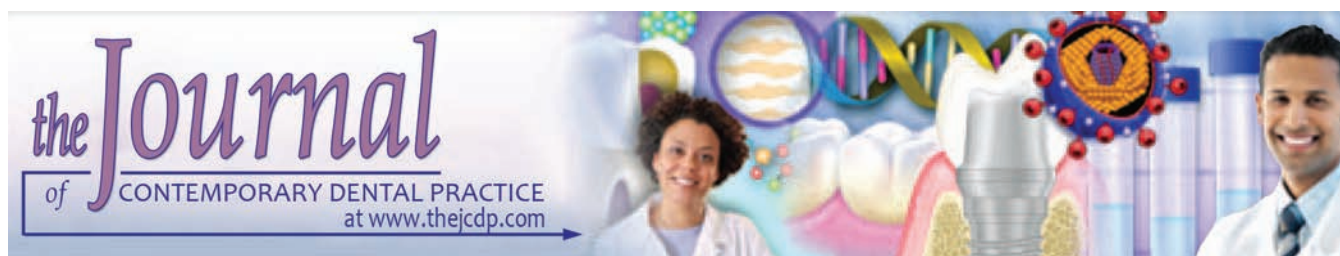**ORIGINAL RESEARCH**

Morphology of the Dentin–resin Interface yielded by Two-step Etch-and-rinse Adhesives with Different Solvents

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ABSTRACT

Aim: The study aimed to analyze the morphology of the dentin–resin interface yielded by two-step etch-and-rinse adhesive systems with different solvents and compositions.

Materials and methods: A total of 32 dentine disks were prepared and randomly assigned to four groups of one-bottle etch-and-rinse adhesive systems containing different solvents: group I, Adper Scotchbond-1XT™ (ethanol/water); group II, XP-Bond™ (tertiary butanol); group III, Prime and Bond NT® (acetone); and group IV, One Coat bond® (5% water). Adhesive systems were applied onto dentin disks, which were then thermal cycled, divided into two hemi-disks (n = 16), and prepared for field-emission scanning electron microscopy to examine the dentin–resin interdiffusion zone. Microphotographs were scanned and data were processed. Data were compared with analysis of variance multivariant test after Kolmogorov–Smirnov and Shapiro–Wilk tests using Statistic Package for the Social Sciences.

Results: The adhesive layer thickness average found was group I: 45.9 ± 13.41 μm, group II: 20.6 ± 16.32 μm, group III: 17.7 ± 11.75 μm, and group IV: 50.7 ± 27.81 μm. Significant differences were found between groups I and IV and groups II and III (p < 0.000).

Groups I (3.23 ± 0.53 μm) and II (3.13 ± 0.73 μm) yielded significantly thicker hybrid layers than groups III (2.53 ± 0.50 μm) and IV (1.84 ± 0.27 μm) (p < 0.003). Group III presented a less homogeneous hybrid layer, with some gaps. Tag length average was greater in groups II (111.0 ± 36.92 μm) and IV (128.9 ± 78.38 μm) than in groups I (61.5 ± 18.10 μm) and III (68.6 ± 15.84 μm) (p < 0.008).

Conclusion: Adhesives systems with different solvents led to significant differences in the dentin–resin interface morphology.

Solvents role in adhesives bond strength should be considered together with the other adhesive system components.

Clinical significance: The adhesive containing tertiary butanol, in addition, seems to originate a good-quality hybrid layer and long, entangled tags and also appears to have greater ability to originate microtags, which may indicate higher bond strength.

INTRODUCTION

Resin bond strength to dentin is the mechanism used to adhere filling materials to the tooth structure and should be strong enough to prevent microleakage, secondary caries lesions, and postoperative sensitivity. To solve the difficulty of bonding an artificial material to a living tissue, an adhesive should promote an intimate contact between the biomaterial (monomer) and the wet hard tissue. To penetrate the dentin structure, the adhesive must be a liquid, but must be converted *in situ* into a solid by polymerization. To allow an easier penetration, dentin is previously conditioned by an acidic gel to remove the smear layer, open dentinal tubules, and to decalcify the underlying dentin. Acid etching also leads to a funnel-shaped appearance of the dentinal tubules due to the demineralization of the superficial peritubular dentin.[1-4]

Penetration of polymerizable monomers of the adhesive into the exposed collagen network will result in the hybrid layer or interdiffusion zone. The resin that gets inside the open tubules and in its lateral branches (canaliculi) will lead to the resin tags and microtags. The

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penetration of the resin through the tubules system/dentin canaliculi has been proven to end up in an anastomosis of the adhesive tags.[3] This network of interconnected adhesive tags is supposed to be an important contributor to the dentin bond strength by some authors.[1-3]

There is little evidence that suggests dental adhesives chemically bond to dentin, but even then the bond strength will probably be low due to that effect.

Hence, resin bond strength to dentin can be considered as the sum of the individual adhesive forces arising from the resin penetration in the partially demineralized intertubular dentin (hybrid layer) and the intratubular penetration by the resin (resin tags).[5] Moreover, due to differences in orientation of the dentinal tubules, often the penetration of the resin tags will lead to a mechanical retention due to their different directions, providing a nonparallel retention.[6]

The canaliculi microtags by a phenomenon called "canaliculi hybridization,"[5] also show hybridization, improving the retention and sealing, thereby diminishing the risk of irritation and pulp sensitivity by infiltration of bacterial products.[4]

However, shrinkage resulting from the adhesive polymerization can cause separation of the tubular walls and allow fluids leakage.[7] On the contrary, resin tag length often surpasses the depth of demineralized dentin by the acids used in the etch-and-rinse or even in self-etch techniques.[8]

The etch-and-rinse adhesive systems contain a primer, whose function is to be the adhesive promoter, assuring the efficient wetness of the exposed collagen fibrils (through the hydrophilic end) and copolymerization with hydrophobic adhesive resin (through the hydrophobic end). In contemporary dental adhesives, the hydrophilic resin monomers are often dissolved in water or volatile solvents, such as ethanol and acetone (and more recently the tertiary butanol). The incorporation of these volatile solvents, also called "water-chasers," aid the water dislodgment from the dentin surface, thereby, facilitating the penetration of resin monomers into nanospaces of the exposed collagen scaffold after demineralization.[9,10] To achieve an adequate hybrid layer, it is important that the dentin is clinically wet (moist), due to the fact that the collagen fibrils network can collapse from excessive drying, inhibiting the interdiffusion of monomers inside it.[11] However, these volatile solvents are technically highly sensitive. In an *in vivo* study, Abdalla and García-Godoy[12] showed that superior performance in resin tags and hybrid layer formation was achieved with a water-based adhesive compared with an acetone-based one, possibly due to higher sensitivity of the latter technique.[13,14] Therefore, resin tags can be longer or shorter

depending on the solvent influence to yield a better or worse penetration of the resin in the dentinal tubules.[6] The low viscosity of the primers and/or adhesive resin is partly due to the dissolution of the monomers in a solvent, which leads to an improvement in its wettability.[15] Also, as shown by scientific evidence, the right solvent can also lead to an increase of the adhesive bond strength.[16]

It is, therefore, important to determine if the variation of the solvents in the two-step etch-and-rinse adhesives could influence the morphology of the dentin–resin interface.

