|                                |                    | One Coat Bond®     |                       |                  |

Sig.(a) = Significance.

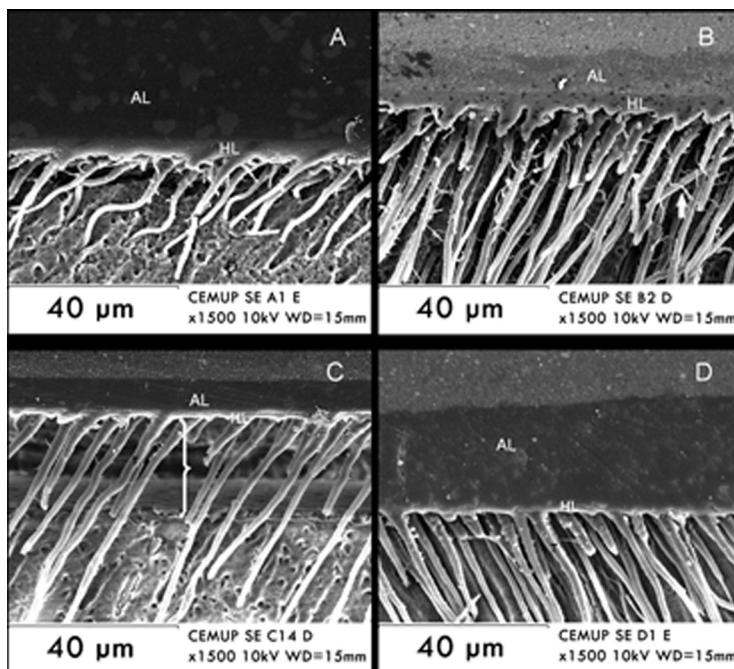
**FIGURE 1** Adhesive layer (AL), hybrid layer (HL), and tags (T) penetrating the dentin (D) visible in all 4 groups – Group A (Scotchbond 1XT™), Group B (XP – Bond™), Group C (Prime&Bond NT®), and Group D (One Coat Bond®). In Group C (Prime&Bond NT) we can easily see the empty space (curly bracket) beneath the hybrid layer (HL) caused by resin bad penetration.

differences between the different pairs: Scotchbond-1XT<sup>TM</sup> and XP – Bond<sup>TM</sup> pair ( $p < 0.003$ ) versus the Prime&Bond NT<sup>®</sup> and One Coat Bond<sup>®</sup> pair ( $p < 0.001$ ), but not between adhesives belonging to the same pair in the case of the Scotchbond-1XT<sup>TM</sup> and XP – Bond<sup>TM</sup> pair. We can also state that there were some statistically significant differences when comparing the same pair in the case of the XP – Bond<sup>TM</sup> and Prime&Bond NT<sup>®</sup> ( $p = 0.003$ ) pair.

### Qualitative Analysis of the Hybrid Layer Using Field Emission Scanning Electron Micrographs (FESEM)

The criterion used for this analysis was the direct observation of the empty spaces present beneath the hybrid layer in the FESEM micrographs.

Figures 1–2 show the four hybrid layer groups and demonstrate that the only adhesive that apparently resulted in a poorer quality hybrid layer



**FIGURE 2** In Group A (Scotchbond 1XT<sup>TM</sup>) it is evident the adhesive layer (AL), the well-marked hybrid layer (HL), and tags (T) penetrates the dentin (D). In Group B (XP – Bond<sup>TM</sup>) the hybrid layer (HL), the tags (T), and microtags (arrow) are evident. In Group C (Prime&Bond NT<sup>®</sup>) the empty space is evident (curly bracket) beneath the hybrid layer (HL) representing weak resin penetration. In that case (Prime&Bond NT<sup>®</sup>), there is a defective hybrid layer. In group D (One Coat Bond<sup>®</sup>) there is the adhesive layer (AL) and the hybrid layer (HL) as well as the resin tags (T). Aside from Group C (Prime&Bond NT<sup>®</sup>), all the hybrid layers of the studied adhesives present good quality.

structure was the Prime&Bond NT<sup>®</sup>. For subjective analysis, all the images captured in the FESEM were analyzed but in this article we only picked up a few, those we considered more illustrative.

