



Influence of Solvents and Composition of Etch-and-Rinse and Self-Etch Adhesive Systems on the Nanoleakage within the Hybrid Layer

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ABSTRACT

Aim: The goal of this study was to evaluate nanoleakage within the hybrid layer yielded by etch-and-rinse and self-etch adhesive systems, with different solvents and compositions.

Materials and methods: Four adhesives were applied onto 20 human dentin disks: group A: Adper Scotchbond 1XT™ (3M ESPE), group B: One Coat Bond® (Coltène Whaledent), group C: AdheSE® (Ivoclar Vivadent) and group D: Xeno-V® (Dentsply). The samples were immersed in aqueous ammoniacal silver nitrate for 24 hour, prepared and observed under field-emission scanning electron microscopy with backscattered electrons. Microphotographs were scanned and data were processed. The mean value and standard deviation were calculated. Kruskal-Wallis and Mann-Whitney tests were used ($p < 0.05$).

Results: All the adhesives showed nanoleakage within the hybrid layer: Adper Scotchbond 1XT™ ($218.5 \mu\text{m} \pm 52.6 \mu\text{m}$), One Coat Bond® ($139.6 \mu\text{m} \pm 79.0 \mu\text{m}$), AdheSE® ($92.7 \mu\text{m} \pm 64.8 \mu\text{m}$) and Xeno-V® ($251.0 \mu\text{m} \pm 85.2 \mu\text{m}$). AdheSE® yielded less nanoleakage than Adper Scotchbond-1XT™ ($p = 0.003$) and than Xeno-V® ($p = 0.007$). No other statistically significant differences were detected.

Conclusion: Two-step self-etch adhesive system (AdheSE®) might contribute for lower nanoleakage deposition and thus better performance in dentin adhesion.

Clinical significance: The two-step self-etch adhesive system showed the lowest nanoleakage deposition compared with the other adhesive systems evaluated, which seems to indicate a better behavior when a restoration is performed in dentin and possibly can lead to a durable adhesion along time.

Keywords: Etch-and-rinse adhesives, Self-etch adhesives, Hybrid layer, Nanoleakage.

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INTRODUCTION

Nanoleakage is considered an important indicator of the sealing ability of restorative materials.^{1,2} 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.³ According to what Nakabayashi, Kojima and Masuhara stated in 1982, hybrid layer is the union zone between the restoring material and the solid dentin.⁴ Nanoleakage can occur not only in the hybrid layer, but also in the adhesive layer,^{1,5} and it is characterized by the infiltration of small molecules or ions in the absence of detectable interface failures.⁵⁻⁸ It is thought that is due to the incomplete infiltration of resin in demineralized dentin, due to shrinkage of restorative material during polymerization, or due to the elution of residual monomer from the adhesive. According to Prati et al, and other authors that studied nanoleakage,^{1,6,9} it results from a combination of mechanisms including the incomplete infiltration of the monomers in the demineralized collagen matrix, the presence of hydrophilic monomers and insufficient removal of solvent or water that remains trapped in the hybrid layer. All of these mechanisms can lead to a porous surface. Furthermore, the presence of water droplets at the margins of the hybrid layer and within the adhesive layer possibly indicates water flow toward the interface, enabling permeability to external oral fluids as well as to enzymes and bacteria.

In etch-and-rinse adhesives, the area of demineralized dentin below the hybrid layer becomes a weak point of adhesion due to matrix metalloproteinases (MMPs), zinc-dependent endopeptidases, that are slowly released from the conditioned dentin, allowing for the hydrolysis of type I

collagen.^{7,10} The incomplete infiltration of resin monomers in the demineralized dentin matrix is thought to be the main reason for the reduction of adhesive bond strengths overtime.^{7,11}

Even though self-etch adhesives simultaneously etch and infiltrate the underlying substrate, a discrepancy between the depth of demineralization and infiltration might also occur for some self-etching adhesives.^{12,13} Nanoleakage within interfaces formed by self-etch adhesives is not solely caused by incomplete resin infiltration into demineralized dentin, but also by areas within the adhesive layer in which water is incompletely removed, resulting in regions of inadequate polymerization and/or hydrogel formation, or hydrophilic domains of acidic monomers that are more prone to water sorption.¹³