This work aims to examine, by high-resolution electronic microscopy analysis, the structure and morphology of the resin–dentin interface yielded by etch-and-rinse adhesive systems with different solvents. In particular, the thickness of the adhesive and hybrid layers and the length of the resin tags after artificial aging by thermal cycling is to be analyzed.

The null hypothesis was that the type of solvent and composition used in two-step etch-and-rinse adhesive systems do not influence the morphology of the resin–dentin interface, the adhesive and hybrid layers thickness as well as the resin tag length.

MATERIALS AND METHODS

This experimental comparative *in vitro* study evaluated the morphology and quantified the thickness of the adhesive and hybrid layers as well as the length of the resin tags, by comparing two-step etch-and-rinse adhesive systems.

A total of 32 caries-free human molars, extracted for periodontal or orthodontic reasons, were used after being disinfected in 0.5% chloramine and stored for no more than 6 months in distilled water (according to ISO/TS 11405, 2003). These teeth were cross-cut with a slow-speed diamond disk (Accuton 2-Struers, Copenhagen, Denmark) to obtain 1-mm thick dentin disks. A standardized smear layer was created with a 600-grit silicone carbide paper on the occlusal dentin surface. Dentin disks were then randomly divided into four different adhesive/solvent groups: group I: Adper Scotchbond-1XT™ (3M ESPE, Seefeld, Germany) (ethanol/water), group II: XP-Bond™ (Dentsply, Konstanz, Germany) (tertiary butanol), group III: Prime and Bond NT® (Dentsply, Konstanz, Germany) (acetone), and group IV: One Coat Bond® (Coltène Whaledent, Altstätten, Switzerland) (solvent free–5% water) (Table 1).

Around 37% phosphoric acid (Ivoclar Vivadent®) was applied for 15 seconds, disks were washed with air/water, and dried or rinsed, and a two-step etch-and-rinse adhesive was then applied according to each adhesive manufacturer's directions.

Table 1: Adhesive composition

Adhesives	Composition
Adper Scotchbond 1XT® (3M-ESPE)*	bis-GMA, HEMA, dimethacrylates, polyalcenoic copolymer, 5 nm diameter 10% of weight silica spherical particles
Lot number: 5 FL	Solvents: Ethanol and water
XP-Bond® (Dentsply) Lot number: 0609000250	Carboxylic acid modified dimethacrylate (TCB resin); PENTA; UDMA; TEGDMA; HEMA; butylated benzenediol (stabilizer); ethyl-4-dimethylaminobenzoate; camphorquinone; functionalized amorphous silica
	Solvent: t-butanol
Prime and Bond NT® (Dentsply) Lot number: 0508000096	Di- and trimethacrylate resins, PENTA, photoinitiators, stabilizers, nanofillers– amorphous silicon dioxide, cetylamine, hydrofluoride
	Solvent: Acetone
One Coat Bond® (Colténe Whaledent) Lot number: 0090783	HEMA, UDMA, HPMA, hydroxypropylmethacrylate, glycerol, methacrylates, methacrylized polyalkenoate, amorphous silica 5% water

*Adper Scotchbond 1XT™ (Europe) is the same as Adper Single Bond Plus™ (USA) and Adper Single Bond-2™ (Latin America, Gulf countries, and the Pacific region including Australia/New Zealand and Hong Kong among others)

Materials were light-cured for 20 seconds with a BluePhase® (Ivoclar Vivadent, Schaan–Liechtenstein) light emission diode curing light at a 1,200 mW/cm² intensity. Two increments of 2-mm hybrid composite (Synergy D6®–Shade A3/D3–Coltène Whaledent, Altstätten–Switzerland) were applied and light-cured for 40 seconds each.

Specimens were stored at 37°C with 100% humidity for 24 hours (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 seconds.

After storage under the same conditions for an additional 24 hours, specimens were fixed in glutaraldehyde and rinsed before being cross-cut in half, creating 64 restored hemi-disks (n = 16). The hemi-disks were polished with a sequence of sandpapers (320, 500, 1,000 and 1,500) and diamond paste 3, 1, and ¼ µm (Kemet® diamond spray, Kapellen–Belgium) on polishing cloths (DP-Nap, Struers, Copenhagen, Denmark). Immediately after that, the hybrid layer was revealed by denaturation and decalcification of the specimens. Finally, they were dehydrated in ethanol and hexamethyldisilazane.[17,18]

The dentin–resin interdiffusion zones of the 16 hemi-disks from each group were observed under field-emission scanning electron microscope (FESEM) (JEOL JSM 6301F, Tokyo, Japan) at 10 kV, using secondary electrons. Electronic microphotographs were then taken at different magnifications and scanned with the energy-dispersive spectroscopy microanalysis system (Oxford Inca Energy 350®–Oxford Instruments, Oxfordshire, United Kingdom). To standardize the measurements for the purpose of quantitative analyses, six microphotographs were taken per specimen (hemi-disk): Two in the left side (one with ×800 magnification and another with ×1500), two in the center (one with ×800 magnification and another with ×1500), and two in the right side (one with ×800 magnification and another with ×1500).

All the microphotographs were evaluated according to an objective evaluation of resin–dentin interface morphology, independently measured at three different points (one in the left side, one in the center, and one in the right side of the hemi-disks) of the specimens 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.

For each specimen, we considered a mean value resulting from the three evaluation points. Values were recorded in an Excel file, and data were processed using Statistic Package for the Social Sciences–version 11.5.

Data were analyzed for normality with Kolmogorov–Smirnov and Shapiro–Wilk tests and a *post hoc* analysis of variance multivariant test. A general linear model was used to compare means. Pairwise comparisons were made between mean values, $p < 0.05$.