## DISCUSSION

It is important to highlight that the *in vitro* studies show some results and conclusions that would not be confirmed in clinical practice (*in vivo*) because, by default, some factors like pulp pressure, dentin moisture, polymerization shrinkage, occlusal chewing forces, and tooth flexibility are not considered [22,23].

Based on previous studies, one may argue that hybrid layer formation and adhesive adaptation evaluation in the demineralized zone after thermocycling can be decisive when evaluating the adhesive's efficiency. The thermocycling-induced artificial aging of dentin-adhesive interfaces has a relative contribution of both chemical (hydrolysis and elution of interface components) and mechanical (repetitive contraction/expansion stress) degradation pathways [24].

In this study, evaluating the hybrid layer's quality is extremely important to the adhesive process and may interfere with durability when exposed to the oral environment. But evaluation is essentially qualitative and, therefore, subjective. The basis for a stable and strong bond is the formation of a homogeneous area, in which the monomers infiltrate completely and fill the collagen scaffold – a good quality hybrid layer [25,26]. When the demineralized dentin (with the collagen fibrils exposed) is protected, or involved by the adhesive, a hybrid layer of good quality is obtained [27]. When this does not happen the result is a bad quality hybrid layer. According to Milia and Santini [25], “the basis of a strong and stable bond is the formation of a homogeneous hybridization zone where the monomers have completely infiltrated and filled the collagen matrix.” It is known that a poor quality hybrid layer can lead to nanoleakage, and the adhesive cannot endure medium- and long-term challenges due to the degradation of the adhesive bonds caused by hydrolysis of suboptimally polymerized hydrophilic resin components and degradation of water-rich, resin-sparse collagen matrices originated by the matrix metalloproteinases (MMPs). The empty spaces present beneath the hybrid layer correspond to unprotected collagen fibrils removed with the 10% sodium hypochlorite during the specimen's preparation. *In vivo*, those unprotected collagen fibrils are susceptible to nanoleakage and hydrolysis, so we clearly can say that the quality of the hybrid layer in that situation is poor. When we have no empty spaces beneath the hybrid layer it means that the resin infiltrated all the demineralized dentin, so the quality of the hybrid layer is good and the probability of better values of adhesion is high.

According to some studies, there is no correlation between the thickness of the hybrid layer and the adhesive strength, suggesting that the quality of the layers is more important than their thickness [7a,7c,28].

The qualitative analysis of the hybrid layer structure using FESEM micrographs implies a certain degree of subjectivity, but it gives an idea of the areas where the resin did not infiltrate the nanospaces and involve the collagen fibrils. By analyzing the figures, we can conclude that Group C did not achieve a good hybrid layer, exhibiting empty spaces beneath it, probably due to the removal of unprotected collagen with sodium hypochlorite (It was used to remove the organic phase — unprotected collagen — after decalcification.) and the thermocycling (Figs. 1 and 2, Group C). The collagen fibrils were not correctly involved by the resin in the Prime&Bond NT<sup>®</sup> application due probably to poor penetration through the dentine because of the technique sensitivity of the acetone-based adhesive.

This can cause bonding issues with time and there is a higher risk of decapsulated collagen hydrolysis and nanoleakage [29–33] leading to an extrapolation that the *in vivo* behavior of this adhesive cannot endure medium and long-term challenges.

All the other adhesive groups achieved good quality hybrid layer formation and we can also speculate on achieving an acceptable long-term performance. The low quality hybrid layers have shorter term durability than the high quality ones, correctly filled with resin [34].

By comparing the results, we can conclude that among the two groups with higher viscosity adhesives in the handling, Group A exhibited the thickest hybrid layer (Figs. 1 and 2, Group A) and that Group D had the lowest average (1.84  $\mu\text{m}$ ) and the lowest dispersion of its values (Variance, 0.075  $\mu\text{m}^2$ ). The molecular weight is also a factor to take into account: Group A (Scotchbond – 1XT<sup>™</sup>) contains Bis-GMA and HEMA monomers in its composition. The molecular weight of Bis-GMA is 512 and the molecular weight of HEMA is 130. Group D (One Coat Bond<sup>®</sup>) contains HEMA, UDMA, and HPMA monomers in its composition. The molecular weight of HEMA is, as mentioned above, 130, UDMA is 471, and HPMA is 144.

