> **Original Research Article**

Dentin Bonding Performance and Nanoleakage Properties of Universal Adhesives in Different Etching Modes

Nurcan Ozakar Ilday[1], Omer Sagsoz[2], Ozcan Karatas[3], Yusuf Ziya Bayindir[4], Verda Turel Rifaioglu[3]

[1]Associate Professor, Department of Restorative Dentistry, Faculty of Dentistry Atatürk University, Erzurum, Turkey
[2]Assistant Professor, Department of Restorative Dentistry, Faculty of Dentistry, Atatürk University Erzurum, Turkey
[3]Research Assistant, Department of Restorative Dentistry, Faculty of Dentistry, Atatürk University Erzurum, Turkey
[4]Professor, Department of Restorative Dentistry, Faculty of Dentistry, Atatürk University, Erzurum, Turkey

*Corresponding Author:
Nurcan Ozakar Ilday
Email: dtnilday@hotmail.com

Abstract: The aim of this study was to compare the microtensile bond strengths (µTBS) and nanoleakage (NL) of four universal adhesives (UAs) applied in two different etching modes, self-etch (se) or etch-and-rinse (er). The occlusal one-third of 48 freshly extracted intact human molars was removed, and the exposed surfaces were treated with one of the four UAs [Single Bond Universal (SBU), Prime Bond NT (PNT), Peak Universal Adhesive (PUA) or All-Bond Universal (ABU)] in se or er mode, with Clearfil SE Bond (CSE) and Adper Single Bond 2 (ASB) as controls (n=8). After composite build-up, specimens were stored in distilled water (37°C/24 h). Composite resin–dentine beams were prepared (0.9 mm^2), and the µTBS test was performed. Data were analyzed using one-way ANOVA and Duncan's test ($\alpha = 0.05$). Additional specimens were processed and examined to assay interfacial NL. The failure patterns of the debonded specimens were evaluated using a stereo electron microscope. One additional tooth was prepared for each group for evaluation of NL. The teeth were immersed in a tracer agent ($AgNO_3$ 50% w/v) for 24 h. After longitudinal sectioning and polishing, the generated interfaces were examined under scanning electron microscope (SEM). Statistical analysis indicated that the addition of an etching step significantly affected the µTBS of all UAs, except for SBU, compared to se application modes (p<0.05). The highest µTBS value (38.84±2.07 MPa) was obtained in the SBU er group, and the lowest (15.96±1.21 MPa) in the ABU se group. While no NL was determined in the SBU adhesive se or er groups, varying levels were detected in the other groups. The results show that UA performance was material-dependent. Application of an etching step prior to UAs increases their bonding effectiveness and reduces levels of NL.
Keywords: microtensile bond strengths, nanoleakage, scanning electron microscope (SEM)

INTRODUCTION

Today's dental adhesive systems enhance long-term bonding while ensuring simplification of the application technique. These adhesive systems available on the market can be classified into two categories: etch-and-rinse (er) and self-etch (se) systems, in three two or one application step versions [1, 2]. In the er systems, the first step involves the application of a phosphoric acid gel (30-40%) to dental hard tissue substrates, resulting in removal of the smear layer, exposure of the collagen fibrils in dentine, and an increase in surface energy in the enamel substrate. The primer is then applied (second step), followed by the bond (third step) resin, separately or in a single solution [3]. These systems, (one- or two-step) employ the use of non-rinse acidic monomers that simultaneously condition and prime dentin without removing the smear layer [4] Both er and se systems form a hybrid layer as a result of resins impregnating the porous enamel or dentin [5].

Se adhesives offer a number of advantages over conventional er adhesives, such as reduction of postoperative sensitivity, involving a less sensitive technique and simplification of bonding procedures. However, the etching effectiveness of these se adhesives on intact enamel, especially that of simplified one-step se adhesives, is still questionable [6, 7]. Application of selective acid etching to enamel before se adhesive application has therefore been recommended in order to enhance the adhesion to enamel, especially when the use of mild-pH se adhesives is intended. [8].

