

# THE INFLUENCE OF DIFFERENT ETCHING MODES AND ETCHING TIME PERIODS ON MICRO-SHEAR BOND STRENGTH OF MULTI-MODE UNIVERSAL ADHESIVES ON DENTIN

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## ABSTRACT

**INTRODUCTION:** Simplifying single-bottle adhesive technology to satisfy clinicians' demand for adhesive procedures, particularly in terms of making the techniques faster, less technically sensitive, and more user friendly, has led to the development of multi-mode universal adhesives, which were designed to bind to tooth structures via the etch-and-rinse, the self-etch, and the selective-etch modes using a single bottle of adhesive solution.

**OBJECTIVES:** The aim of this study was to determine the dentin bonding ability of multi-mode universal adhesive systems in relation to different etching modes and etching time periods using micro-shear bond strength analysis.

**MATERIAL AND METHODS:** Three multi-mode universal adhesives and one etch-and-rinse adhesive were assessed in this study. Micro-shear bond strengths ( $\mu$ SBS) to dentin were obtained in etch-and-rinse (etching 5 s/10 s/15 s) and self-etch modes. For each test condition, 10 specimens were prepared for each group. A scanning electron microscope (SEM) was used to examine representative bonded specimens, treated dentin surfaces, and the resin/dentin interface for each test condition.

**RESULTS:** Multi-mode universal adhesives showed the lowest  $\mu$ SBS results in etch-and-rinse mode for 10-s and 15-s etching ( $p < 0.05$ ). Universal adhesives and self-etch adhesives showed similar  $\mu$ SBS values in self-etch mode ( $p > 0.05$ ).

**CONCLUSIONS:** Analysis concluded that phosphoric-acid etching for time periods greater than 5 s significantly reduced the efficacy of bonds when using multi-mode universal adhesives.

**KEY WORDS:** universal adhesive, self-etch adhesive, etch-and-rinse adhesive, micro-shear bond strength.

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## INTRODUCTION

The prognosis of adhesive restorations depends mainly on adhesive bond strength and the durability of the adhesive interface [5]. Innovations in adhesive technology lead to the development of a new generation of adhesive systems. Currently, dental adhesive systems are divided

into two main groups named as “etch-rinse” or “self-etch” strategies. In addition, these systems can be classified according to the application stages: two- or three-step systems for those using acid etching, and for self-etching it can be classified in one- or two-step systems [18, 36].

In etch-and-rinse adhesion strategy, the first step includes demineralisation with phosphoric acid; this

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increases the adhesive penetration. After the demineralisation, the upper inter-tubular matrix changes and nanoscale porosities form with the underlying collagen fibrils. The following step is the conditioning of the dentin surface with a primer, and the final step is the application of adhesive resin. These two materials can be applied either separately or in a single solution [18]. In addition to the fact that this technique involves several steps, one of the disadvantages of etch-and-rinse adhesive systems is that the collagen fibres collapse as the demineralised dentin dries; this can lead to a reduction in bond strength [29]. Therefore, it is important to keep the etched dentin moist for effective resin infiltration into the demineralised areas [11, 19]. In particular, the definition of an “ideal moisture” content depends on various factors: (1) clinician experience [25]; (2) the “directions for use” recipe of the material [12]; and (3) the solvent type present in the composition [22]. However, standard guidelines have not been established with respect to how moisture should be retained, and this remains a significant challenge for clinicians and researchers.

Unlike etch-and-rinse adhesives, self-etch adhesives do not involve a divided etching step because they involve an aqueous mixture of acidic functional monomers, usually phosphoric-acid esters [36], and the degree of moisture affects them less than etch-and-rinse adhesives [30]. Therefore, previous authors have claimed that this approach is more user friendly and less technically sensitive, and results in a more reliable clinical performance [20, 32]. Nevertheless, phosphoric acid is still often recommended for etching of the enamel, particularly in the selective-etch technique when cavity margins end in the enamel [16]. However, when low-viscosity acid is used, accidental etching may occur on the dentin surface during the enamel-etching process, and this etching procedure is generally considered to be unsuitable for use on the dentin substrate for self-etch adhesives [33].

Manufacturers often want to produce user-friendly products with fewer components, which require less technical precision and produce faster results. These systems are single bottles and can be used as either total-etch, selective-etch, or self-etch [8]. These new materials are highly versatile and provide clinicians with various bonding strategies.

When using the total-etch method, it is necessary to apply acid to dental tissues for a certain period of time. Many studies have been published on different etching periods and their relative effect on dentin when using etch-and-rinse adhesive systems [39]. However, there is a lack of information on the appropriate etching period for dentin when using multi-mode universal adhesives in etch-and-rinse mode.

## OBJECTIVES

The aim of the current research was to compare the bond strength with various etching modes and time periods using three multi-mode universal adhesives with two self-etch adhesives and an etch-and-rinse adhesive on dentin.

## MATERIAL AND METHODS

This study was planned and conducted under the approval of faculty Ethics Committee (Ankara University, Faculty of Dentistry 36290600/14).

## TOOTH SELECTION AND PREPARATION

In total, 170 extracted sound human third molars were used for this experiment ( $N = 170$ ). After removing residual tissue tags, extracted teeth were stored in 0.9% saline solution, and specimens were prepared for experimentation within two weeks. The occlusal surfaces of extracted teeth were removed with a cutting machine under water-cooling (Micracut 201, Metkon, Turkey) to obtain flat dentin surfaces and then embedded in a self-cured acrylic resin (Vertex Self Curing, Vertex Dental, Netherlands). The occlusal parts of the teeth were polished with silicon carbide paper (600-grit) under water-cooling (P1000-P4000 Metkon, Gripo 2v Grinder-Polisher, Turkey) to create a uniform smear layer.

All surfaces were then carefully verified using a stereomicroscope at 25 $\times$  magnification (M3Z, Leica Microsystems, Wetzlar, Germany) to ensure the absence of pulpal exposure and residual enamel.

## BONDING PROCEDURES

Three multi-mode universal adhesives (Single Bond Universal [SBU] [3M ESPE, USA], All Bond Universal [ABU] [Bisco Inc., USA], and Clearfil Universal Bond [CUB] [Kuraray Noritake Dental Inc., Japan]), one two-step self-etch adhesive (Clearfil SE Bond [CSE] [Kuraray Noritake Dental Inc., Japan]), one one-step self-etch adhesive (Clearfil S3 Bond Plus [CS3] [Kuraray Noritake Dental Inc., Japan]), and one etch-and-rinse adhesive (Adper Single Bond-2 [ASB2] [3M ESPE, USA]) were selected for this study. The compositions of these adhesives are listed in Table 1.