Nanoleakage is always present in the adhesive interfaces produced by the current and available commercial adhesives in the market.¹⁴ The hydrophilicity of these systems makes them prone to absorb water, within the hybrid layer and the adhesive layer. The more hydrophilic the material, more prone it is to water sorption.¹⁴ Furthermore, a discrepancy between demineralization and infiltration of resin monomers is also observed for self-etch systems as they contain acidic but nonpolymerizable monomers.¹⁵

Solvents carry monomers into dentin and seem to play a major role in the formation of a high quality hybrid layer, and consequently in dentin adhesion, reducing the risk of nanoleakage phenomenon appearance.

All adhesive systems have solvents in their composition. These solvents can be inorganic like water, or organic, with high volatility, like acetone or ethanol. More recently emerged the tertiary butanol for this application.

In order to achieve an adequate hybrid layer, it is important to keep dentin clinically moist, since the collagen fibrils network can collapse with excessive drying, preventing monomer diffusion in dentin.¹⁶ The diffusibility of the monomers is a key factor in dentin hybridization. To this end, organic solvents such as acetone or ethanol, chemicals known as 'water chasers', solubilize the resinous components of the adhesive system, increasing the wettability of the dentin by the adhesives, helping to remove water from the collagen network, promoting hydrophilic monomer infiltration into collagen nanospaces and opened tubules, and increasing the adhesive bond strength.¹⁷⁻²²

On the other hand, adhesive systems containing only acetone as solvent are more sensitive to variations in humidity of the substrate, and may lead to lower bond strengths both in the presence of little or excess water in dentin.^{23,24} In this case, it would be advantageous to use water-based adhesives, for optimal hybridization and tubular

sealing due to its relative insensitivity to moisture content of the dentin and capability to rewet dentin.

It should also be noted that, the water absorption by the hydrophilic monomers within the hybrid layer and the adhesive layer might contribute to the degradation of the bond strength overtime,⁸ since a permeable hybrid layer appears to be highly susceptible to slow hydrolysis by water.

This phenomenon can directly contribute to the degradation of resins by the extraction of unpolymerized monomers or small oligomers overtime.²⁵

Immersion in water of polymerized adhesive systems, by long periods, may cause a marked reduction on the longevity of adhesive restorations since the hybrid layer will be degraded and the adhesive bond strengths will be weakened.^{26,10}

In research studies, ammoniacal silver nitrate (AgNO_3) has been used to identify regions filled with water, regions not impregnated with polymer and the deterioration of the hydrophilic polymers in the hybrid layer and adhesive.²⁵ This presence of the silver then can be disclosed by studies with transmission electron microscopy (TEM) and scanning electron microscopy (SEM) although these do not display high-resolution images of silver particles (granules). Later, with the emergence of FESEM (Field Emission In-lens SEM), high resolution imaging became possible.

The present work aimed to evaluate, by means of FESEM, the presence of nanoleakage in the hybrid layer for different adhesive systems. The null hypothesis was that the type of solvent and composition used in two-step etch-and-rinse, two-step self-etch and all-in-one self-etch adhesive systems do not influence the presence of nanoleakage in the hybrid layer.

MATERIALS AND METHODS

The general approach of the study included FESEM evaluation of ammoniacal silver nitrate nanoleakage in the hybrid layer.

After appraisal and approval by an Ethics Committee, we conducted this evaluation.

Sample Preparation

Twenty caries-free human molars ($n = 20$), 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). Teeth were crosscut, into 1 mm thick dentin disks (each tooth yielded one disk), with a slow-speed diamond saw (Accuton 2-Struers, Copenhagen, Denmark) and a standardized smear layer was created, on the occlusal surface, with 600-grit silicon carbide (SiC) paper (for 1 minute under cooling with distilled water).

Restorative Procedures

Specimens were randomly allocated into 4 different adhesive/solvent groups (5 disks per group). Group A: Adper Scotchbond 1XT™-3M ESPE, Seefeld – Germany (ethanol/water), group B: One Coat Bond®-Coltène Whaledent, Altstätten – Switzerland (solvent free – 5% water); group C: AdheSE®-(Ivoclar Vivadent, Schaan – Liechtenstein) (water) and group D: Xeno-V®-Dentsply, Konstanz – Germany (water/tertiary-butanol) (Table 1).