New adhesives that can be applied in either er, se or selective-etch protocols after deciding on the most suitable modality for a specific cavity preparation have been recently introduced with the intention of eliminating complications and providing a single product suitable for all situations [9]. These adhesive

systems are known as 'universal adhesives,' 'multimode adhesives' or 'multi-purpose adhesives.'

The term "nanoleakage" (NL) was introduced by Sano *et al.* in 1995 [10] to describe a specific type of leakage involving poor infiltration of adhesive resin into the demineralized dentin leaving nano-spaces (estimated at approximately 20 to 100 nm in width) in the hybrid layer, in which unprotected collagen fibrils might be prone to degradation from oral fluids and bacterial enzymes. This demineralized but not fully hybridized dentin layer can be considered a weak point in the adhesion mechanism that might allow dentinal fluid to slowly permeate the interface, and this is believed to degrade the adhesive resin. This leakage permits tracer penetration to occur in the absence of interfacial gaps. The penetration of silver nitrate along gap-freemargins can be observed using either scanning (SEM) or transmission electron microscopy (TEM) [10, 11].

Although the NL values for various adhesive systems and the effect of NL on bond strength have been evaluated in previous studies [12-14]. little is still known about the bond strength of these new UAs and their NL patterns. This in vitro study was intended to evaluate the bond strengths and NL patterns of recently introduced UAs.

The null hypotheses were that (1) the application mode (in se or er) of UAs may affect their μTBS, and (2) that it may affect their NL properties.

The purpose of this study was to compare the microtensile bond strength and NL pattern into dentine of four commercial UAs applied in two different etching modes (se or er) compared to two-step er and two-step se adhesive.

MATERIALS AND METHODS
Tooth selection and preparation
Forty-eight freshly extracted intact human molars were collected after obtaining informed consent from patients under a protocol approved by the local Ethics Committee Review Board (2015/12). Teeth were then disinfected in 0.5% chloramine and stored in distilled water at 4 °C for no longer than 1 month until use. The teeth were cleaned with a slurry of pumice and water and rinsed thoroughly with tap water. The occlusal one-third of teeth was removed using a water-cooled low-speed saw (Isomet 1000, Buehler, Lake Bluff, IL, USA), without any additional pressure. The dentin surfaces were polished by wet grinding with #320-grit SiC paper for 1 minute in order to obtain a sufficiently large flat surface, and then rinsed and dried with an air-water syringe.

Experimental design
The teeth were randomly assigned into six groups (n = 8) based on the different adhesive protocols. Two-step er adhesive [Adper Single Bond Plus Adhesive (ASB)] and two-step se adhesive [Clearfil SE Bond (CSE)]were selected as control materials. The exposed surfaces were treated with one of the four UAs [Single Bond Universal (SBU), Prime Bond NT (PNT), Peak Universal Adhesive (PUA) or All-Bond Universal (ABU)] in se or er mode, applied strictly in accordance with the manufacturer's instructions. Composition and manufacturer's instructions are given in Table 1. Composite build-ups were made with the nanohybrid composite Grandio (Voco, Cuxhaven, Germany; Shade A3; batch number: 1402202) in two layers using a rubber cylindrical mold with a 4-mm internal diameter and height of 4 mm. The dentin adhesives and resin composite were polymerized with a light-emitting diode (LED) Elipar Free Light (3M ESPE, Seefeld, Germany) for 20 seconds for each layer. The light intensity was checked periodically with a radiometer (model 100; Demetron, Danbury, CT, USA) as 1200-1250 mW/cm^2. After composite build-up, specimens were stored in distilled water (37°C /24 h). The specimens were sectioned longitudinally in the mesio-distal and buccal-lingual planes across the bonded interface, using a water-cooled slow-speed diamond saw to obtain 20 composite resin–dentin sticks with a cross sectional area of approximately 0.9 mm^2. These were then fixed with cyanoacrylate adhesive (Zapit Dental Ventures of America, Corona, CA, USA) to the grips of a microtensile device (Micro Tensile Tester, T-61010 K, Bisco, U.S). The μTBS was derived by dividing the force imposed at time of fracture by the bond area (mm^2) to obtain the μTBS in MPa. All the specimens from each group were used for μTBS evaluation.