The teeth were divided into six groups and randomly assigned to one of the six adhesives. Adhesives were then applied to occlusal dentin surfaces using different etching modes and periods ( $n = 10$ ) as follows:

- self-etching mode,
- etch-and-rinse mode (5-s etching),
- etch-and-rinse mode (10-s etching),
- etch-and-rinse mode (15-s etching).

**TABLE 1.** Adhesive composition and application procedure, as described in safety data sheets and instructions

Adhesive materials		Chemical composition	Self-etch technique	Etch-and-rinse technique	Manufacturer
Single Bond Universal	MDP, Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Filler, Ethanol, Water, Initiators, Silane	MDP, bis-GMA, HEMA, ethanol, water, initiators	1. Applied the one coat adhesive with disposable applicator to the entire sample surface, rubbed it in for 20 s. 2. Air dried the solvent with an air syringe 5 s. 3. Light cured for 10 s.	1. Applied etchant for 5 s/10 s/15 s. 2. Rinsed for 10 s and dried with cotton pellets. 3. Applied adhesive as for the self-etch mode.	3M ESPE, St. Paul, MN
All Bond Universal	MDP, bis-GMA, HEMA, ethanol, water, initiators	MDP, bis-GMA, HEMA, ethanol, water, initiators	1. Applied two separate coats of adhesive with disposable applicator to the entire sample surface, rubbed it in for 20 s. 2. Air dried the solvent with an air syringe 5 s. 3. Light cured for 10 s.	1. Applied etchant for 5 s/10 s/15 s. 2. Rinsed for 10 s and dried with cotton pellets. 3. Applied adhesive as for the self-etch mode.	Bisco Inc., Schaumburg, IL, USA
Clearfil Universal Bond	10-MDP, BISGMA, HEMA, ethanol, hydrophilic aliphatic dimethacrylate, colloidal silica, camphorquinone, silane coupling agent, accelerators, initiators, water	10-MDP, BISGMA, HEMA, ethanol, hydrophilic aliphatic dimethacrylate, colloidal silica, camphorquinone, silane coupling agent, accelerators, initiators, water	1. Applied the one coat adhesive with disposable applicator to the entire sample surface, rubbed it in for 10 s. 2. Air dried the solvent with an air syringe 5 s. 3. Light cured for 10 s.	1. Applied etchant for 5 s/10 s/15 s. 2. Rinsed for 10 s and dried with cotton pellets. 3. Applied adhesive as for the self-etch mode.	Kuraray, Okayama, Japan
Clearfil SE Bond	Primer: MDP, HEMA, Hydrophilic dimethacrylate, Water Adhesive: MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, di-Camphorquinone, N,N-Diethanol-p-toluidine, Silanated colloidal silica	Primer: MDP, HEMA, Hydrophilic dimethacrylate, Water Adhesive: MDP, Bis-GMA, HEMA, Hydrophobic dimethacrylate, di-Camphorquinone, N,N-Diethanol-p-toluidine, Silanated colloidal silica	1. Applied the one coat primer with disposable applicator to the entire sample surface, left in place for 20 s. 2. Air dried the solvent with an air syringe. 3. Applied adhesive to the entire sample surface and then created a uniform film using a gentle air stream. 4. Light cured for 10 s.	N.A.	Kuraray, Okayama, Japan
Clearfil S3 Bond Plus	MDP, HEMA, Bis-GMA, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Colloidal silica, di-Camphorquinone, Accelerators, Initiators, Water	MDP, HEMA, Bis-GMA, Hydrophilic aliphatic dimethacrylate, Hydrophobic aliphatic methacrylate, Colloidal silica, di-Camphorquinone, Accelerators, Initiators, Water	1. Applied the one-coat adhesive with disposable applicator to the entire sample surface, left in place for 10 s. 2. Air dried the solvent with an air syringe 10 s 3. Light cured for 10 s.	N.A.	Kuraray, Okayama, Japan
Adper Single Bond-2	bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(taconic) acids, 10% by weight of 5 nm-diameter spherical silica particles	bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and poly(taconic) acids, 10% by weight of 5 nm-diameter spherical silica particles	N.A.	1. Applied etchant for 5 s/10 s/15 s. 2. Rinsed for 10 s. 3. Blotted excess water. 4. Applied two consecutive coats of adhesive for 15 s with gentle agitation. 5. Air-dried the solvent with an air syringe for 10 s. 6. Light cured for 10 s.	3M ESPE, St. Paul, MN