Each adhesive system was applied in two layers over the dentin disks according to manufacturers' instructions and light cured for 20 seconds (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/cm² (BluePhase, Ivoclar Vivadent, Schaan, Liechtenstein).

Nanoleakage Assessment

After bonding, the specimens were placed in a greenhouse (Hemmet, Schwabach, Germany) at 37°C temperature, 100% humidity and then vertically cut with a microtome (Accuton 2-Struers, Copenhagen, Denmark) in 0.9 mm thick slabs, originating 10 specimens per group (n = 10). Each slab was then coated with two layers of nail polish, except 1 mm around the adhesive interface. Specimens were allowed to rehydrate in distilled water for 20 minutes before immersing in 50 wt% aqueous ammoniacal silver nitrate (pH 9.5) for 24 hours. According to the protocol described by Tay et al, 2002,⁸ specimens were then immersed in a photo-developer solution for 8 hours (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-and washed in water for 1 minute.

Fixation was performed by immersion in a solution of 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.4) for 12 hours at 4°C. Specimens were then rinsed three times in 20 ml of 0.2 M buffered sodium cacodylate (pH 7.4) for 1 hour followed by distilled water for 1 minute. Surface polishing was achieved with decreasing abrasive grits (600, 800, 1200) of silicon-carbide paper and diamond spray (Kemet-diamond spray) of 2 and 1 µm on polishing cloths (DP-NAP, Struers, Copenhagen, Denmark) followed by ultrasound bath in 100% ethanol for 10 minutes, and demineralization in 0.5% silica free phosphoric acid for 1 minute to remove surface debris. After that procedure, the specimens were dehydrated in ascending concentrations of ethanol in water: 25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes and 100% for 60 minutes and dried by immersion in hexamethyldisilazane for 10 minutes according to the protocol described by Perdigao et al 1995.²⁷

Specimens Preparation for FESEM Observation

Specimens were sputter-coated with Au-Pd (JEOL Fine Coat Ion Sputter JFC-1100, Tokyo, Japan) and observed in FESEM (JEOL JSM 6301F, Tokyo, Japan) with a backscattered electron detector and accelerating voltage of 15 kV. The software used to detect silver was the EDS Microanalysis System (Oxford Inca Energy 350®-Oxford Instruments, Oxfordshire-United Kingdom).

For the analysis of nanoleakage within the hybrid layer, six images were taken with different magnifications (400× and 2000×; sometimes at 1000× for water-tree observation) per specimen (right, center and left). The total length of the hybrid layer and the ammoniacal silver nitrate impregnation extension were measured in the 400× images using the 'offline' mode software (Oxford Inca Energy 350®-Oxford Instruments, Oxfordshire-United Kingdom). The nanoleakage length within the hybrid layer observed in the

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

Adhesives	Composition
* Adper Scotchbond 1XT™ (3M-ESPE, Seefeld – Germany) Batch number: N198228	Bis-GMA, HEMA, dimethacrylates, polyalcenoic copolymer, 5 nm diameter 10% of weight silica spherical particles Solvents: ethanol and water
One Coat Bond® (Coltène Whaledent, Altstätten – Switzerland) Batch number: 0222825	HEMA, UDMA, HPMA, hidroxypropylmethacrylate, glycerol, methacrylates, polyalkenoate methacrylized, amorphous silica 5% water
AdheSE® (Ivoclar Vivadent, Schaan – Liechtenstein) Batch number: Primer (1): M02841 Bonding Agent (2): L56767	AdheSE Primer: dimethacrylate, phosphonic acid acrylate, initiators and stabilizers in an aqueous solution AdheSE Bond: HEMA, dimethacrylate, silicon dioxide, initiators and stabilizers
Xeno-V® (Dentsply, Konstanz – Germany) Batch number: 1002000450	Bifunctional acrylate, acidic acrylate functionalized phosphoric acid ester, acrylic acid, water, tertiary butanol, initiator, stabilizer

Bis-GMA: bisphenol A glycidyl methacrylate; HEMA: hydroxyethylmethacrylate; UDMA: urethanedimethacrylate; HPMA: hydroxypropylmethacrylate; *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)

three images at 400×, per specimen, was averaged in order to obtain a single value to represent each tooth.