RESULTS

Mean values of adhesive layer thickness for the 16 specimens with three measurements each were 45.9 µm (±13.41) in group I, 20.6 µm (±16.32) in group II, 17.7 µm (±11.75) in group III, and 50.7 µm (±27.81) in group IV (Graph 1). Mean values of hybrid layer thickness for the 16 specimens with three measurements each were 3.23 µm (±0.53) in group I, 3.13 µm (±0.73) in group II, 2.53 µm (±0.50) in group III, and 1.84 µm (±0.27) in group IV (Graph 1). Mean values of tag lengths for the 16 specimens with three measurements each were 61.5 µm (±18.10) in group I, 111.0 µm (±36.92) in group II, 68.6 µm (±15.84) in group III, and 128.9 µm (±78.38) in group IV (Graph 1).

Adper Scotchbond-1XT™ and One Coat Bond® achieved better adhesive layer thickness average values. Adper Scotchbond-1XT™ and XP-Bond™ achieved better hybrid layer thickness average values. XP-Bond™ and One Coat Bond® achieved better tag length average values. Despite no extreme values of doubtful credibility, One Coat Bond® shows the largest dispersion (amplitude quartile range: 110.48 mm).

Table 2 illustrates the comparison of the adhesive layer thickness average when analyzed in pairs (two by two), their differences, and statistical significance. According to the table, when the "adhesive layer thickness average"
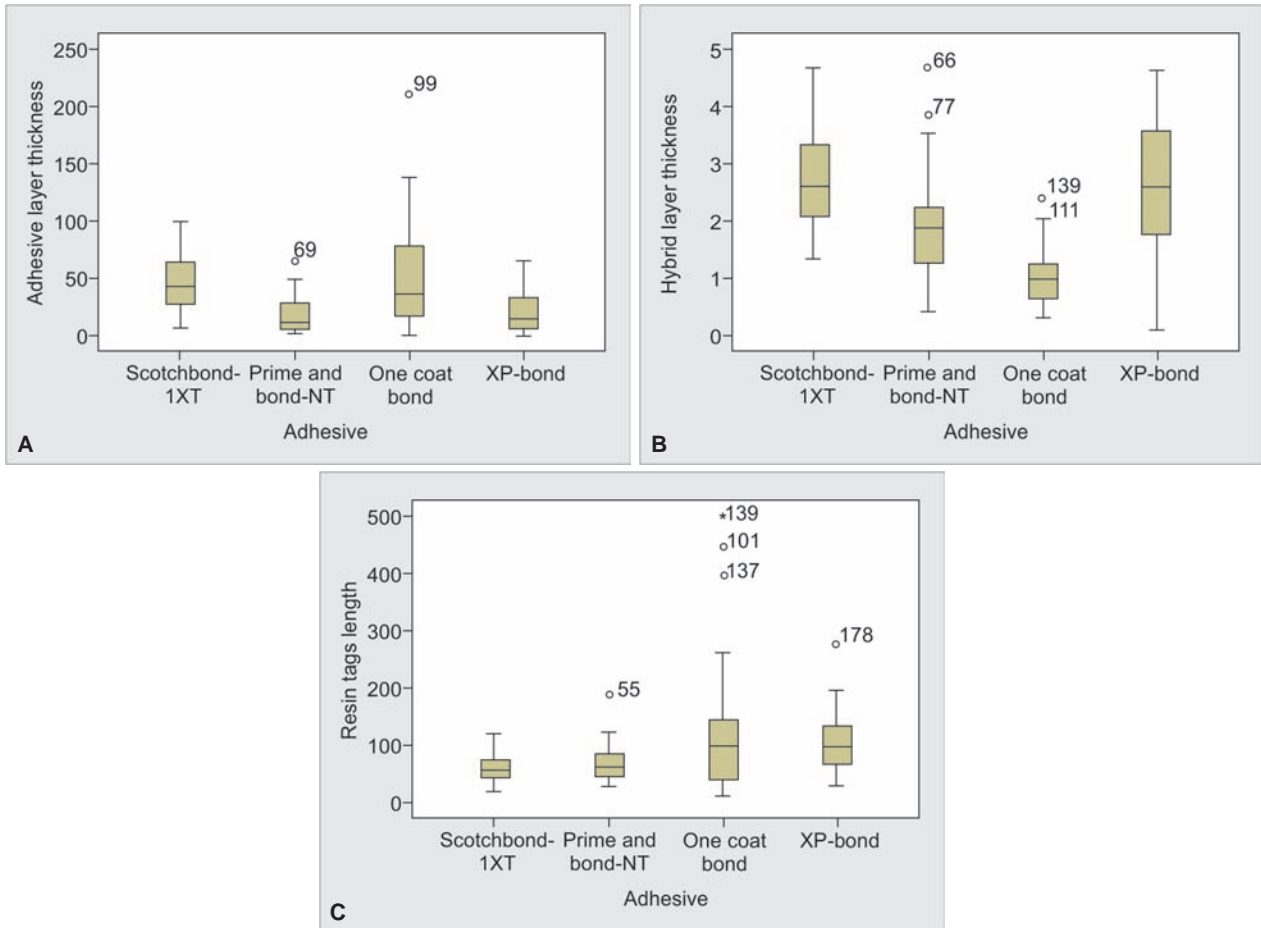
Graphs 1A to C: Adhesive layer, hybrid layer, and tag length average–comparison of the four adhesives

is analyzed, there are significant differences between the pair of adhesives composed by the Adper Scotchbond-1XT™ and One Coat Bond® and the pair composed by XP-Bond™ and Prime and Bond NT® (p < 0.000).

Table 3 illustrates the comparison of the hybrid layer thickness average when analyzed in pairs (two by two),

their differences, and statistical significance. According to the table, when the "hybrid layer thickness average" is analyzed, there are significant differences between the pair of adhesives composed by the Adper Scotchbond-1XT™ and XP-Bond™ and the pair composed by One Coat Bond® and Prime and Bond NT® (p < 0.003).