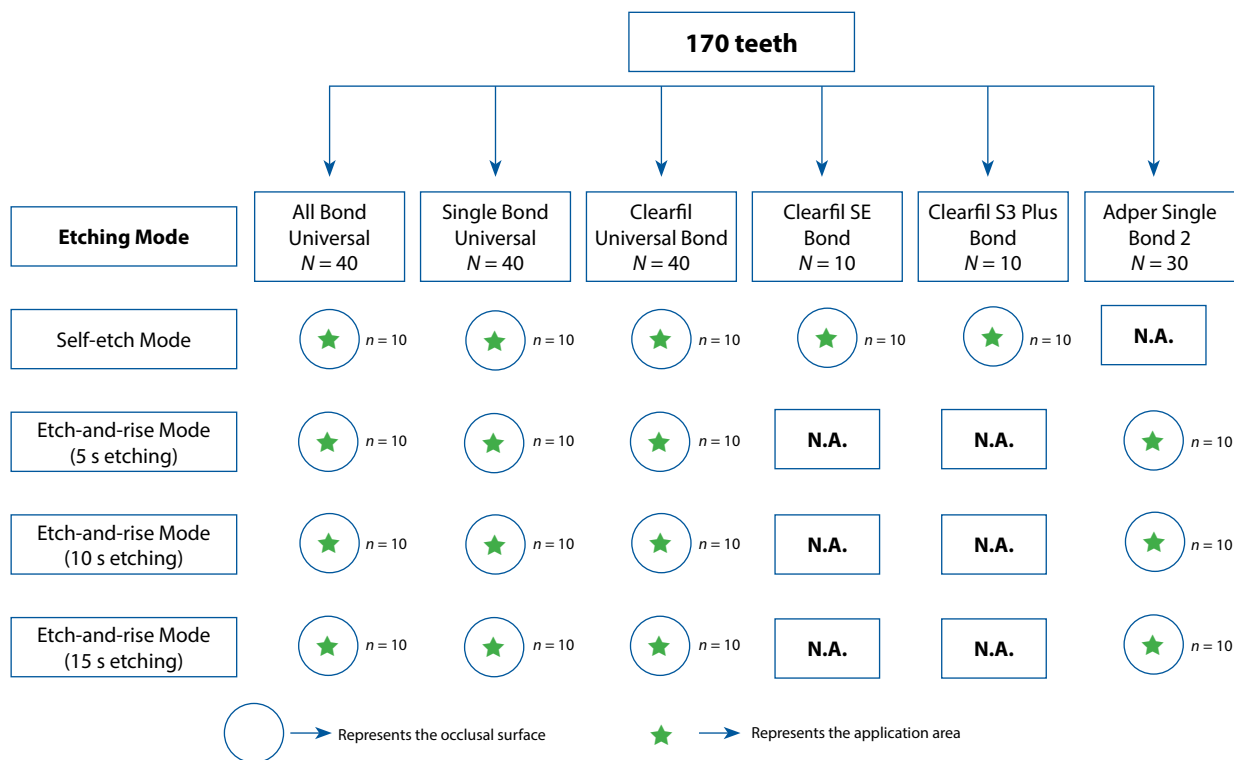


FIGURE 1. Study design

For the etch-and-rinse mode, a 35% phosphoric acid etchant (K-ETCHANT Syringe, Kuraray Noritake Dental Inc., Okayama, Japan) was used for etching occlusal dentin surface for 5 s/10 s/15 s. Then, etched dentin surfaces were rinsed for 10 s. The water present on the rinsed dentin surface was removed according to the manufacturer’s instructions for each respective adhesive. For the self-etch mode, no etchant was used, and the tested adhesives were applied to the dentin surface according to the manufacturer’s instructions. The experimental design is illustrated in Figure 1.

Afterwards, a polyethylene tube (0.5 mm in height and of 0.8 mm internal diameter [Unomedical, Conva-Tec Limited, UK]) was placed on the bonding area and cured by light for 10 s with a LED-curing unit (SDI Radium Plus, SDI Limited, Australia). Efforts were made to ensure that light intensity was  $\geq 1000$  mW/cm<sup>2</sup> using a Hilux Ledmax curing lightmeter (Benlioglu Dental, Turkey). Then, resin composite (Clearfil Majesty Esthetic, Kuraray Noritake Dental Inc., Okayama, Japan) was condensed into the tube with a composite filling instrument (Polyfill, 1051/95, Carl Martin GmbH, Solingen, Germany). The tube was then light cured for 20 s, and following this procedure the polyethylene tube was removed and each specimen was examined under a stereomicroscope (M3Z, Leica Microsystems, Wetzlar, Germany) at 25× magnifications to verify that no interfacial gaps, bonding defects, or air bubble inclusions were present. Then, all specimens were subjected to a thermo-cycle process (5-55°C, 10,000 cycles).

## MICRO-SHEAR BOND STRENGTH AND FAILURE ANALYSIS

Each bonded interface was subjected to a  $\mu$ SB test in a universal testing machine (Z1010, Zwick, Ulm, Germany) using a chisel-shaped blade to deliver a force parallel to the bonded surface, loaded at a crosshead speed of 1 mm/min until failure. The load at debonding was recorded, and  $\mu$ SBS  $\sigma$  was calculated using the load at failure F (N) in the adhesive area A (mm<sup>2</sup>):  $\sigma = F/A$ .

Further, the deboned areas were examined under stereomicroscope at 25× magnification (M3Z, Leica Microsystems, Wetzlar, Germany). Failures were classified as: (1) cohesive failure exclusively in dentin, (2) cohesive failure exclusively in the resin composite, (3) adhesive failure at the interface and mix failure at the adhesive/dentin interface, which included cohesive failure of the dentin and/or resin composite, and (4) adhesive material.

## SCANNING ELECTRON MICROSCOPY ANALYSIS

Three samples were prepared for each group in the same manner as for the bonding procedures, but tubes with 5 mm diameter were used. The embedded specimens were cut into two halves longitudinally in a mesial/distal direction using a low-speed cutting machine. After 24 h, the surfaces of the cut halves were polished with 600-, 800-, and 1200-grit abrasive paper. The specimens were then etched with 35% phosphoric acid for 5 s, immersed

in 5% sodium hypochlorite for 5 min, rinsed with distilled water, and then dried in a desiccator cabinet for two days. Surfaces were then sputter-coated with 5-nm gold/palladium particles (EMS150R S Sputter Coater, Electron

Microscopy Sciences, Hotfield, USA). The ultra-structures of all dentin-adhesive interface specimens were then observed with SEM (FEI Inspect F50 FEQ, Thermo Fisher Scientific Inc., Oregon, USA).

**TABLE 2.** Micro-shear bond strength ( $\mu$ SBS) values (MPa) for the different adhesive systems tested. Mean values with the same letters are not significantly different ( $p > 0.05$ )

Adhesive system	Etching mode	$\mu$ SBS mean	SD
Single Bond Universal	5-s etching	25.35(a)	5.9
	10-s etching	22.55(b)	3.9
	15-s etching	20.05(b)	3.7
	Self-etch	30.25(a)	5.7
All Bond Universal	5-s etching	31.4(a)	4.5
	10-s etching	18.7(b)	5.1
	15-s etching	16.85(b)	2.6
	Self-etch	30.75(a)	3.8
Clearfil Universal Bond	5-s etching	28.25(a)	5.6
	10-s etching	20.9(b)	5.2
	15-s etching	14.7(b)	3.3
	Self-etch	31.2(a)	4.0
Clearfil SE Bond	Self-etch	29.4(a)	3.3
Clearfil S3 Bond Plus	Self-etch	27.3(a)	4.4
Adper Single Bond-2	5-s etching	17.75(b)	4.5
	10-s etching	28.8(a)	4.8
	15-s etching	33.45(a)	2.9