STATISTICAL ANALYSIS

Kruskal-Wallis and Mann-Whitney tests were used (confidence level set at 95%; $p < 0.05$) with SPSS (Statistic Package for Social Sciences) (IBM SPSS statistics V.19).

RESULTS

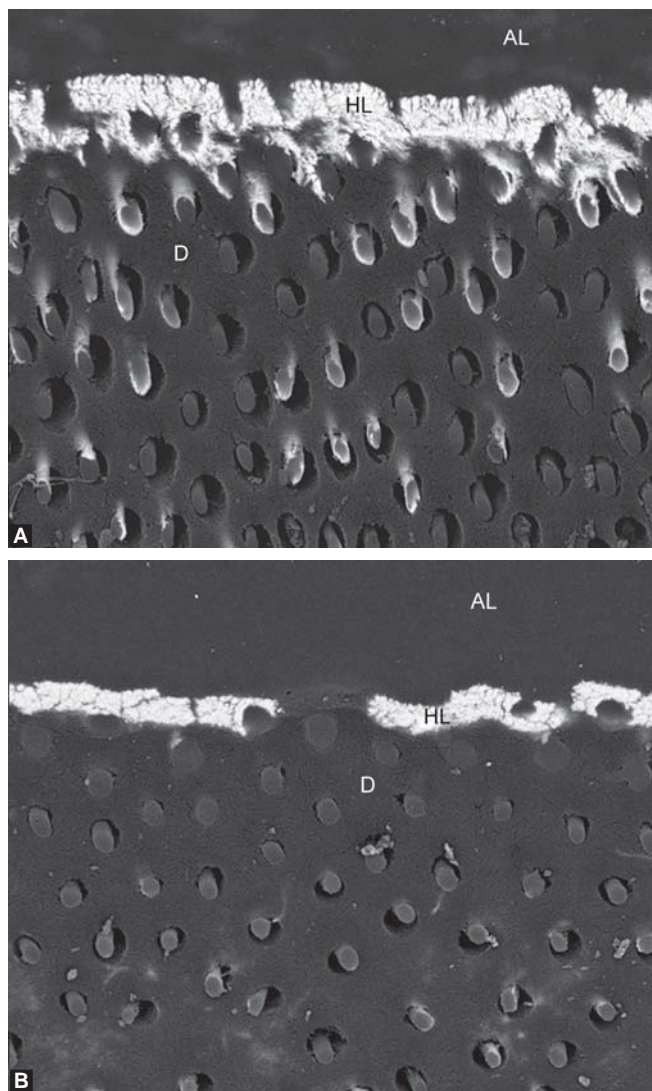
Images, at 400× magnification, showed a total length of the hybrid layer of 305 μm . The small number of specimens and the values distribution led to application of nonparametric methods. All adhesives yielded nanoleakage within the hybrid layer. Kruskal-Wallis test confirmed the existence of statistically significant nanoleakage differences among the four groups ($p = 0.004$).

According to Mann-Whitney test AdheSE[®] yielded less nanoleakage than Adper Scotchbond-1XT[™] ($p = 0.003$) and than Xeno-V[®] ($p = 0.007$). No other statistically significant differences were detected (Table 2, Figs 1, 2 and Graph 1). Water-trees were visible in the group B (One Coat Bond[®]) FESEM images (Figs 3 and 4).

DISCUSSION

The nanoleakage pathway is thought to initiate at the interface between the adhesive-hybrid layer, the weakest point within the dentin-restoration junction^{28,29} or within a hybrid layer which was not perfectly impregnated by the bonding resin.^{2,30}

Samples prepared with etch-and-rinse adhesive system (Adper Scotchbond-1XT[™], One Coat Bond[®]) and with one-step self-etch system (Xeno-V[®]) evaluated in this study, revealed an increased flow of silver ions in water filled channels within the adhesive layer leading to the appearance of droplets at the dentin-resin interface. According to Tay et al, there are two modes of nanoleakage expression: the spotted pattern and the reticular mode.⁸ The reticular mode of the nanoleakage pattern – in particular the silver deposits that were oriented perpendicular to the surface of the hybrid layer – is the morphological manifestation of water-treeing, a well-known phenomenon,⁸ visible in the FESEM micrographs (Figs 3 and 4).