Table 2: Comparison of adhesive layer thickness average

Dependent variable	(I) Adhesive	(J) Adhesive	Mean difference (I–J)	Significance
Adhesive layer thickness	XP-Bond	XP-Bond		
		Prime and Bond NT	2.373	0.65
		Scotchbond 1-XT	−25.328	<0.000
		One Coat bond	−27.255	<0.000
	Prime and Bond NT	XP-Bond	−2.373	
		Prime and Bond NT		
		Scotchbond 1-XT	−28.201	<0.000
		One Coat bond	−30.128	<0.000
	Scotchbond and 1-XT	XP-Bond	25.32S	<0.000
		Prime and Bond NT	28.201	<0.000
		Scotchbond 1-XT		
		One Coat bond	−1.B27	0.768
	One Coat bond	XP-Bond	27.255	<0.000
		Prime and Bond NT	30.128	<0.000
		Scotchbond 1-XT	1.927	0.768
		One Coat bond		

Table 3: Comparison of hybrid layer thickness average

Dependent variable	(I) Adhesive	(J) Adhesive	Mean difference (I–J)	Significance
Hybrid layer thickness	XP-Bond	XP-Bond		
		Prime and Bond NT	0.599	0.003
		Scotchbond 1-XT	−0.095	0.62
		One Coat bond	1.321	<0.000
	Prime and Bond NT	XP-Bond	−0.599	0.003
		Prime and Bond NT		
		Scotchbond 1-XT	−0.694	0.001
		One Coat bond	0.722	0.001
	Scotchbond 1-XT	XP-Bond	0.095	0.62
		Prime and Bond NT	0.694	0.001
		Scotchbond 1-XT		
		One Coat bond	1.416	<0.000
	One Coat bond	XP-Bond	−1.321	<0.000
		Prime and Bond NT	−0.722	0.001
		Scotchbond 1-XT	−1.416	<0.000
		One Coat bond		

Table 4: Comparison of resin tag length average

Dependent variable	(I) Adhesive	(J) Adhesive	Mean difference (I–J)	Significance
Resin tag length	XP-Bond	XP-Bond		
		Prime and Bond NT	42.383	0.008
		Scotchbond 1-XT	49.544	0.002
		One Coat bond	−17.857	0.255
	Prime and Bond NT	XP-Bond	−42.383	0.008
		Prime and Bond NT		
		Scotchbond 1-XT	7.15	0.512
		One Coat bond	−60.241	0
	Scotchbond 1-XT	XP-Bond	−49.544	0.002
		Prime and Bond NT	−7.15	0.512
		Scotchbond 1-XT		
		One Coat bond	−67.401	0
	One Coat bond	XP-Bond	17.857	0.265
		Prime and Bond NT	60.241	0
		Scotchbond 1-XT	67.401	0
		One Coat bond		

Table 4 illustrates the comparison of resin tag length average when analyzed in pairs (two by two), their differences, and statistical significance. According to the table, when the "tag length average" is analyzed, there are significant differences between the pair of adhesives composed by the XP-Bond™ and One Coat Bond® and the pair composed by Adper Scotchbond-1XT™ and Prime and Bond NT® (p < 0.008), but not between the adhesives of the same pair.

FESEM Images–Dentin–resin Interface Morphology Analysis

Qualitative Analysis of Hybrid Layer using FESEM Microphotographs

The criteria used for this analysis were the direct observation of the empty spaces present beneath the hybrid layer in the FESEM microphotographs. Figures 1 to 5 show the four hybrid layer groups and demonstrate that the only adhesive that apparently resulted in a poorer quality hybrid layer structure was the Prime and Bond NT®. For subjective analysis, all the images captured in the FESEM were analyzed. The images also show the adhesive layer and the resin tags.

DISCUSSION

In this study, bond strength evaluation was not performed since the main aim of this study was the evaluation of dentin–resin interface morphology and its relation to the solvents and general composition present in two-step etch-and-rinse adhesives. Furthermore, the aim of this study was to investigate differences in several types of solvents present in the adhesives and not to experiment different application techniques.

The evaluation of the adhesive systems performance on shear/tensile/microtensile bond strengths is of
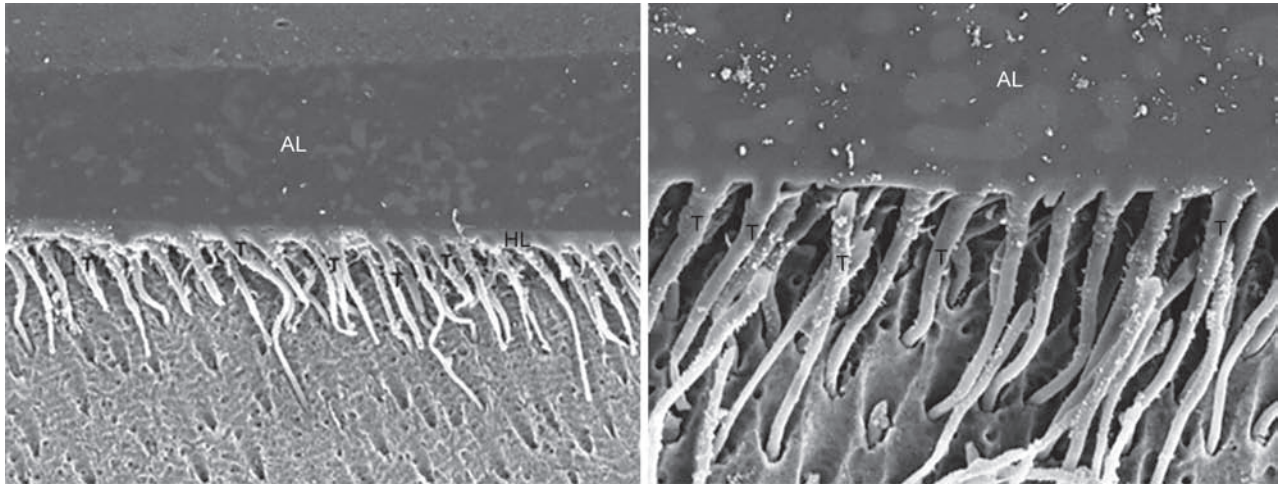
Fig. 1: Scotchbond-1XT™ (3M) FESEM images at ×800 and ×1,500 magnifications: Resin tags (T), hybrid layer (HL), and adhesive layer (AL) can be observed. A high-quality hybrid layer is present
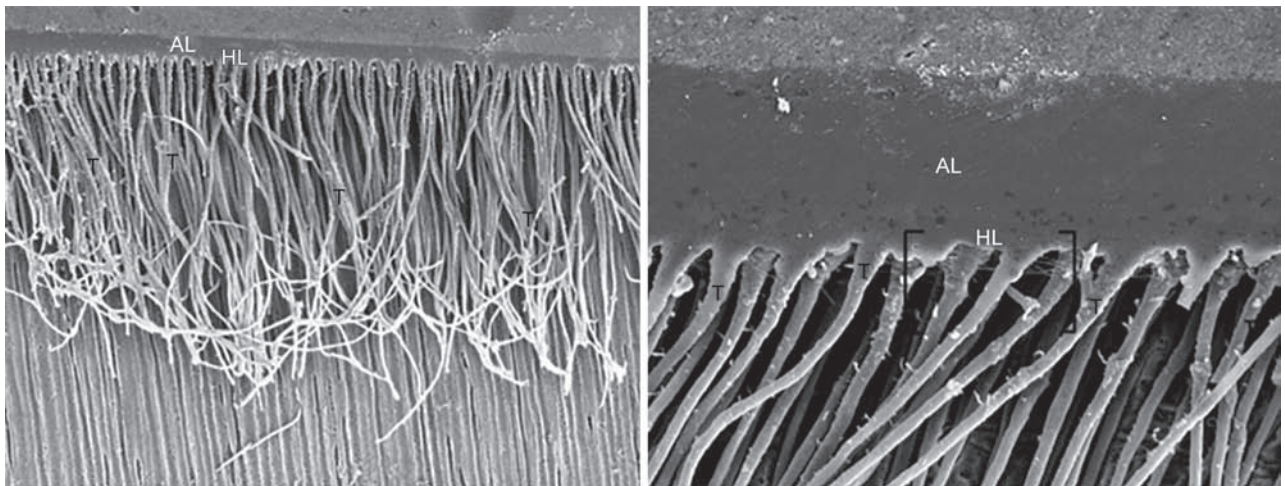