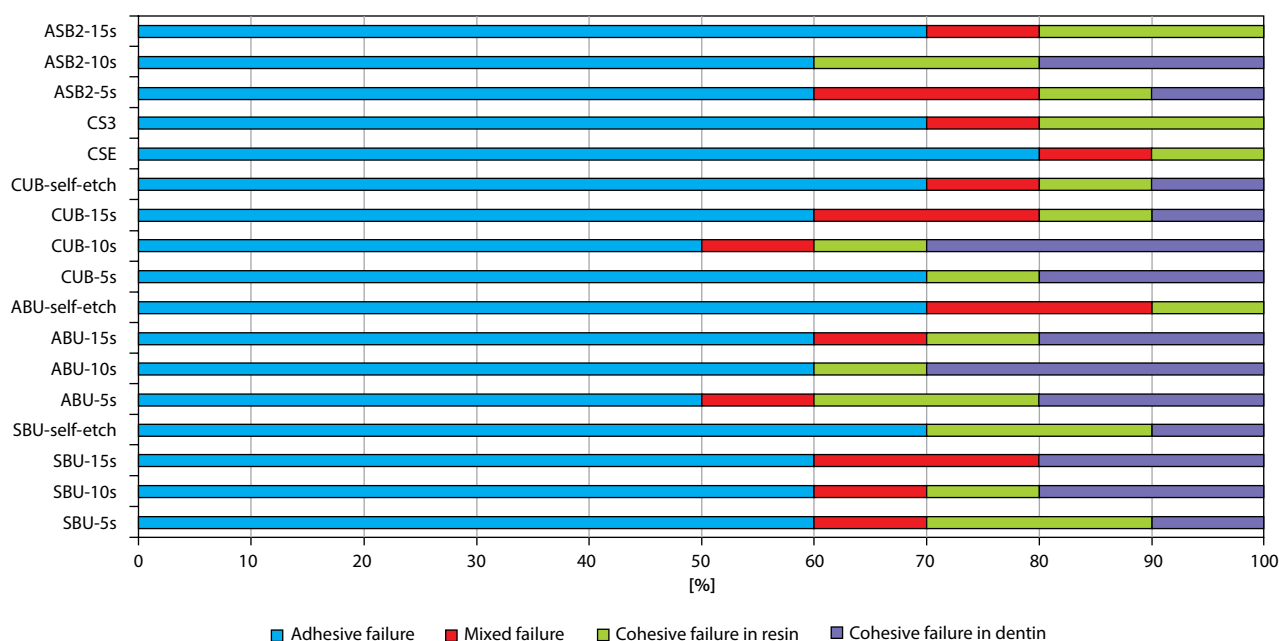
### STATISTICAL ANALYSIS

The statistical program GraphPad Prism (ver. 6.0) was used to analyse the data. The Kolmogorov–Smirnov test was used to confirm normal data distribution ( $\alpha = 0.05$ ). Obtained data were then analysed by one-way analysis of variance, and the Tukey HSD post hoc test was used for multiple comparisons. The level of significance was set at  $p < 0.05$ .