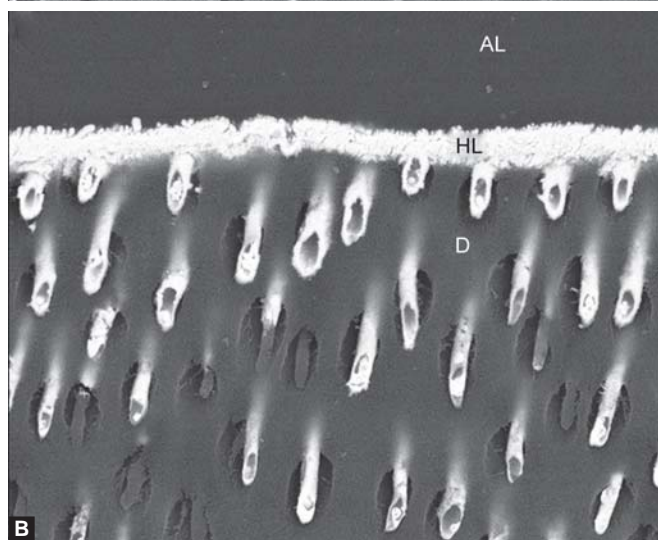
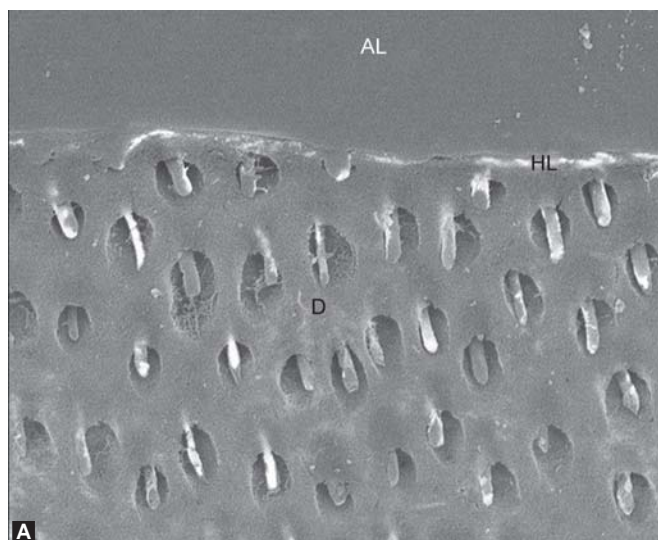
Figs 1A and B: FESEM images at 2000× magnification of groups A and B. It is possible to observe the adhesive layer (AL), the hybrid layer (HL) impregnated with ammoniacal silver nitrate (greater amount in the group A – Adper Scotchbond-1XT[™] compared to group B - One Coat Bond[®]) and the underlying dentin (D)

This phenomenon is probably responsible for the deterioration of the polymers, since the presence of water in the nanochannels can directly contribute to the degradation of resins by extraction of unpolymerized monomers or small oligomers overtime.^{25,31}

Several factors can influence the development of nanoleakage, including the individual chemical constituents, the type of solvent (water vs acetone/ethanol), as well as

Table 2. Mean and standard deviation values obtained for nanoleakage length in the hybrid layer for all the adhesives tested.