Probably due to the resin monomer high molecular weight, this group adhesive had a lower penetration and compressed the collagen network, causing a less thick hybrid layer [35]. Curiously, the hybrid layer values from Group D are compatible with the results by Breschi *et al.* in which the One Coat Bond<sup>®</sup> thickness ranges from 1.4 to 2.1  $\mu\text{m}$  [36]. Group D systematically presents lower values of the hybrid layer thickness.

Solvents can also play an important role in the resin penetration along the dentin, because they are the monomer carriers. In Group A, the presence of ethanol and water might have caused its diffusion in the dentin, showing a greater probability to increase its adhesion. However, it can only be confirmed when adhesive bonding tests are performed, which were not investigated in this study. Therefore, the simultaneous presence of a solvent

with high vapor pressure such as alcohol or even acetone and water, can influence the adhesive performance, especially when applied in teeth with some dryness, like the endodontically treated ones. Based on scientific evidence it is known that endodontically treated teeth become drier because they have water loss due to the removal of the vital pulp and the loss of positive pulpar pressure which can compromise the penetration of the water-chaser solvents like acetone or ethanol into the dentinal tissue. These types of solvents depend on the presence of water in the dental tissues to penetrate by chasing it (“water-chasers”) and displacing it. This is the main reason that the dentin should be moist and not dry after rinsing off the phosphoric acid from the application site.

Therefore, acetone alone cannot re-expand the collagen scaffold if it is collapsed [37–39]. This theory is corroborated by an *in vitro* study of Mohan and Kandaswamy [9]. According to these authors, in cases of moisture variance of the substrate, the acetone-based adhesive (Prime&Bond NT<sup>®</sup>) achieved worse performance when compared with adhesives containing alcohol and water solvents (Single Bond<sup>TM</sup>, 3M ESPE, St Paul, MN, USA) or just with water (Syntac Single Component<sup>®</sup>, Ivoclar Vivadent, New York, USA). However, in an *in vivo* study by Abdalla and Garcia-Godoy [23] a higher performance was achieved in terms of hybrid layer structure and resin tags with a water-based adhesive compared with an acetone-based adhesive. This might be due to the acetone-based adhesive’s greater sensitivity to the technique [40–42].

The monomer diffusivity also plays an important role in hybridization. The solute’s diffusivity (resinous monomers), as Nakabayashi and Takarada stated [43], can depend on its molecular weight [22].

Another important factor that can influence an adhesive’s penetration into the dentin is the application period along with its viscosity [44].

It is now possible to state that, when we know in advance which of these adhesives are going to be used in the dentin, their behavior towards the formation and thickness of the hybrid layer can be predicted, when compared in pairs. Exceptions to this statement are the One Coat Bond<sup>®</sup> and the Prime&Bond NT<sup>®</sup> in hybrid layer thickness, because in this case if the adhesive is not known, its behavior in the dentin can be predicted individually, since none of the minimum and maximum values are within each other’s value range. It is important to mention that the average value was considered in order to avoid biased results.

According to the results achieved in this study, the adhesive without an organic solvent (One Coat Bond<sup>®</sup>) resulted in the lowest hybrid layer thickness. On the other hand, the adhesive with ethanol and water (ScotchBond 1 XT<sup>TM</sup>) caused a higher thickness of the hybrid layer, followed by the adhesive with a tertiary-butanol based solvent (XP – Bond<sup>TM</sup>). Lastly, the acetone-based adhesive (Prime&Bond NT<sup>®</sup>) yielded the poorest hybrid layer, not in thickness but in terms of quality. The etch-and-rinse adhesive

Prime&Bond NT<sup>®</sup> must be used with caution, because the hybrid layer might not have the structure needed for long-term performance.

## CONCLUSIONS

Within the limitations of this study, the following conclusions can be drawn:

1. The type of solvent present in the two-step etch-and-rinse adhesives influenced the hybrid layer's morphology, thickness, and quality.
2. Although the tested hypothesis was confirmed with this study, this hypothesis has to be further substantiated using more groups of dentinal adhesives, a nanoleakage evaluation, and bond strength tests.

## CLINICAL RELEVANCE

This study suggests caution when choosing an acetone-based etch-and-rinse adhesive due to its greater sensitivity to the technique which can result in an incapacity of the adhesive to endure medium- and long-term challenges in the oral cavity.

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