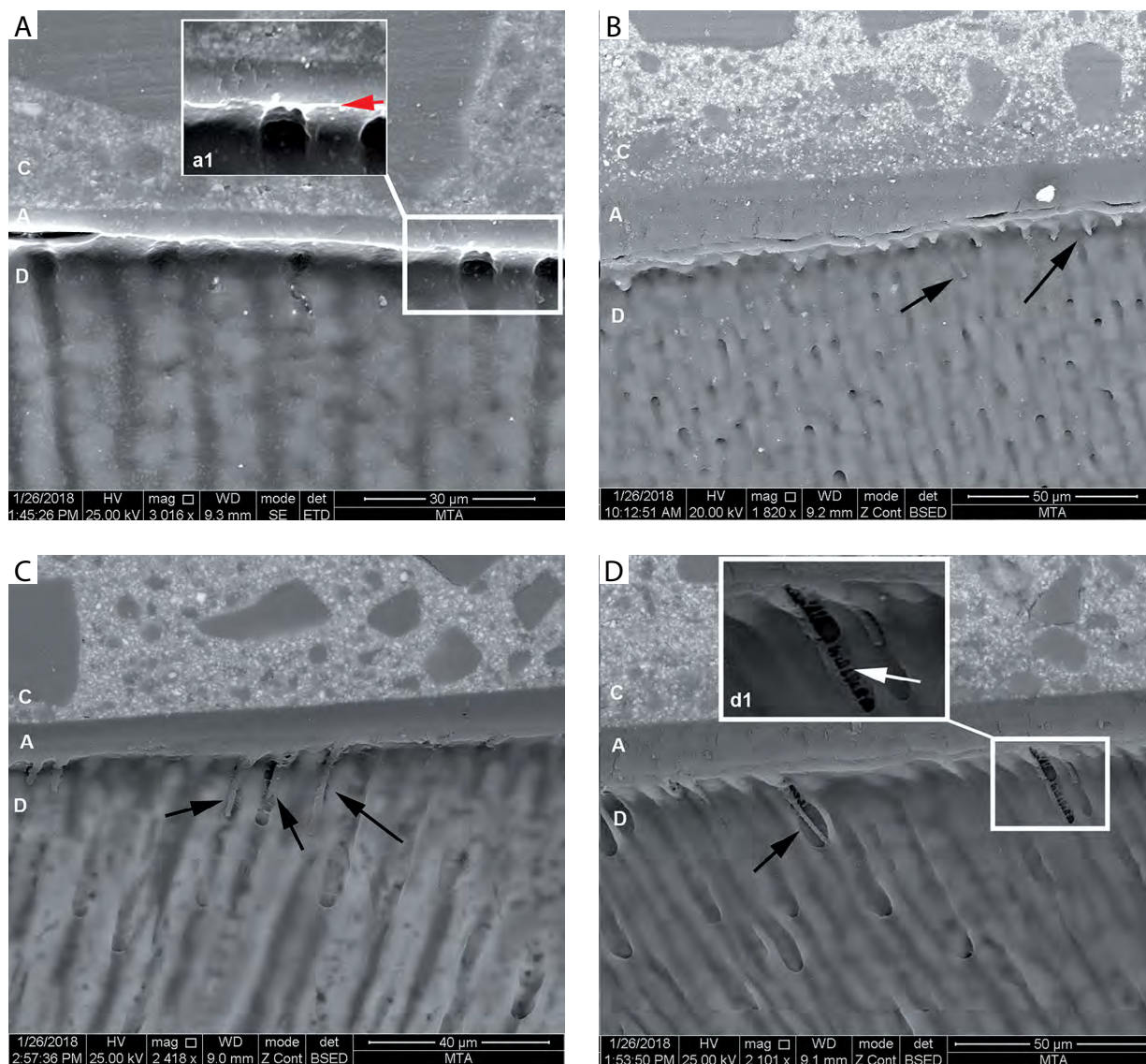
### RESULTS

The effects of acid etching modes and times on  $\mu$ SBS are shown in Table 2. Multi-mode adhesives (SBU, ABU, CUB) showed higher bond strength values in self-etch and 5-s etch-and-rinse modes than in 10-s and 15-s etch-and-rinse modes ( $p < 0.05$ ). In self-etch mode, all of the tested adhesives (SBU, ABU, CUB, CSE, CS3) showed similar  $\mu$ SBS results ( $p > 0.05$ ). The etch-and-rinse adhesive (ASB2) showed significantly higher  $\mu$ SBS values when used with 10-s and 15-s etching modes than with 5-s etching mode ( $p < 0.05$ ).

The most common failure in all groups was the adhesive type (Figure 2). For all adhesives tested in etch-and-rinse mode, SEM images demonstrated funnel- or cylindrical-shaped resin tags, along with lateral ramifi-



**FIGURE 2.** Failure mode distribution. Adhesive failures were the most common form of failure in each group (SBU – Single Bond Universal, ABU – All Bond Universal, CUB – Clearfil Universal Bond, CSE – Clearfil SE Bond, CS3 – Clearfil S3 Bond Plus, ASB2 – Adper Single Bond-2)



**FIGURE 3.** Representative scanning electron microscopy (SEM) images of the resin-dentin interface bonded with Single Bond Universal adhesive in different etching modes. A) In self-etch mode, continuous hybrid layer (a1 – red arrow) with no resin tags. B-D) In etch-and-rinse mode with 5-s, 10-s, and 15-s etching. Funnel-shaped resin tags (black arrow) with lateral ramifications (white arrow). C – composite, A – adhesive, D – dentin

cations. In contrast, resin tags were not observed in any of the adhesives applied in self-etch mode, except two-step CSE bonding (Figures 3-7).

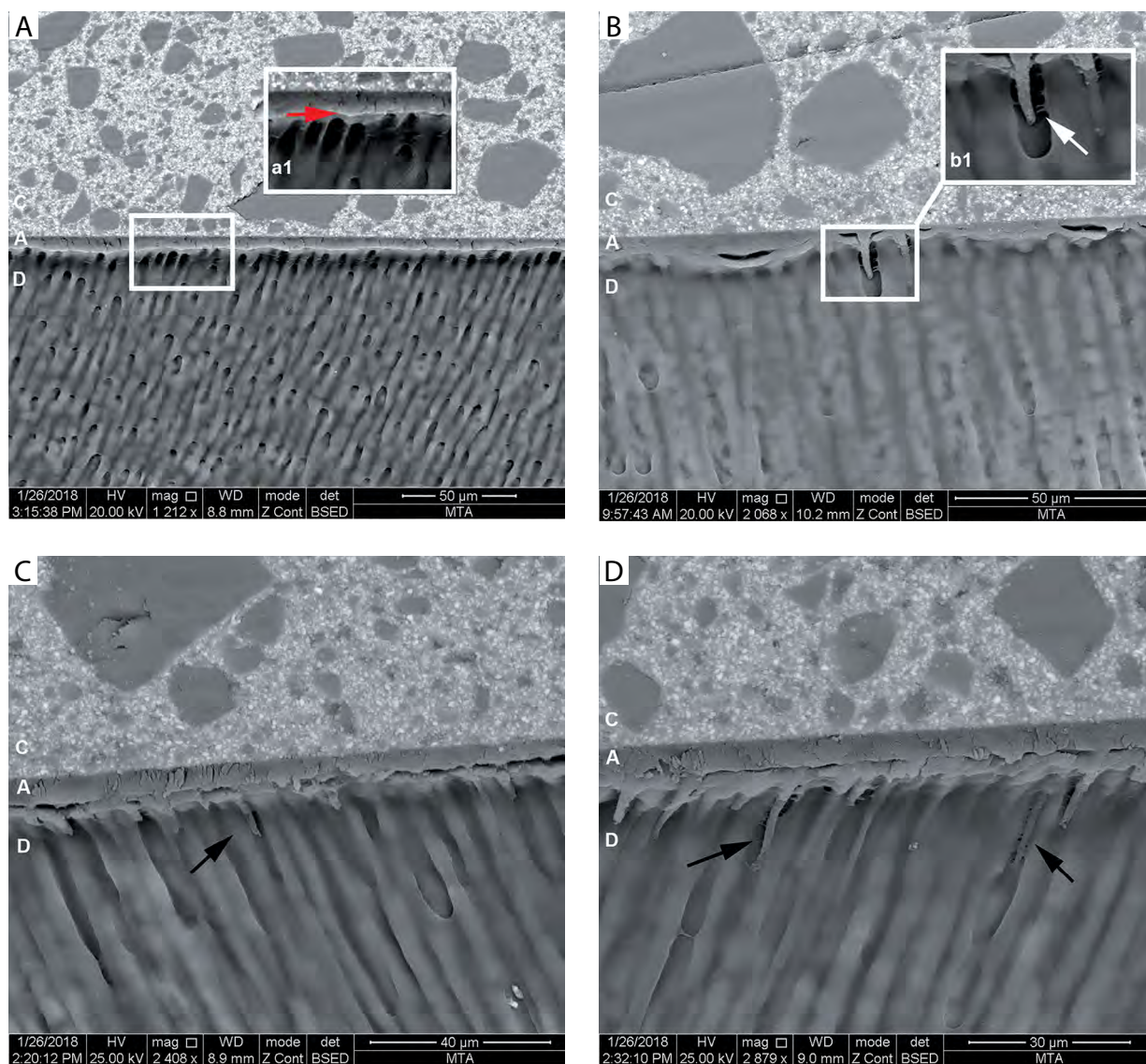
## DISCUSSION

The launch of the universal adhesives was a notable development in adhesive dentistry. These adhesive systems can be used for different substrates with different application techniques and were composed as an “all-in-one” approach from the “one-step self-etch” systems. Versatile usability allows clinicians to modify the technique according to different clinical situations.