Groups	Adhesive	Number of specimens	Mean nanoleakage length \pm standard deviation (μm)
A	Adper Scotchbond-1XT [™]	10	218.5 \pm 52.6
B	One Coat Bond [®]	10	139.6 \pm 79.0
C	AdheSE [®]	10	92.7 \pm 64.8
D	Xeno – V [®]	10	251.0 \pm 85.2



Figs 2A and B: FESEM images at 2000 \times magnification of groups C and D. It is possible to observe the adhesive layer (AL), the hybrid layer (HL) impregnated with ammoniacal silver nitrate (much smaller amount in the group C—AdheSE[®] when compared to group D—Xeno-V[®]), and the underlying dentin (D)

other additives and the mode of application (moist *vs* dry).³² HEMA is known to prevent phase separation of dental adhesive blends.³³ All the adhesives evaluated in this study have HEMA in their composition, except Xeno-V[®], so the hydrophobic resin component within the residual water is prone to separate into resin globules and water blisters, what is called a resin material's phase separation.^{33,34} In this process, once terbutanol starts to evaporate, the solvent-monomer balance is broken, with water separating from the other adhesive ingredients. When the adhesive is cured, these water blisters become entrapped in the adhesive layer, probably jeopardizing the bonding effectiveness.³⁵ On the other hand, large-molecular-weight hydrophobic components do not successfully infiltrate the thinner spaces created by the acidic monomers, possibly due to the lack of HEMA.

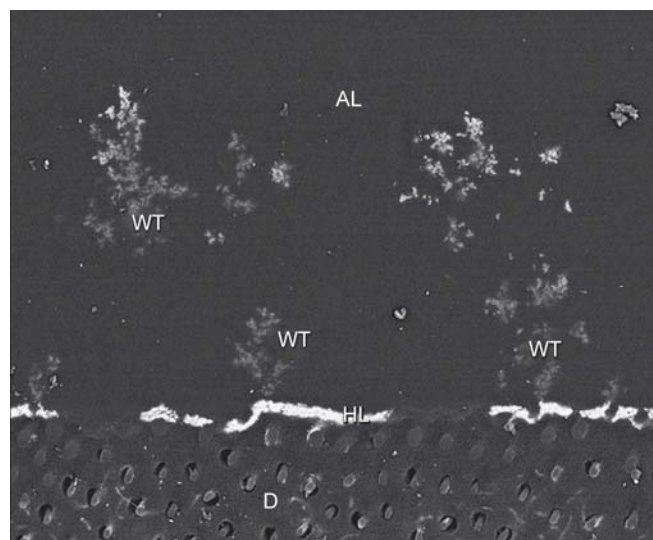


Fig. 3: Group B (One Coat Bond[®]) FESEM image magnification at 1000 \times —water-trees (WT) are visible resulting from movement of water through the adhesive layer (AL) and the hybrid layer (HL)

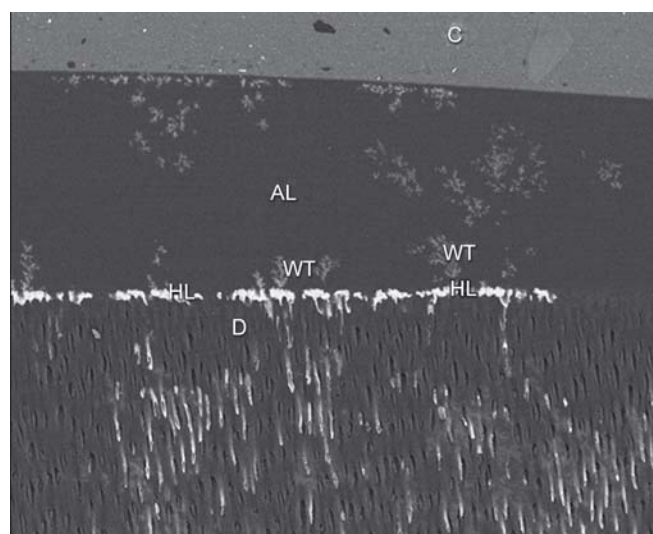
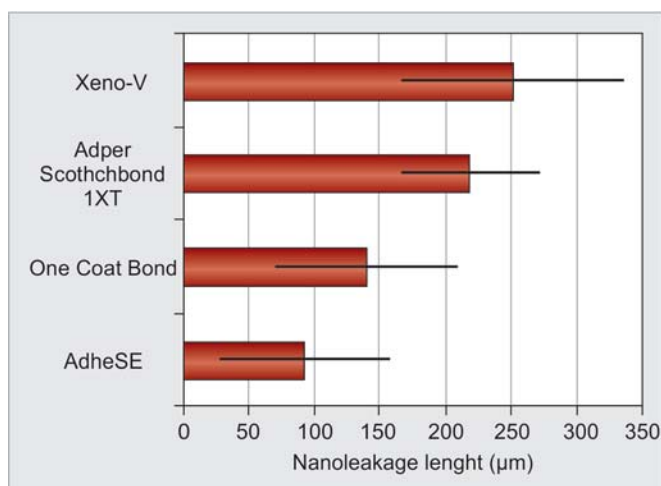