Fig. 2: XP-Bond™ (Dentsply) FESEM images at ×400 and ×1,500 magnifications: Resin tags (T) can be observed quite intertwined, and the hybrid layer (HL) and the adhesive layer (AL) can also be seen. With magnification of ×1,500, there is a zone between the curly brackets that has been magnified to ×7,000 and in which there are perfectly observable microtags. A high-quality hybrid layer is present
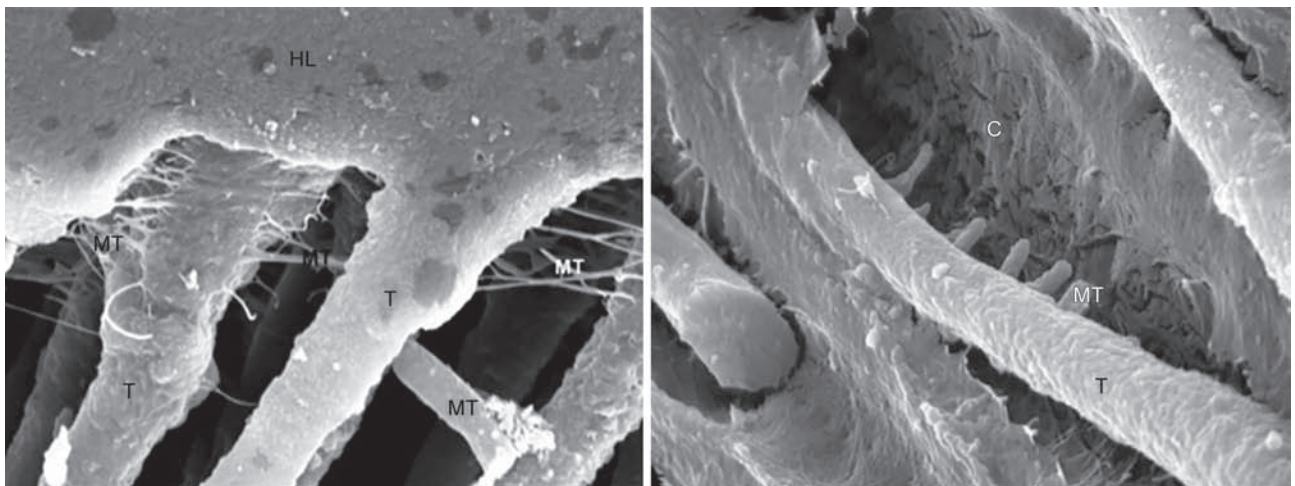