Analysis of failure mode by light microscopy
After microtensile testing, the dentin sides of fractured specimens were evaluated for each sample using a standard stereo microscope (OlympusSZ61, Tokyo, Japan) at X50 magnification. These were then classified as one of three different types; 'adhesive' (at the dentin-resin interface) 'cohesive' (entirely within the dentin substrate or resin composite) or 'mixed' (at the dentin-resin interface including failure into one of the substrates) as shown in Table 3. Samples broken cohesively in dentin or with glue covering the adhesive layer as well as pre-testing failure (PTF) samples were excluded from mean μTBS calculation.

Nanoleakage evaluation
One additional tooth was prepared for each group for evaluation of NL patterns. After composite build up, root apices were sealed with a cyanoacrylate adhesive (Super Bonder TM, 3M, São Paulo, SP, Brazil) and the teeth were coated with two layers of nail varnish up to approximately 0.5 mm from the restoration margins. The specimens were immersed in a 50% (w/v) silver nitrate solution in darkness for 24 h, rinsed under running water for 5 min, immersed in a photo-developing solution and exposed to a fluorescent light for 8 h [15]. After being removed from the photo-

developing solution, the specimens were again rinsed under running water for 5 min, and sectioned in the buccolingual plane using a low-speed diamond saw. The sectioned surfaces were polished with increasingly fine diamond pastes to a high gloss with abrasive disks followed by diamond pastes down to 0.1 µm particle size. The specimens were ultrasonicated in distilled water for 5 minutes, air dried, mounted on aluminum stubs and sputter-coated with Au-Pd. NL patterns were observed under scanning electron microscopy (SEM; JEOL 6400, Tokyo, Japan) in back scattered electron mode at x3000 magnification.

pH measurement

The acidity (pH) of the adhesive systems was measured using a digital pH meter (HI2020, Hanna Instruments, Ann Arbor, MI, USA). Three readings were taken in darkness at room temperature (23° C) and a mean value was calculated (Table 4).

Statistical analysis

The results were analyzed by calculating the mean and standard deviation for each adhesive system. The Kolmogorov–Smirnov test at $\alpha = 0.05$ was applied to confirm the normal distribution of the results. The data were subjected to one-way ANOVA followed by Duncan's multiple range tests. µTBS data were analyzed using statistical software (SPSS 18.0, SPSS Inc, Chicago, IL, USA).

RESULTS
Microtensile bond strength

The µTBS test results are shown in Table 2. ANOVA statistical analysis indicated that the addition of an etching step significantly affected the µTBS of all UAs, except for SBU, compared to their se application modes ($p < 0.05$). The highest µTBS value after 24-h water storage was obtained in the SBU er (38.84 ± 2.07 MPa), and the lowest (15.96 ± 1.21 MPa) in the ABU se. Values in the ASB and CSE (control groups) were 34.03 ± 1.09 MPa and 29.21 ± 1.37 MPa, respectively.

Failure mode analysis

Fractographic analysis under light microscope ($\times 100$ magnifications) revealed a predominance of failures in the adhesive type. Fractographic analysis under light microscope ($\times 100$ magnifications) revealed a predominance of failures in the adhesive type, except for SBU se, SBU er and AS, the in which the dominant failure type was mix failure.

Nanoleakage evaluation

SEM images representing the adhesive/dentin (A/D) interfaces (Figure 1) revealed variations in the detected interaction layers between the tested adhesive systems.