Fig. 4: Group B (One Coat Bond[®]) FESEM image magnification at 400 \times —water-trees (WT) are visible and at the top of the image, the layer of composite resin (C) as well as some impregnation of ammoniacal silver nitrate under the hybrid layer, at the resin tags

Concerning the functional monomers and their molecular weight, Adper Scotchbond 1XT[™] contains Bis-GMA (512 Da) and HEMA (130 Da), One Coat Bond[®] contains HEMA (130 Da), UDMA (471 Da) and HPMA (144 Da) and AdheSE[®] contains HEMA in the bonding resin.³⁶ The differences in composition and molecular weight of monomers among the adhesives used in this study probably influenced their penetration into the intertubular dentin spaces, even when we compare etch-and-rinse and self-etch adhesives between them. Further, AdheSE[®] also includes bis-acrylamide, a more hydrolytically stable monomer than other hydrophilic monomers used in dentin adhesive formulations.³⁷



Graph 1: Mean value (μm) for the impregnation of ammoniacal silver nitrate in the hybrid layer per 305 micrometers and standard deviation (lines)

Concerning the solvents in the adhesives compositions, the organic solvents (acetone, ethanol) have greater difficulty in penetrating a more dehydrated dentin substrate, since they are water chasers they depend on the presence of moisture to penetrate and carry the resin monomer,²³ displacing water.

The present study was performed *in vitro*, therefore, samples have no positive pulp pressure. Dentin permeability is usually measured by means of hydraulic conductance which involves forcing a fluid through the dentin by applying pressure. If the pulpal tissue pressure fall to zero, during the application of an adhesive system to dentin, there is potentially no outward fluid flow.³ This fact can compromise the correct infiltration of monomers for the Xeno-V[®] adhesive (with butanol and water as solvent) and Adper Scotchbond-1XT[™] (with water and ethanol as solvent).

The addition of solvents to resin comonomer blends, decrease their viscosity and increase the wetting characteristics, although it is unlikely to remove more than half of the solvent by evaporation. In the case of Xeno-V[®] and Adper Scotchbond-1XT[™] adhesive systems, although the manufacturer recommends the evaporation of ethanol solvent prior to light curing, this may be difficult, therefore, remnants of ethanol can restrict the conversion of soluble adhesive monomers to insoluble polymers, or may become entrapped as a separate phase during polymerization.³⁵ After polymerization, the residual solvent will be replaced by water that will likely plasticize the polymer and reduce its mechanical properties.³⁸ This can be observed in the Xeno-V[®] (with butanol and water as solvent) and Adper Scotchbond-1XT[™] (with water and ethanol as solvent) adhesives, that had the highest nanoleakage values also explained by the presence of water in the composition which

probably lead to water sorption and the possibility of organic solvents have not been correctly evaporated.

The main aim of the present study was the evaluation of the extent of the hybrid layer's nanoleakage, comparing different types of solvents and composition present in etch-and-rinse and self-etch adhesive systems, applied according to the manufacturer's instructions. Bond strength evaluation was not performed. Nonetheless, a high hydrolysis prone hybrid layer can lead to decline of adhesive bond strengths over time. It also is shown by some studies^{7,10} that a hybrid layer with a high nanoleakage rate will have unprotected collagen fibers, which would be easily degraded by metalloproteinases (MMPs), resulting in bond strengths degradation. Further studies should include nanoleakage evaluation of the same adhesive systems over a longer period of time than was used in the study reported here.