Fig. 3: Left side–XP-Bond™ (Dentsply)–FESEM image at ×7,000 magnification: Hybrid layer (HL), resin tags (T), and the microtags (MT) in detail. Right side–FESEM ×10,000–tag (T) and microtags (MT) inside a dentinal tubule surrounded by the collagen scaffold (C)
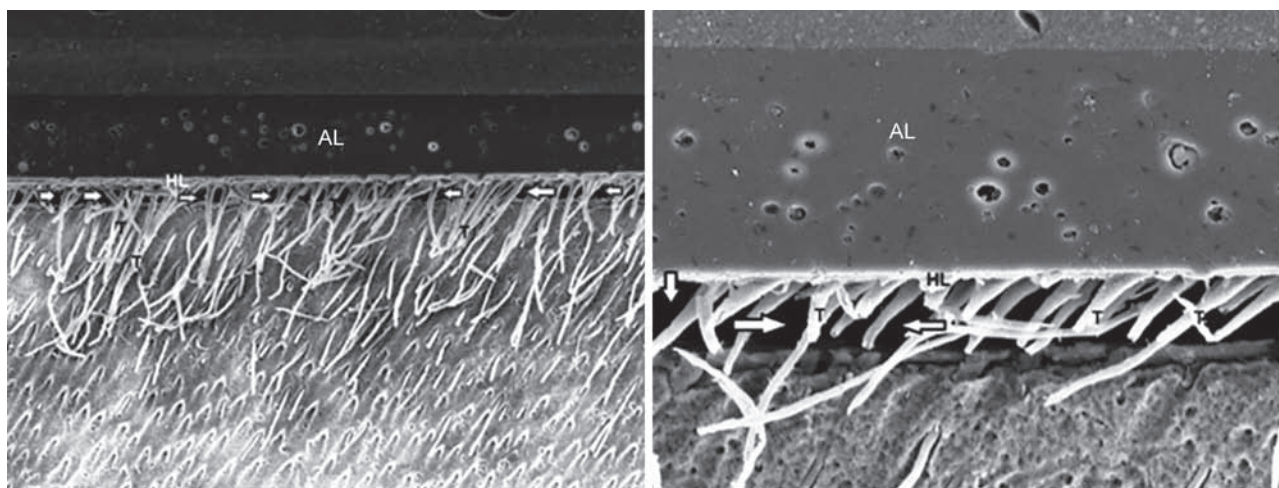
Fig. 4: Prime and Bond NT® (Dentsply) FESEM images at ×500 and ×1,500 magnifications: Resin tags (T), the hybrid layer (HL), and adhesive layer (AL). Prime and Bond NT® presented a more inconsistent hybrid layer, with empty spaces underneath the hybrid layer (arrows)
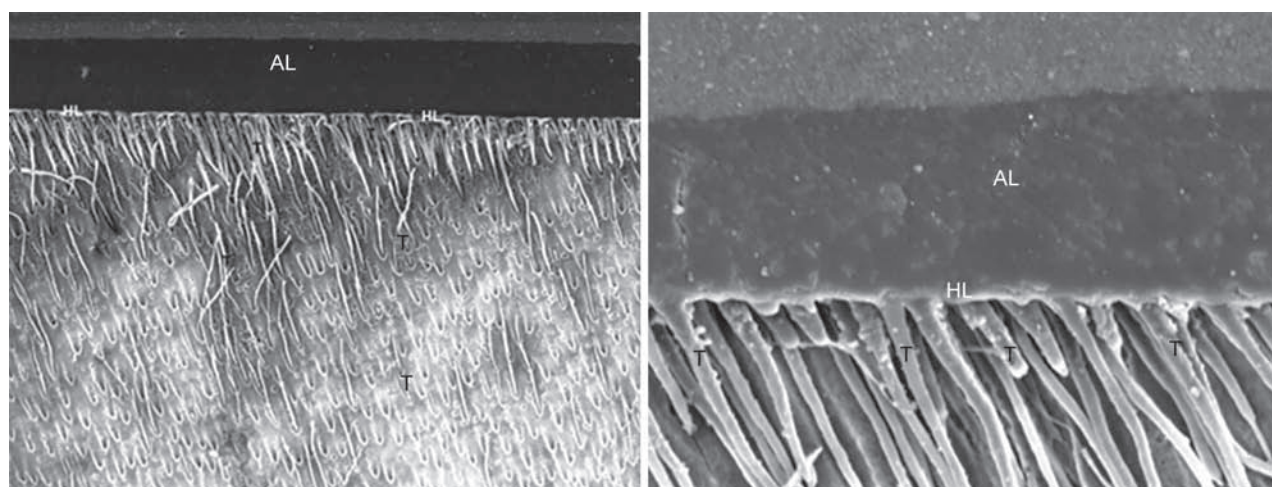

Fig. 5: One Coat bond® (Coltène Whaledent) FESEM images at ×400 and ×1,500 magnifications: Resin tags (T), hybrid layer (HL), and adhesive layer (AL). A high-quality hybrid layer is present

utmost importance for safe, clinical use. However, one of the ways to understand the reasons of their different behaviors is to analyze the interface between the adhesive layer and the dentin.

The etch-and-rinse adhesive systems used in this study were chosen according to the solvent to have a representative of each possible solvent present in these systems. Accordingly, as can be observed in Table 1, Adper Scotchbond-1XT™ contains water and ethanol, XP-Bond™ contains tertiary butanol, Prime and Bond-NT® contains acetone, and One Coat Bond® contains 5% water in its composition.

Analyzing the morphology of the resin–dentin interface, the adhesive and hybrid layers thickness as well as the resin tag length, it is possible to see the way adhesive systems differ and the role of each of their components in those parameters.

The role of the adhesive layer thickness of etch-and-rinse systems in adhesive bonding and preventing gaps

and nanoleakage are well recognized, highlighting its importance in the adhesion process.

Zheng et al[19] showed that the solvent could be more easily removed from the thinner rather than the thicker layers of adhesive thus, originating higher adhesive bond strength with thinner adhesive layers. Moreover, Cho and Dickens[20] demonstrated that an adhesive agent without a solvent originated the highest adhesive bond by increasing the thickness of the adhesive layer.

With respect to the adhesive layer thickness average, statistically significant differences were only found between the pair of adhesives composed by the Adper Scotchbond-1XT™ (45.9 μm ± 13.41) and One Coat Bond® (50.7 μm ± 27.81) *vs* the pair composed by Prime and Bond-NT® (17.7 μm ± 11.75) and XP-Bond™ (20.6 μm ± 16.32) ($p < 0.000$). No statistically significant differences were found between adhesives of the same pair.

Koike et al[21] concluded that a thicker adhesive layer (about 10 μm), by applying and polymerizing twice the

adhesive (using Adper single Bond™, 3M), proved to be advantageous in preventing gaps in the marginal composite resin restorations in dentin cavities. A study by transmission electron microscopy showed that the adhesive bonds increased on each layer placed until the fourth layer and nanoleakage decreased with each layer placed, becoming insignificant after placing four or more layers of adhesive.[22]

The number of layers applied can also have an influence on the restoration behavior because thicker adhesive layers can act as an elastic intermediate layer between the walls of the cavity preparation and the adjacent composite. Therefore, the adhesive could withstand the shrinkage stress of polymerization and absorb the stress produced by thermal cycles and occlusal loads.

When using adhesives without fillers, thick layers are not recommended because these materials have low mechanical properties, which can lead to fractures in the adhesive layer and, as they are generally not radiopaque, the radiolucency can be interpreted by the clinicians as a gap or recurrent caries at the margin of the restoration. The incorporation of small amounts of filler may be a solution to this problem.

Manufacturers and clinicians should consider the limitations of curing the adhesive without filler particles. According to Velazquez et al,[23] adhesives with little or no filler content may be more sensitive to the inhibition of polymerization by oxygen when applied in thin layers. Higher molecular weight may also be important in reducing the oxygen inhibition in polymerization and increasing the bond strength.[23]

It can, therefore, be stated that with respect to the adhesive layer thickness average, the thicker it is, the greater the risk it will be for resin degradation, which may lead to a lower durability of the restoration unless it contains a filler.

Thus, the adhesives Adper Scotchbond-1XT™ and One Coat Bond®, interestingly adhesives with water as solvent (although the former also contain ethanol as comonomer), show statistically higher adhesive layer thickness, which may be because of a more viscous consistency.