While no NL was determined in the SBU adhesive se and er groups, varying levels of were detected in the other groups (Figure 1).

pH measurement

The pH values of the adhesives are shown in Table 4. ASB exhibited the highest pH (4.2) and the Peak SE primer the lowest (1.3).
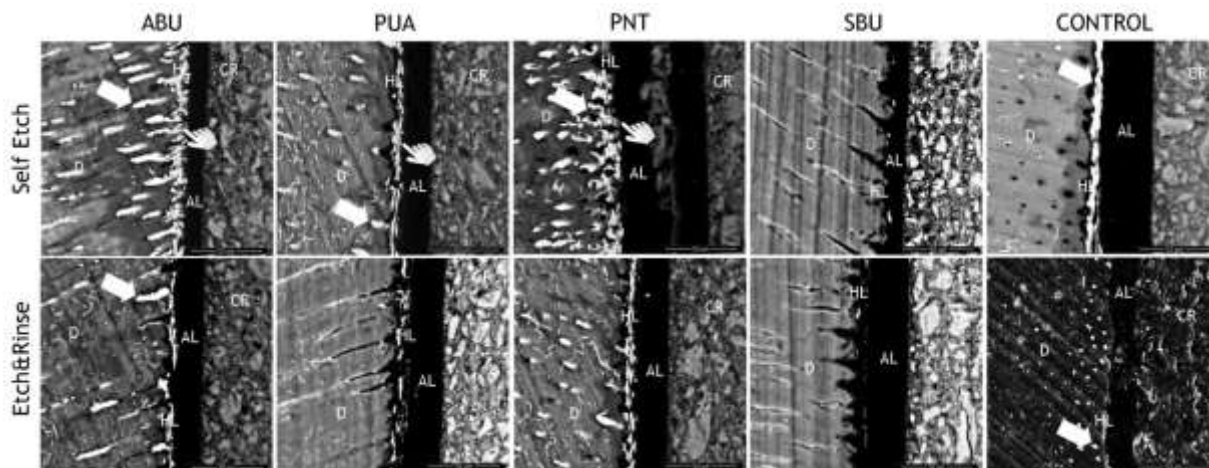

Fig-1: Representative SEM photomicrograph (magnification×3000) of the resin–dentin interfaces of each universal adhesive system

[(Single Bond Universal (SBU), Prime Bond NT (PNT), Peak Universal Adhesive (PUA) or All-Bond Universal (ABU)] in self-etch or etch &rinse mode, with Clearfil SE Bond (CSE) and Adper Single Bond 2 (ASB)]. The images reveal silver nitrate deposition (white arrows) mainly at the bottom and also within the hybrid layer. For SBU, NL was not observed in the er and se strategy. For PNT and PUA, lower NL was observed in the er strategy compared with se strategy. CSE Control self etch) bond exhibited minor silver deposits visible along the hybrid layer with a lower amount of NL. ASB (control etch & rinse) demonstrated silver deposits along the adhesive layer. ABU exhibited highest silver deposition at the HL and AL. (white arrows). In the PUA se, PNT se and ABU se strategy observed that most silver nitrate uptake occurred inside the dentinal tubulus and throughout the entire thickness of the HL (white arrows) forming the so-called ''water trees'' (white pointers). NL, nanoleakage, CR composite resin, D dentin, HL (Hybrid Layer), AL (Adhesive Layer)

Table 1: Adhesive system, etching mode, manufacturer, composition and application procedure for the adhesive systems used according to the manufacturers' instructions