An important feature of two-step self-etch systems (like AdheSE[®] used in this study) that might contribute for the better results when compared with one-step systems (like Xeno-V[®] used in this study), is the hydrophobic resin layer that is placed over the primer, which might reduce water sorption,³⁹ in the two-step system. Therefore, the two-step self-etch adhesives may contribute to the sealing of the bonding interface⁴⁰ and such an effect might prevent water treeing and promote bonding longevity.⁸

The latest developed single-step self-etch adhesive systems combine etching, priming and bonding into one bottle,⁴¹ such as Xeno-V[®]. This adhesive system presents a shorter clinical application time and reduction in technique sensitivity. Although they are marketed as simplified, a more complex chemistry is necessary to blend hydrophilic and hydrophobic monomers, solvents, water and additives¹³ and the benefit of saving time may be achieved at the expense of compromising the durability of resin-dentin bonds.⁸ These one-step adhesives are believed to be more acidic and contain a larger portion of hydrophilic component, which may not be totally polymerized during light curing period recommended by manufacturers. Incomplete hydrophobic sealing will cause the incomplete polymerized hydrophilic resin component to elute from the adhesive and hybrid layer in the short term, exposing the collagen fibers to the enzyme attack.^{1,42} A great impregnation of ammoniacal silver nitrate in the hybrid layer of group D (Xeno-V[®]) can be observed in Figure 2B. Samples in this group yielded the highest nanoleakage mean (251.0 μm) compared to other groups.

Because one-step self-etch adhesives can act as permeable membranes^{43,44} the application of a hydrophobic coating layer is essential to improve the durability of the bonding⁴⁵ as shown by Sillas Duarte Jr et al (2009) in a study with etch-and-rinse and self-etch adhesives.⁴⁶

It is also known that the movement of fluid through the dentin with adhesive is significantly higher in etch-and-rinse than the in the self-etch adhesive system.⁴⁷ Hashimoto et al⁴⁷ compared the fluid movement across the resin-dentin interface by using another self-etch product and they concluded that, due to the milder acid-etching effects of self-etching primers, which only modify the smear layer rather than remove it, it may reduce outward fluid flow and result in superior dentin sealing due to retained hybridized smear plugs with the tubules.¹ We can clearly verify this statement by observing Figure 2A of group C (AdheSE[®]), in which can be observed a lower impregnation of ammoniacal silver nitrate in the hybrid layer and consequently lower mean of the same (92.7 μ m) compared to other groups, thus indicating better sealing, less presence of water and consequently a lower hydrolysis of hybrid layer, which will probably yield acceptable adhesive bond strengths over time. Probably due to the reasons described above, this reasoning does not relate to Xeno-V[®]. According to Reis et al, two-step self-etch adhesive systems have the lowest failure rate,³⁹ which confirms the findings of our study when comparing the AdheSE[®] with Xeno-V[®].

Clinically, it is preferred that dental adhesives systems are hydrophilic during application, then become hydrophobic after application and completely seal the restoration margins for a significant time. Many clinicians hope that there is one agent suitable for any case of direct resin restoration. On the other hand, one should not underestimate the complexity of the real oral condition. Base on the theory that dentin permeability will greatly affect the bond strength, as well as the sealing ability of resin materials,⁴⁸ the variation of dentin depth is believed to cause more intricate nanoleakage.¹

CONCLUSION

Within the limitations of this study, it could be concluded:

1. All adhesives used in this study showed nanoleakage within the hybrid layer, under the conditions described in the methods.
2. The two-step self-etch adhesive system (AdheSE[®]-Ivoclar Vivadent) seems to have very acceptable behavior, concerning nanoleakage, when adhesion is performed in dentin.
3. Two-step self-etch adhesive systems might contribute to better results when compared with one-step and two-step etch-and-rinse systems, probably because the hydrophobic resin layer that is placed over the primer might reduce water sorption.
4. According to the results of this study, we reject the null hypotheses; there were differences between the adhesive systems studied that most probably are related to their composition. Nonetheless further studies are needed to corroborate it.

CLINICAL SIGNIFICANCE

The two-step self-etch adhesive system showed the lowest nanoleakage deposition compared with the other adhesive systems evaluated, which seems to indicate a better behavior when a restoration is performed in dentin and possibly can lead to a durable adhesion along time.

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