The findings of the current research showed that all of the tested multi-mode universal adhesives exhibited

a homogeneous behaviour with different etching modes and time periods. Higher  $\mu$ SBS values were detected while multi-mode universal adhesives were applied in the self-etch mode and exhibited similar  $\mu$ SBS results with one-step system, two-step self-etch system, and etch-and-rinse system (in 10-s and 15-s etching).

It may be reasonable to assume that the universal and self-etch adhesives showed similar  $\mu$ SBS values in the current investigation because of the similarities in their chemical formulas. All the adhesives tested, except for Adper Single Bond2, contained a functional 10-methacryloyloxydecyl dihydrogen phosphate monomer (MDP). MDP can chemically bond to the tooth structure; for this reason, a large number of manufacturers produce MDP containing adhesive

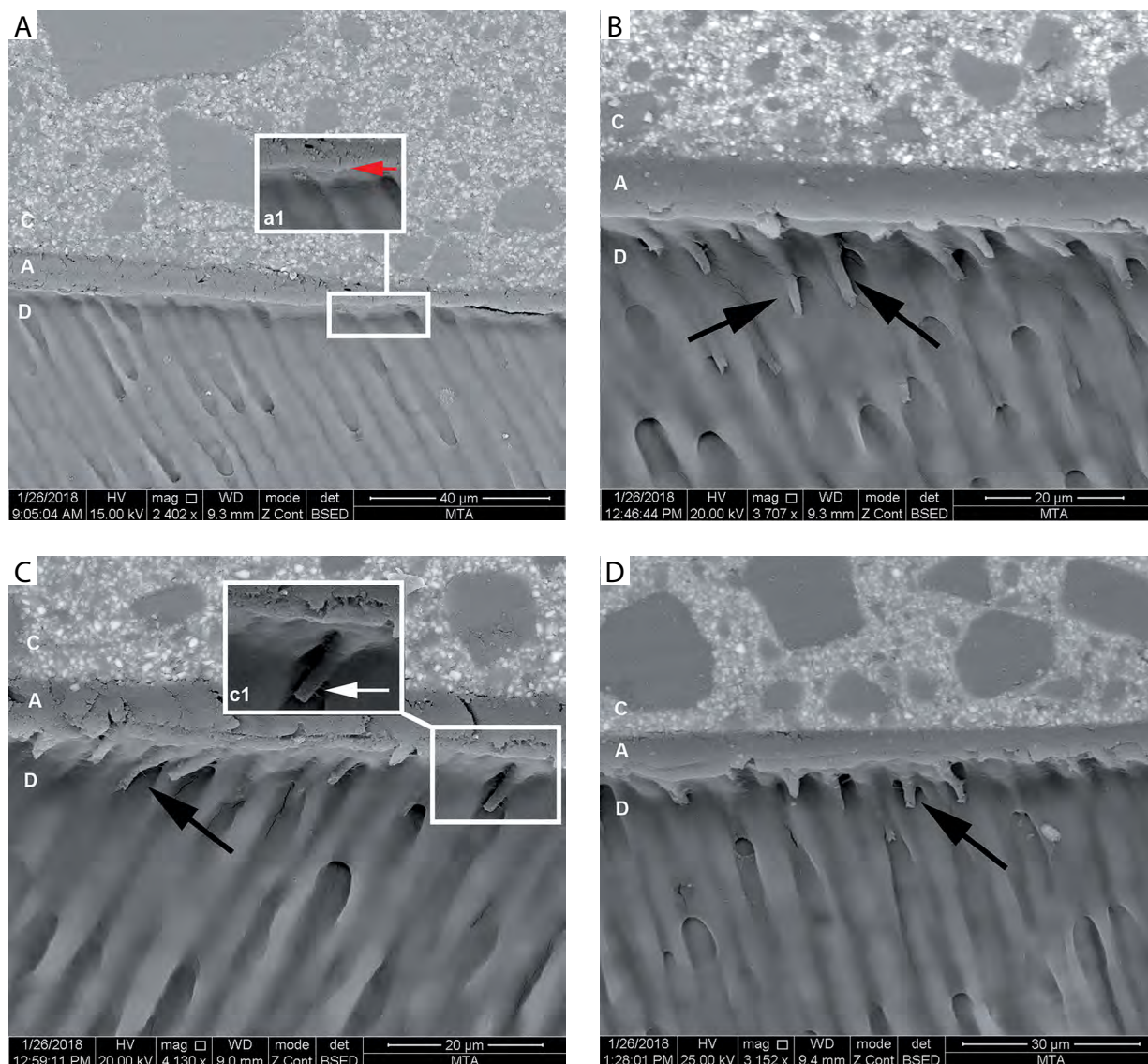


**FIGURE 4.** Representative scanning electron microscopy (SEM) images of the resin-dentin interface bonded with All Bond Universal adhesive in different etching modes. A) In self-etch mode, continuous hybrid layer (a1 – red arrow) with no resin tags. B-D) In etch-and-rinse mode with 5-s, 10-s, and 15-s etching. Cylindrical-shaped resin tags (black arrow) with lateral ramifications (b1 – white arrow). C – composite, A – adhesive, D – dentin

systems. Such adhesives can also self-assemble into nano-layers [39] and exhibit high hydrophobicity, which can support the hybrid layer formed from hydrolysis [38].