Adhesive System/code/ Manufacturer (Batch number)	Etching mode	Composition	Application procedure	
			Self-etch application	Etch-rinse application
Adper Single Bond Plus Adhesive ASB 3M ESPE, St Paul, MN, USA (N499125)	Two-step etch & rinse	Adhesive: water, ethanol, BisGMA, HEMA, UDMA, Bisphenol A, glycerolate, dimethacrylate Etchant: Water, 35%, phosphoric acid, syntetic amorphous silica.		1. Apply etchant for 15 sec 2. Rinse for 10 sec 3. Blot excess water 4. Apply 2–3 consecutive coats of adhesive for 15 sec with gentle agitation 5. Gently air dry for 5 s 6. Light polymerize for 10s.
Clearfil SE Bond CSE Kuraray Medical Inc., Tokyo, Japan (01630A)	Two-step self-etch	*Primer*: MDP, water, HEMA Hydrophilic aliphatic dimethacrylatedl-Camphorquinone N, N Di ethanol-p-toluidine *Bond*: MDP, Bis-GMA, HEMA Hydrophobic aliphatic dimethacrylate dl-Camphorquinone, N,N-Diethanol-p-toluidine Colloidal silica	1. Apply primer to tooth surface and leave in place for 20 sec 2. Dry with air stream to evaporate the volatile ingredients 3. Apply bond to the tooth surface and then create a uniform film using a gentle air stream 4. Light polymerize for 10 sec.	
Single Bond Universal SBU 3M ESPE, St. Paul, MN, USA (D48832/ 537256)	Multi-mode	Etchant: 35% phosphoric acid MDP, BISGMA HEMA, DMA, polyalcenoic acid copolymer, filler, ethanol, water, initiators, silane	1. Apply adhesive to tooth surface by scrubbing action for 20 sec. 2. Dry the adhesive for 5 sec. 3.Light cure for 10 sec.	1. Apply etchant for 15 sec. 2. Rinse for 10 sec. 3. Apply adhesive using a scrubbing action for 20 sec. 2. Dry the adhesive for 5 sec. 3. Light cure for 10 sec.
Prime & Bond NT PNT Dentsply/Caulk, Milford, DE (1311001049)	Multi mode	PENTA, UDMA, T-resin (cross-linking agent), D-resin, (small hydrophilic molecule), butylated hydroxytoluene, ethyl 4-dimethylaminobenzoate, cetilamine hydrofluoride, acetone, silica nanofiller	1. Initial use of Peak SE requires activation of the two components separated in the syringe 2. Application of the Peak SE with microbrush for 20 sec using continuous scrubbing on dentin. 3. Thin/dry for 3 sec using an air/water syringe or high volume suction directly over preparation 4. Apply a puddle coat of Peak LCBond and gently agitate for 10 sec. 5. Thin/dry for 10 sec	1. Apply etchant for 15 sec. 2. Wash etching acid for 10 sec. 3. Apply adhesive and dry to evaporate solvent. 4. Light-cure for 10-20 sec.

				using ¼ to ½ air pressure 6. Light-polymerize for 10 sec.
Peak Universal Adhesive PUA Peak LC Bond and Peak SE Primer, Ultradent Products Inc., South Jordan, UT, USA (B8ZG1)	Multi mode	Etchant: 35% phosphoric acid (Ultra etch) Peak SE Primer: ethyl alcohol, methacrylic acid, HEMA Peak LC Bond resin: ethyl alcohol, HEMA	1. Apply two separate coats, scrubbing the preparation with a microbrush for 10–15 sec per coat. Do not light polymerize between coats 2. Evaporate excess solvent by thorough air-drying for at least 10 sec. 3. Surface should have a uniformly glossy appearance. 4. Light cure for 10 sec.	1. Apply etchant for 20 sec. 2. Rinse for 5 sec. 3. Air dry for 2 sec. 4. Apply a puddle coat of Peak LC Bond with gentle agitation for 10 sec. 5. Dry for 10 sec using ¼ to ½ air pressure 6. Light-polymerize for 10 sec.
All-Bond Universal ABU Bisco Schaumburg, USA (1400001841)	Multi mode	Etchant: 35% phosphoric acid 10-MDP, HEMA BISGMA, ethanol		1. Apply etchant for 15 sec. 2. Rinse thoroughly 3. Remove excess water with absorbent pellet or high volume suction for 1–2 sec. 4. Apply adhesive. 5. Light polymerize for 10 s.