The hybrid layer quality is extremely important to the adhesive process and may interfere with durability when exposed to the oral environment. The basis for a stable and strong bond is a good-quality hybrid layer with a homogeneous hybrid area, in which the monomers infiltrate completely and fill the collagen scaffold.[24-26] When this does not happen, the result is a bad-quality hybrid layer, which can lead to nanoleakage and the adhesive cannot stand medium- and long-term challenges due to the degradation of the adhesive bonds. The cause is hydrolysis of suboptimally polymerized hydrophilic resin components and degradation of water-rich, resin-sparse collagen matrices originated by the matrix metalloproteinases. When there are empty spaces beneath the hybrid layer of a sample, it corresponds to unprotected collagen fibrils removed with 10% sodium hypochlorite during the specimens' preparation. *In vivo*, those unprotected collagen fibrils are suitable to nanoleakage and hydrolysis, so it can be stated that the quality of the hybrid layer in that situation is poor. When there are 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 on adhesion is higher.

According to some authors, there is no correlation between the hybrid layer thickness and adhesive strength, suggesting that the quality of the layers rather than their thickness is more important.[27-30]

By analyzing Figures 1 to 5, it can be concluded that groups I, II, and IV achieved a good-quality hybrid layer, and it can been expected an acceptable long-term performance.[30] Group III did not achieve a good hybrid layer, exhibiting empty spaces beneath, probably due to the removal of unprotected collagen with thermocycling and the sodium hypochlorite (Fig. 4, Group III). The collagen fibrils were not correctly involved by the resin in Prime and Bond NT® application due probably to poor penetration through the dentine because of the technique sensitivity of the acetone adhesive. This can cause bonding problems with time, and there is a higher risk of DE capsulated collagen hydrolysis and nanoleakage,[31-35] leading to a speculation that the *in vivo* behavior of this adhesive cannot bear medium- and long-term challenges.

With respect to the hybrid layer thickness average, Adper Scotchbond-1XT™ (3.23 μm ± 0.53 and XP-Bond™ (3.13 μm ± 0.73) were statistically thicker than the pair composed by Prime and Bond-NT® (2.53 μm ± 0.50) and One Coat Bond® (1.84 μm ± 0.27) (p < 0.003). Group IV exhibited the thinner hybrid layer (1.84 μm), which is in line with the results of Breschi et al,[36] where the One Coat Bond® thickness ranges from 1.4 to 2.1 μm.

Solvents can also play an important role in the resin penetration along the dentin because they are the monomer carriers. Group I showed the thickest hybrid layer, probably because ethanol and water might have caused its diffusion into dentin, showing a greater probability to increase its adhesion as water can re-expand the collagen scaffold and ethanol can carry the monomers. However, it can only be confirmed when shear bond tests are performed, which was not the purpose of this study.

On the contrary, acetone alone cannot reexpand the collagen scaffold if this is collapsed.[37-39] This theory is corroborated by an *in vitro* study of Mohan and Kandaswamy.[4] According to these authors, in case of

moisture variance of the substrate, the acetone-based adhesive (Prime and Bond NT®) achieved worse performance when compared with adhesives with alcohol and water solvents (Single Bond®, 3M ESPE, St Paul, USA) or just with water (Syntac Single Component®, Ivoclar Vivadent, New York, USA). Furthermore, an *in vivo* study of Abdalla and García-Godoy[12] showed a higher performance of the hybrid layer structure and resin tags with a water-based adhesive compared with an acetone-based adhesive. This might be due to acetone-based adhesive's greater sensitivity to the technique.[14,20,40]

According to the results of this study, the adhesive without organic solvent (One Coat Bond®) resulted in the lowest hybrid layer thickness. On the contrary, the adhesive with ethanol and water (Adper Scotchbond 1 XT™) caused a higher hybrid layer thickness, followed by the adhesive with a tertiary butanol-based solvent (XP-Bond™). Finally, the acetone-based adhesive (Prime and Bond NT®) yielded the poorest hybrid layer, not in thickness, but in terms of quality. The etch-and-rinse adhesive Prime and Bond NT® must be used with caution because the hybrid layer might not result in the structure needed for a long-term performance.

The hybrid layer is the main structure responsible for the adhesion in superficial dentin, but in deep dentin, the resin tags are viewed as the main mechanism for adhesion.[4] According to Mohan and Kandaswamy,[4] due to differences in direction of the dentinal tubules toward the pulp, often the penetration of the resin may be in different routes, promoting nonparallel retention, only changeable by fracture of the tags. These are also firmly bonded to the tubular walls promoting sealing and decreasing the risk for pulpal sensitivity and irritation, as shown in Figure 2 (left side). It seems that the resin tag contribution to the dentin adhesion is low if they are not hybridized themselves to the walls of the tubules.[4]

Since resin adhesion to tooth structure is essentially mechanical, longer and more numerous tags and lateral branches (microtags) will more likely lead to higher bond strength. However, even though suggested by other authors,[1] this statement should be further confirmed with bond strength tests.

According to Chappell et al,[3] microtags are resin anastomoses resulting from the primer or adhesive entry or both in the lateral canals originating a communication with the adjacent tubules.[3]

Comparisons between the different adhesive systems are usually done by the evaluation of their shear/tensile/microtensile bond strengths or even by microscopic morphological analysis, as was the case of this work. However, one cannot forget that these studies are conducted in the laboratory, *in vitro*, so the results

and findings may not always transpose to their clinical behavior (*in vivo*) because, as a rule, some factors, such as pulpal pressure, dentinal fluid, the conditions leading to polymerization shrinkage, and tooth flexure are not taken into account.[41] The lack of pulpal pressure, also found in endodontically treated teeth, can compromise the penetration of the water-chasers solvents like acetone or ethanol into the dentinal tissue. It is also recognized that resin tags formed *in vivo* are generally shorter than those formed *in vitro* as dentinal tubules are filled with fluid, which can reduce the penetration of resin *in vivo*.[42]

Despite these factors, there is a constant need for the scientific evidence arising from these studies since it is an area in permanent evolution.[12]

With respect to the tag length average, statistically significant differences were only found between the pair of adhesives composed by the XP-Bond™ (111.0 μm ± 36.92) and One Coat Bond® (128.9 μm ± 78.38) *vs* the pair composed by Adper Scotchbond-1XT™ (61.5 μm ± 18.10) and Prime and Bond-NT® (68.6 μm ± 15.84) ($p < 0.008$). No statistically significant differences were found between adhesives of the same pair.