Etching dentin with phosphoric acid is a controversial issue. Some studies [17, 27] have shown that a reduced etching period (7 s) provided significantly higher dentin bond strength than a conventional etching period (15 s) for etch-and-rinse adhesives. In contrast, Sanabe *et al.* [24] found no significant differences between 7-15-s acid etching after 24-h water storage but noted that bond strength values decreased significantly for 15-s acid etching after 6 and 12 months of water storage. Studies with self-etch adhesives [33, 34] have also shown that the etching of dentine substrate could

decrease bond strength for self-etch systems. Phosphoric acid can decalcify dentine to a higher degree than a self-etch adhesive is planned to penetrate. Incomplete resin penetration to the demineralised dentine tissue by the adhesive resin was therefore considered the major factor responsible for the reduction in bond strength [3, 34]. In the present study, etching over 5 s led to a significant reduction in bond strength for all the universal adhesives tested. These results are critical because clinicians mostly restore cavities, which contain both dentine and tissues, in daily clinical practice. In this state, it can be proposed that when using universal adhesives, acid etching should be applied solely to the enamel. This outcome is in parallel with the results of previous studies, which have investigated



**FIGURE 5.** Representative scanning electron microscopy (SEM) images of the resin-dentin interface bonded with Clearfil Universal Bond in different etching modes. A) Self-etch mode, continuous and relatively thin hybrid layer (a1 – red arrow) and no resin tags. B-D) In etch-and-rinse mode with 5-s, 10-s, and 15-s etching. Upper portion of cylindrical-shaped resin tags (black arrow) with lateral ramifications (c1 – white arrow). C – composite, A – adhesive, D – dentin

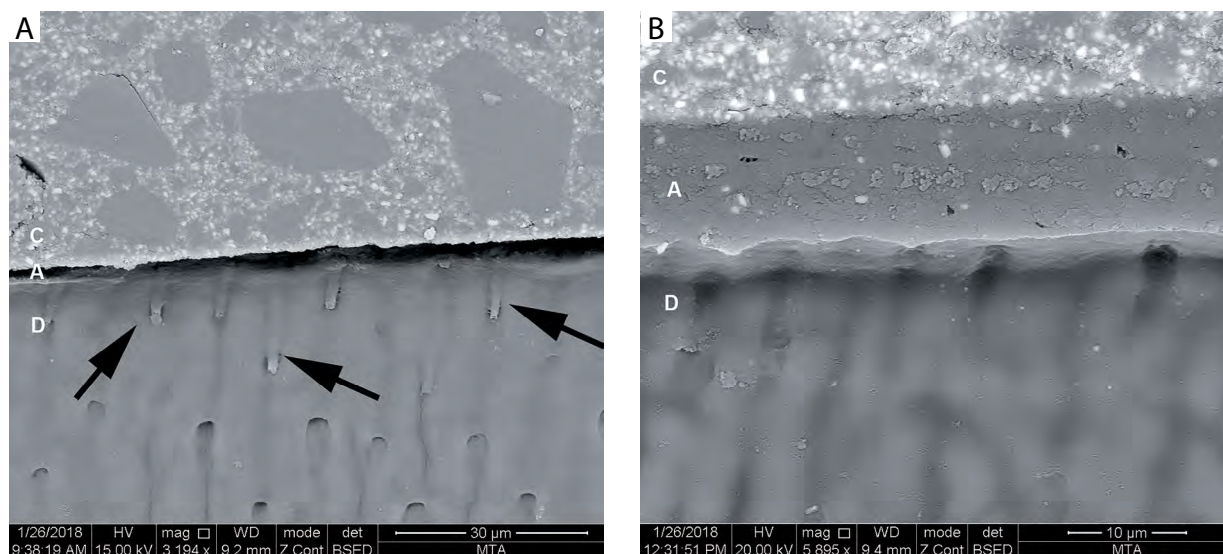
bond strength on enamel and dentin after pre-etching of universal adhesives [1, 21].

Takamizawa *et al.* [28] investigated the effects of different acid-etching times on the shear bond and fatigue strength of self-etch adhesives to dentin. Researchers found that one of the tested adhesives showed a reduction in dentin bond strength with an extended etching period, and that the group with 15 s of etching exhibited significantly lower shear bond strength and shear fatigue strength values than the group without pre-etching. The other self-etch adhesives did not show a significant difference.

Wagner *et al.* [37] compared the micro-tensile bond strength of universal adhesives in etch-and-rinse and self-etch modes. In contrast to the results of our study, these authors found no significant difference in terms

of the effects of etching modes. The major difference between these two studies was the artificial aging period. In the study by Wagner *et al.*, the samples were subjected to artificial aging for 5000 cycles. In the current study, specimens were subjected to 10,000 cycles, and this extended period might have caused the observed reduction in bond strength values. Gale and Darvell [7] concluded that when considering long-term bonding efficacy, 10,000 cycles are equivalent to approximately 1 year of *in vivo* function. The artificial aging effect generated by thermo-cycling can develop in either of the two forms: high temperature may stimulate hydrolysis of components at the interface and cause extraction of breakdown products or poorly polymerised resin oligomers [9], or repetitive contraction/expansion stresses are generated





**FIGURE 6.** Representative scanning electron microscopy (SEM) images of the resin-dentin interface bonded with self-etch adhesives. A) Clearfil SE Bond. Upper portion of cylindrical-shaped resin tags (black arrow) without lateral ramifications. B) Clearfil S3 Bond Plus. Approximately 10  $\mu\text{m}$  thick adhesive layer with no resin tags. C – composite, A – adhesive, D – dentin

at the tooth-biomaterial interface because of different thermal contraction/expansion coefficients of the restorative material and the tooth tissue [7].

Hanabusa *et al.* [8] tested immediate (24-h water storage)  $\mu\text{TBS}$  to dentin using a self-etch adhesive with different protocols and found no significant differences between the effects of self-etch and etch-and-rinse mode. Although there was no reduction in bond strength, their ultra-structural analysis showed clear signs of low-quality hybridisation when the universal adhesives were deployed in etch-and-rinse mode. Therefore, these authors reported that zones in which resin had infiltrated poorly may have influenced the mechanical stability of adhesive bonding, and that the adhesive interface may have been more vulnerable to biodegradation. Furthermore, although phosphoric acid etching did not reduce the immediate bonding strength in dentin, some researchers have recommended that universal adhesives should be used only in the self-etch mode on the dentin [8, 23]. The differences in results between the current study and previously published studies may be due to the fact that low-quality hybridisation areas in the adhesive interface may have been affected during the 10,000 cycles of artificial aging.