Bis-GMA: Bisphenol A diglycidyl methacrylate, HEMA: 2-Hydroxyethyl methacrylate, UDMA: urethane dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate, PENTA: Penta-acrylate ester DMA, dimethacrylate; MDP: 10-Methacryloyloxydecyl dihydrogen phosphate

Table 2: Microtensile bond strength (µTBS) values (standard deviations) of the different experimental groups

Adhesive System	Etching mode	µTBS (MPa)
ASB	Etch&rinse	34.03 (1.09)[B]
CSE	Self etch	29.21 (1.37)[C]
SBU se	Self etch	37.01 (2.07)[A]
SBU er	Etch&rinse	38.84 (2.07)[A]
PNT se	Self etch	22.05 (2.13)[E]
PNT er	Etch&rinse	29.88 (2.91)[C]
PUA se	Self etch	18.78 (2.14)[F]
PUA er	Etch&rinse	26.22 (1.46)[D]
ABU se	Self etch	15.96 (1.21)[F]
ABU er	Etch&rinse	29.98 (2.05)[C]

*Similar capital letters in the column are not statistically significant different.

Table 3: Number (%) of fracture types in µ TBS samples as analyzed by stereo-microscopy

Adhesive system	Failure Mode		
	Adhesive	Cohesive	Mix
ASB	5 (25)	4 (20)	11 (55)
CSE	16 (80)	2 (10)	2 (10)
SBU se	5 (25)	3 (15)	12 (60)
SBU er	7 (35)	3 (15)	10 (50)
PNTse	13 (65)	4 (20)	3 (15)
PNT er	12 (60)	3 (15)	5 (25)
PUAse	16 (80)	2 (10)	2 (10)
PUAer	11 (55)	5(25)	4 (20)
ABUse	13 (65)	1 (5)	6 (30)
ABU er	11 (55)	5 (25)	4 (20)

Table 4: pH values of the tested adhesive systems

Adhesive System		pH
ASB		4.2 ± 0.01
CSE	primer	2.0 ± 0.02
	bond	2.5 ± 0.03
SBU		2.6 ± 0.04
PNT		2.1 ± 0.06
PUA	Peak SE Primer	1.3 ± 0.03
	Peak LC Bond resin	2.0 ± 0.01
ABU		2.5 ± 0.03

DISCUSSION

UAs have been described by some manufacturers as ideally a single-bottle, no-mix, adhesive system that can be used in er, se, or selective-etch mode, depending on the specific clinical situation and personal preferences of the clinician [16]. The present study evaluated the µTBS and NL patterns of four UAs systems (SBU, PNT, PUA and ABU), a two-step etch/rinse adhesive (ASB) and a two-step self/etch adhesive (CSE).

Phosphoric acid etching significantly increases the bond strength of one-step se adhesives to enamel [7, 10]. Selectively etching enamel combined with se adhesives can therefore be recommended in order to achieve effective and durable bonding to tooth enamel by increasing the bonding surface area [17, 18]. Previous studies have shown that preliminary etching of enamel significantly increases bond strength for multimode adhesive and two-step se adhesive [19]. However, this has been shown to be unsuitable for deep dentin cavities, because accidental dentin etching may occur during the selective enamel-etching process, when a low viscosity etchant is used [20-23]. Inadvertent pre-etching of dentin is a clinical risk which may compromise bonding efficacy [21]. The effect of premeditatedly etching dentine with phosphoric acid prior to the application of se adhesives has been investigated in previous studies. The results are controversial and material-dependent. van Landuyt *et al* [24] and Hanabusa *et al* [19] concluded that adding a preliminary etching step is beneficial for enamel but should be avoided for dentin as it does not reduce the bond strength and may even compromise the bonding stability. Giulio Marchesi *et al* .[23] indicated that improved bonding effectiveness of the tested universal adhesive system on dentin can be obtained when the adhesive is applied using the se approach. Another study reported that the addition of an etching step did not significantly affect the µTBS of any of the UAs compared to their se application mode [25]. Munoz *et al* [3] reported that when UAs were tested using the self-etch or etch-and-rinse strategy on dentine, they were inferior to the two-step etch-and-rinse or a two-step etch-and-rinse adhesive with respect in terms of at least one of the properties tested (microtensile bond strength, NL or degree of conversion.In contrast, Taschner *et al* [22] advocate the use of one-step adhesives on etched