Regarding the morphological analysis of tags and microtags, in group I the presence of not very long tags and the presence of some microtags are visible (Fig. 1). Group II is perfectly visible to the existence of long and entangled tags and the presence of a significant number of microtags (Figs 2 and 3). In group III, the tags are slightly longer than in group I and microtags are practically absent (Fig. 4). Finally, in group IV, the observed tags are the longest among all groups, although fewer in number than in group II. In this group, the microtags are also practically absent (Fig. 5).

These differences may be related to surface wettability and application technique as well as to chemical composition of the resins, in particular, their viscosity and solvents, which are important for the diffusivity of the adhesive into dentin as they are "carriers" of the monomers.[43]

The presence of ethanol and water in Adper Scotchbond-1XT™ could have contributed to its higher diffusion into dentin since moisture inside dentinal tubules pulls ethanol to the interior carrying the resin, evaporating in the end, and leaving the resin inside. However, this happened only partially in this case probably due to the absence of a positive pulpal pressure, but the water present in the system could help dentin rehydration and increase the wettability of the adhesive.[4] Even though they have different vapor pressures, ethanol and acetone act in a similar way.[14] Vapor pressure is 2,330 Pa in water, 4,133 Pa in *t*-butanol, 5,900 Pa in ethanol, and 23,300 Pa in acetone.[44]

According to Perdigão and Frankenberger,[40] the simultaneous inclusion of water and an organic solvent may result in some infiltration in demineralized dry dentin, yielding a technically less sensitive procedure when compared with adhesives exclusively with the organic solvent, especially when applied to teeth with low moisture as are the teeth in this study, since the solvent is unable to reexpand the collagen matrix if it is collapsed.[37]

This theory is supported by an *in vitro* study of Mohan and Kandaswamy[4] in which, although obtaining best results with an acetone-based solvent adhesive (Prime and Bond-NT®, Dentsply) in moist dentin, the variation in substrate humidity leads to worst performances in hybrid layer thickness and length of the resin tags compared with adhesives containing organic solvent and water (single Bond™, 3M) and only water (Syntac Single Component®, Ivoclar-Vivadent).

Water in excess can dramatically affect the adhesive performance of acetone-based adhesives to a greater degree than in alcohol-based ones.[13,14] Interestingly, on the contrary, according to Jacobsen and Söderholm,[45] the water-based adhesives can result in lower adhesive bond strength when compared with adhesives with alcohol or acetone since excess water can jeopardize adhesive polymerization. The moisture amount of the substrate is, therefore, extremely important for dentin hybridization and tag formation.[37]

The diffusivity of the monomers plays an important role in the phenomenon of hybridization and tag formation. The ideal situation occurs when there is a high permeability of the substrate and a high diffusion of the resin monomers as stated by Nakabayashi and Takarada.[46] In this particular aspect, it is known that the molecular weight of the monomers may have some influence[41] since, by increasing the molecular weight, the monomer mobility and its penetrability are reduced.[47] It is further known that the bisphenol A glycidyl methacrylate (bis-GMA) has a molecular weight of about 512 Da, urethane dimethacrylate (UDMA) 471 Da, triethylene glycol dimethacrylate (TEGDMA) about 286 Da, and 2-hydroxyethyl methacrylate (HEMA) 130 Da.[41] As can be observed in Table 1, Adper Scotchbond-1XT™ has bis-GMA, HEMA, and UDMA, the XP-Bond™ presents phosphoric acid-modified acrylate resin (PENTA), butan-1,2,3,4-tetracarboxylic acid, di-2-hydroxyethylmethacrylate ester (TCB), UDMA, TEGDMA, and HEMA, Prime and Bond-NT® contains PENTA and One Coat Bond® has HEMA, hydroxypropyl methacrylate (HPMA), UDMA. The UDMA has a higher molecular weight than HEMA and is more flexible than bis-GMA due to the long aliphatic intermediate chain, and to the two polymerizable methacrylate groups, which contribute to the formation of a three-dimensional network polymer.[14] This flexibility may have had an influence on good penetration of the adhesive XP-Bond™ and One Coat Bond® because both contain UDMA in their composition and interestingly were those which were more infiltrated into dentin forming the longer tags. However, Adper Scotchbond-1XT™, despite a good morphology, originated shorter tags than XP-Bond™ and One Coat Bond®, perhaps because it contains not only UDMA, but other high molecular weight monomers: Bis-GMA. According to Perdigão et al,[42] HEMA and PENTA monomers are essentially hydrophilic; bis-GMA, UDMA, and TEGDMA have a more hydrophobic behavior. This may also have had a great importance in our study since the specimens had less moisture content, which may partially explain the worst results achieved by Prime and Bond NT®.

Regarding One Coat Bond®, excellent infiltration of dentin to form long tags may be explained by the simultaneous action of two small hydrophilic monomers, HEMA and HPMA, combined with the water present in its constitution that would increase penetration in a pulp pressure-free specimen.

Another important factor that can influence the penetration of the adhesive in dentin is its viscosity. The less viscous adhesive could penetrate deeper dentin due to its increased fluidity. This could have been one reason for the XP-Bond infiltrate dentin better than Adper Scotchbond-1XT™ but it is not true in relation to One Coat Bond® (which is more viscous) and to Prime and Bond NT® (more fluid) probably because in these systems, the monomeric composition and type of solvent had a greater influence in resin infiltration than the viscosity itself. The fact that one has water and the other has acetone could have played a stronger role in the resin infiltration of these two adhesive systems.[28]

This study should be further substantiated using other dental adhesives like self-etching adhesive systems. Bond strength tests should also follow this study to assess if, and in what manner, morphological differences in the adhesive layer, hybrid layer, and tags are reflected in bond strengths.

CONCLUSION

Within the limitations of this *in vitro* study, it could be concluded that:
- Adhesives systems with different solvents led to significant differences in the adhesive and hybrid layer thickness and resin tag length.
- Adhesive systems with alcohol showed thinner hybrid layers and adhesive systems with water show thicker adhesive layers.
- The quality of Prime and Bond NT® hybrid layer was poor.

- Solvents role in adhesives bond strength should be considered together with the other adhesive system components.
- According to the results of this study, the null hypothesis was rejected: The type of solvent and composition used in two-step etch-and-rinse adhesive systems have influenced the morphology of the resin–dentin interface, the adhesive and hybrid layer thickness as well as the resin tag length. Nonetheless, further studies are needed to corroborate it.

CLINICAL SIGNIFICANCE

The adhesive containing tertiary butanol, in addition, seems to originate good-quality hybrid layer and long, entangled tags and also appears to have greater ability to originate microtags, which may indicate higher bond strength.

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