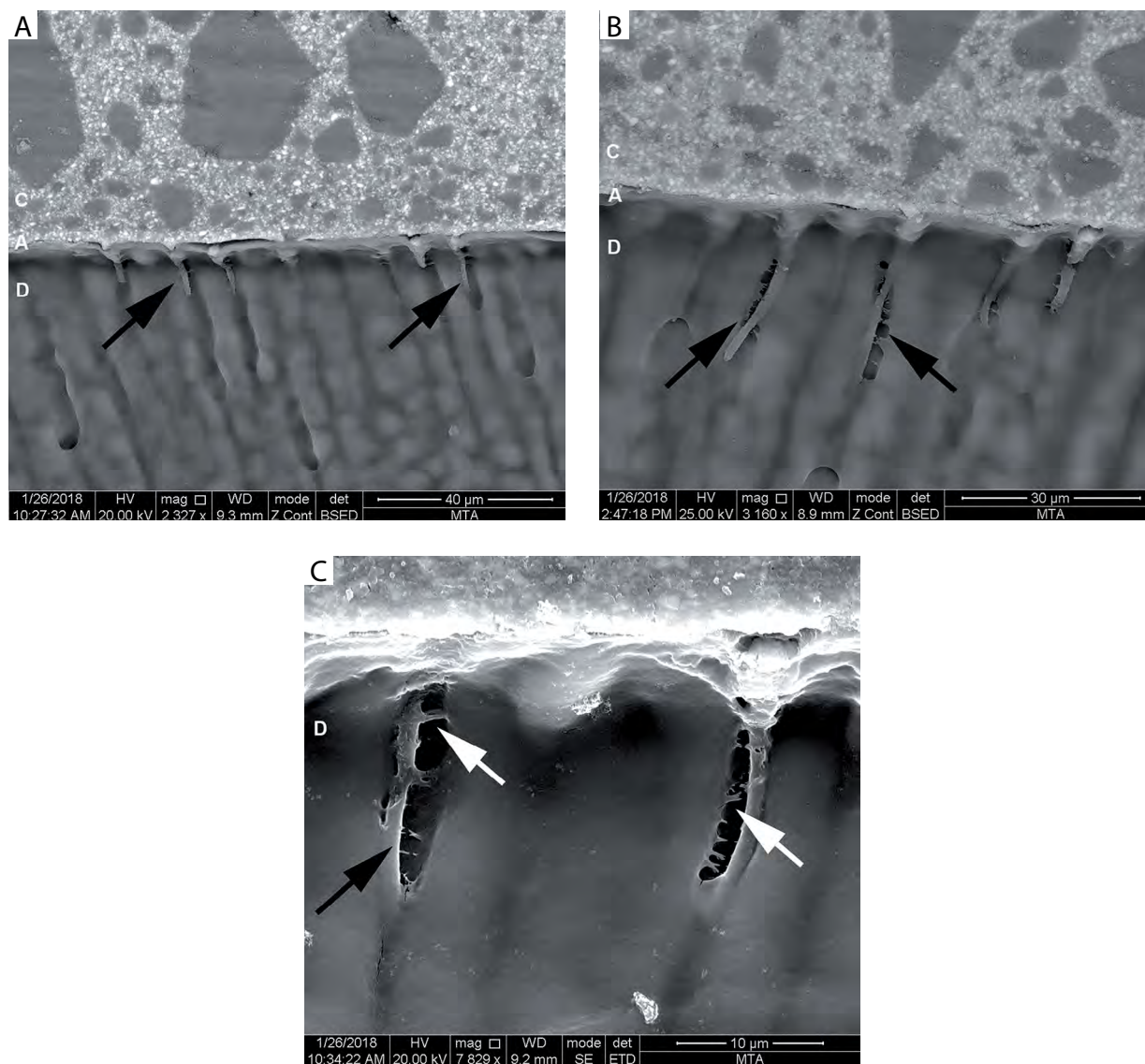
The SEM results obtained during the present study showed that the pH and etching mode affected the morphology of the resin-dentin interface. After the etching procedure (pH  $\sim 0.1$ ), a thick, funnel-shaped hybrid layer was produced. On the other hand, for self-etch mode, a thin, continuous hybrid layer was produced without resin tags except CSE (pH of SBU = 2.7, ABU > 3, CUB = 2.3, CSE = 2, CS3 = 2.3) [6]. This finding correlates with many previous investigations, which have indicated that the thickness of the hybrid layer depends

on the applied acidity, but the bond strength depends on the quality and not on the thickness [13].

For long-term success of composite resin restorations, strong and stable bonding is required. Long-term clinical trials are considered the gold standard for evaluating the bonding quality [14]. However, conducting long-term clinical studies is difficult because there are variables such as operator differences, substrate differences, and follow-up and ethical issues [26]. Therefore, *in vitro* studies may be more effective in providing useful information for the potential clinical success of dentin adhesives.

Even though several studies have reported excellent bonding performance of dental adhesives over the immediate- and short-term [2, 10], some researchers have suggested that results in the long- and short-term studies are not correlated because of the rapid degradation of the hybrid layer [3-5]. Thus, in the current study, all samples were subjected to thermal cycling for aging to evaluate the long-term bonding performance of multi-mode universal adhesives.

In order to estimate the clinical performance of adhesive materials, laboratory bond strength assessments are commonly performed. In order to prevent premature failure caused by microcracks and structural defects, the  $\mu\text{SBS}$  test, which did not require a trimming operation, was used in this study [15, 35]. Another advantage of  $\mu\text{SBS}$  is its homogenous stress distribution. As a result, more adhesive-type failure and more reliable data can be obtained than in macro tests [31]. Failure analysis showed that most of the failures were partially or completely in the adhesive interface, which increased the validity of the results.



**FIGURE 7.** Representative scanning electron microscopy (SEM) images of the resin-dentin interface bonded with Adper Single Bond-2 in different etching periods. A-C) With 5-s, 10-s, and 15-s etching, respectively. Funnel-shaped resin tags (black arrow) with lateral ramifications (white arrow). C – composite, A – adhesive, D – dentin

## CONCLUSIONS

Within the limitations of the present study, the use of an etchant before adhesive application significantly improved the pattern of dentine penetration; however, phosphoric acid treatment for > 5 s caused a significant reduction in  $\mu$ SBS results. In clinical conditions, it is very difficult to manage time for the acid-etching process. It is also difficult to apply the etchant to a specific tissue area. This study showed that even a 5-s extension of the etching time can change the  $\mu$ SBS. In the etch-and-rinse mode, clinicians should consider that the etching time period does not extend when using universal adhesives. For selective-etch mode, clinicians should also consider that the etchant does not overflow the dentin surfaces.

## CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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