dentin because of the increased bond strength obtained compared with their application onto smear-layer-covered dentin, regardless of storage conditions. In our study, µTBS testing showed that all of universal adhesives systems exhibited increased resin–dentin bond strengths when used in the er mode, following the manufacturers' directions. The first null hypothesis was thus accepted..The most rational explanation for the increased µTBS is an increase in dentin porosity, resulting in increased resin interlocking and micro-mechanical retention for all UAs [21].

In addition to the application mode, compositional differences between adhesives may also play an important role in adhesive performance. The highest µTBS value was obtained in the SBU er(38.84 ± 2.07 MPa). The presence of polyalkenoic acid copolymer (Vitrebond Copolymer) in the composition of SBU (Table 1) may explain the higher µTBS in this group in the se and er modes. Primary chemical bonding occurs through the formation of ionic bonds between the carboxyl groups of the polyalkenoic acid and calcium of hydroxyapatite that remain around the exposed surface. This has been proved for polyalkenoic acids applied to hydroxyapatite [26, 27]. This chemical bonding mechanism explained by adhesion decalsification concept. According to this concept, the less soluble the calcium salt of an acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate [28]. SBU, CSE and ABU contain MDP capable of chemically interacting with hydroxyapatite by the formation of stable nano-layering at the adhesive interface [23]. The formulation of SBU includes MDP polyalkenoic acid copolymer, which may explain the good performance of SBU in er and se modes. These molecules may compete by binding to the calcium in hydroxyapatite [16, 29, 30].

The most common failure mode in this study was adhesive failure. A mixed failure pattern was predominantly observed in SBU se and SBU er and in ASB. This failure mode may be due to the relatively higher bond strength to dentin in these groups exhibiting high µTBS values [31, 32].

The principle of bonding to dental hard tissues is essentially based on micromechanical interlocking of the adhesive resin with dentin/enamel surfaces. While

bonding to enamel is dependent on the micromechanical retention to the etched substrate, that to dentin relies on hybridization with the exposed collagen mesh [32]. Even if all se adhesives rely on the same bonding mechanism, they still differ from each other in many other aspects, such as acidic resin monomer composition, water content and acidity. Indeed, adhesive acidity influences the ability of the system to interact with the underlying enamel and dentin [23]. Generally, se adhesives are divided into three groups based on their acidity levels; mild (pH>2), moderate (1<pH<2) and strong (pH<1) [34]. The adhesives (PNT and PUA) used in this study have low ph values. It may be claimed that adhesive systems with higher pH values are less effective in solubilizing thick smear layers and demineralizing solid dentin surfaces for hybridization than adhesives with lower pH values. This is still a subject of debate. Previous studies have reported no correlation between hybrid layer thickness and bond strengths [35, 36].

Our results agree with those of previous studies, in that the quality of the hybrid layer is more important than its thickness. Previous studies have shown variations in thickness between specimens prepared from different dentin substrates using the same adhesive system [37].

Silver nitrate solution 50% (w/v) (AgNO3) is the most used tracer for evaluation of the NL phenomenon. This solution can penetrate the dentin due to the small silver ion size (0.059 nm in diameter) [12]. Despite difficulties associated with the technique, previous studies have indicated that 50% silver nitrate solution is effective in detecting NL within the hybrid layer [8]. In this study, a 50% (w/v) silver nitrate solution was used as a tracer to evaluate the NL phenomenon.

The NL pathway, the weakest point within the hybrid layer, is probably responsible for the deterioration of polymers since it may permit dentinal and oral fluid to slowly permeate the interface [38]. Several factors can influence the occurrence of NL, including the individual chemical components, the type of solvent (water, acetone or ethanol) as well as other additives and type of application (dry or moist) [39]. In order to achieve an optimal hybrid layer it is important to keep the dentin moist, because the collagen network can collapse with excessive drying prevent monomer diffusion to the dentin surface [40]. In clinical terms, it is preferred that dental adhesives be hydrophilic during application and then become hydrophobic after application and completely seal the restoration margins for an extended period. UAs must also contain water, for dissociation of the acidic functional monomers, inherent in all these systems, that makes self-etching possible. Some water is needed, although too much can degrade the chemistry of these systems, contribute to phase separation of monomers, reduce shelf-life, and

make complete evaporation during the air-drying step difficult. Residual water after air-drying can result in incomplete adhesive polymerization, increased hydrolysis after polymerization and in a generally compromised adhesive interface [14].

ABU exhibited the poorest performance in terms of µTBS, which may be attributed to the application procedures involved. In line with the manufacturer's instructions for ABU, adhesive was used two coats, the first of which was not light polymerized. Studies have reported the use of adhesives in more than one coat increases bonding strength [41, 43]. Intermediate adhesive layers have not been polymerized in some of these studies [41, 42] using more than one coat of adhesive, while each coat has been individually polymerized in order studies [43].

The results of previous studies show that an excess of adhesive layer thickness can compromise the strength and the quality of adhesion [44]. In another study, Ito et al [45] indicated that bond strengths increased with the number of coatings, up to three layers, especially if each layer is light cured. NL of silver tended to decrease with each coat in both adhesive systems. In the line with the instructions by manufacturer of the ABU used in this study two layers were applied, but no polymerized was performed after first layer, only after the application of the second layer. ABU se and er exhibited silver deposition within the hybrid layer and the dentin tubules (Figure 1). A previous study reported that the number of coats did not affect bond strength values, and that a higher number of coats produced a thicker layer of adhesive than one single application, which may lead to a different degree of hydrolytic degradation [46]. Increased NL and decrease of bonding strength may be attributed to the use of the two-layer adhesive system. Further research is necessary to support this possibility.

In this study, the results of SEM analysis of the ultrastructure of adhesive bond between composite and dentin also showed a significantly better marginal adaptation of composite restorations when the er technique was used compared to these technique, apart from the SBU se and er groups. These groups exhibit sparse silver nitrate deposition at AL, while no deposition was determined in the HL. The second null hypothesis was thus accepted. All adhesive systems employ acetone, ethanol, water, or a combination of these as solvents for their particular monomers. Adding ethanol or acetone into UA formulations enhances resin wetting and infiltration of tooth tissues and also aids in water removal and evaporation during the air-drying step [14]. The high diffusion of ethanol, together with a high water displacement capacity, may result in better SBU contact with the dentin, thus avoiding the development of NL. In addition, SBU contains polyalcenoic acid comonomer, a molecule responsible for chemical adhesion [39].

The PUA se, PNT se, PNT er, ABU se and ABU er specimens exhibited extensive silver deposit in the hybrid zone and inside the dentinal tubules (Figure 1.) Water tree formation from the hybrid layer to the adhesive layer was identified in these groups. CSE specimens exhibited silver nitrate deposits in the HL. In these specimens, the NL pattern represents discontinuous islands of silver deposits within the HL and AL which may be related to areas of incomplete water removal from dentin-resin interfaces, instead of incomplete adhesive infiltration [39]. PNT specimens exhibited extensive silver deposits in the hybrid zone and inside the dentinal tubules. (Figure1). This finding is in agreement with Duarte et al.'s study [39]. and may be attributed to UDMA and PENTA being high molecular weight monomers that also possibly reduce the ability of PNT to diffuse through demineralized dentin.

CONCLUSIONS

Within the limitations of this study, we conclude that additional acid etching may be acceptable in UAs. The degree of the increase in microtensile bond strength and the degree of decrease in NL was product-dependent and based on individual compositions. Further research is needed to determine the long-term bonding ability and clinical performance of UA systems.

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