



## Effect of Selective Dentin Etching and Ethanol Wet Bonding on Micro-Shear Bond Strength of Adhesive

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### Abstract

**Aim of the study:** This in vitro study aimed to evaluate the impact of selective dentin etching for 3 seconds and ethanol pre-treatment on the micro-shear bond strength ( $\mu$ SBS) of Scotchbond Plus Universal (SBU), a hydrophilic adhesive containing 2-hydroxyethyl methacrylate (HEMA).

**Material and method:** Forty-eight sound premolars had their occlusal enamel ground to create flattened midcoronal dentin. Teeth were divided into four groups of 12 samples. In Group A (control), SBU plus was applied without prior treatment. Group B involved selective dentin etching with 37% phosphoric acid for 3 seconds. Group C treated dentin surfaces with 100% ethanol for 60 seconds. Group D combined 3-second phosphoric acid etching with 60-second ethanol pre-treatment. The adhesive resin was applied then light cured. Teeth were restored with Filtek Z350 (3M ESPE, USA) composite rods (1mm diameter). After composite placement, samples were stored in distilled water at 37°C for 24 hours and thermocycled for 500 cycles.  $\mu$ SBS was measured using a universal testing machine. Data were analyzed using Shapiro-Wilks test, one-way ANOVA and Tukey statistical test.

**Results:** ANOVA revealed significant differences among the groups ( $P < 0.001$ ). Group D (selective dentin etching + ethanol pre-treatment) showed the highest  $\mu$ SBS at 57.16 MPa. The number of mixed failures was higher when dentin surfaces were treated with selective etching for 3 seconds and 100% ethanol.

**Conclusion:** In the conditions of the present study, the dentin treated using a 3-second phosphoric acid etchant and rewetted using 100% ethanol for a period of 60 seconds demonstrated a significant increment in the  $\mu$ SBS of the Scotchbond Universal Plus adhesive.

**Keywords:** Ethanol-wet bonding; Micro-Shear bond strength; Scotchbond Universal plus; Selective dentin etching; Universal adhesive

### Introduction

Dentin is layered with a covering of enamel, both of which have varying constituents and properties; hence, the bonding mechanisms differ (Goldberg M, et al., 2011). Dentin bonding is complicated because of the intricate histology. The universal bonding kit should be a single bottle system and works well in both total etch, self-etch, and selective systems (Voinot J, et al., 2024). In the total-etching procedure, the acid is harsh and etches the

surface using a strong acid; thereafter, the resin is introduced to the area and hardened. Self-etching materials have acidic Monomers that do not require the etching process (Van Meerbeek, et al., 2011). Dentin etching with phosphoric acid for 15 seconds removes mineral content, exposing collagen fibers for adhesive infiltration (Pashley et al., 2011). In self-etching, universal adhesives partially dissolve the smear layer and preserve hydroxyapatite for chemical bonding to the functional monomer (Van Meerbeek, 2011). Universal adhesives in



self-etch mode provide superior, long-term bonding compared to the etch-and-rinse method, with reduced etching time improving resin penetration (Chen et al., 2010). Selective etching for 3 sec helps preserve hydroxyapatite crystals, maintaining the hydroxyapatite surrounding collagen is crucial for preventing hydrolysis and early bond breakdown (Stape et al., 2018) and enhances bonding effectiveness thus improve bonding (Li et al., 2016).

Although the bonding strength of commonly used adhesives (fourth to eighth generation) satisfies clinical requirements, their durability and bonding interface quality are subpar. Effective monomer infiltration and bonding to collagen fibers are hindered by dentin's high hydrophilicity. In order to increase strength, hydrophilic monomers such as HEMA, PENTA, and MDP have been added; however, their combination with hydrophobic monomers decreases resin penetration, resulting in problems such as separation, water trees, lower polymerization rates, nano-leakage, and a water-absorbing interface. Research to improve the quality and durability of the bonding interface is ongoing as a result of plasticization and degradation (Liu et al., 2011; Hattar et al., 2015). Ethanol Wet Bonding (EWB) technology was created to enhance resin monomer infiltration and lessen bonding degradation because ethanol is an efficient solvent for hydrophobic monomers (Sadek et al., 2010). Although EWB improves bonding strength and durability, its dehydration step takes three to four minutes and requires a gradient of ethanol concentrations (50%, 70%, 80%, 95%, and 100%), which lowers clinical efficiency (Ekambaram et al., 2014). Consequently, more straightforward ethanol application techniques are being

investigated. Dentin adhesives are frequently used in clinical settings, but their bonding stability varies. It is still unknown how bonding efficacy is affected by a clinically acceptable ethanol dehydration time. According to some research, bonding is not improved by using 100% ethanol for 20 seconds or a minute (Pei et al., 2012). However, Sauro et al. (2009) contend that applying 100% ethanol for one or five minutes yields good bonding results and that ethanol/water exchange can be finished in thirty seconds. This is due to the fact that ethanol takes the place of water, maintaining the integrity of collagen fibers and improving resin penetration. The duration of ethanol application has a major impact on bonding results.

There are conflicting findings from recent research on simplified ethanol wet bonding (EWB) in total-etch adhesives. Li et al in 2012 and Yang et al in 2016 have found that 1-minute ethanol-wet bonding improves or maintains bonding strength compared to water-wet bonding, while other studies (Guimar~aes et al., 2012; Ayar et al., 2014; Yesilyurt et al., 2015) found no significant difference, suggesting the need for further research into optimal ethanol application times. In this experiment, the adhesive chosen utilize ethanol as the solvent. Research conducted by Sartori et al., 2015 demonstrated that ethanol-based bonding technology yields superior outcomes when combined with adhesives that use ethanol as the solvent. As a result, the adhesive selected for this study are eight-generation universal adhesives SBU Plus, which are commonly employed in clinical practice today.

This study aims to enhance adhesive-dentin bond strength and stability over time

by combining selective dentin etching (SDE) and ethanol pre-treatment with Scotchbond Plus Universal (SBU) adhesives containing 2-hydroxyethyl methacrylate (HEMA) using  $\mu$ SBS test. The goal is to utilize the synergistic effects of these techniques to achieve higher bond strength. To our knowledge, no previous studies have incorporated these methods together into any adhesive system. Therefore, this is the first study of its kind.

We hypothesize the null hypothesis [1] that SDE and EWB exhibited no effect on  $\mu$ SBS of SBU plus adhesive. [2] There is

**Materials and Methods:**

Study protocol was approved by the Research Ethics Committee, Mustansiriyah University, College of Dentistry (study number Muopr35).

**Teeth Collecting:** Forty-eight sound human upper premolars were extracted for orthodontic reasons, were obtained from patients aged 14 to 20 years. Each tooth was inspected under a 6X magnifying loupe to ensure they were intact and free from caries and cracks. Subsequently, they were cleaned using a rubber cup with pumice and rinsed thoroughly with distilled water applied via a triple syringe. These teeth were preserved in a 0.1% (weight/volume) thymol solution (CAROLINA, Burlington, USA) to inhibit fungal and bacterial contamination (Al-Obaidi and Jasim., 2023).

**Acrylic Block Construction:** A custom silicone cubic mold measuring 1.5 cm on each side was used to create acrylic blocks. The cemento-enamel junction was marked, and then an additional mark 2 mm below the cemento-enamel junction, indicating the insertion depth of the teeth in

no difference in SDE for 3 sec followed by application of 100% ethanol for 1min on micro-shear bond strength after application of scotchbond universal plus adhesive.

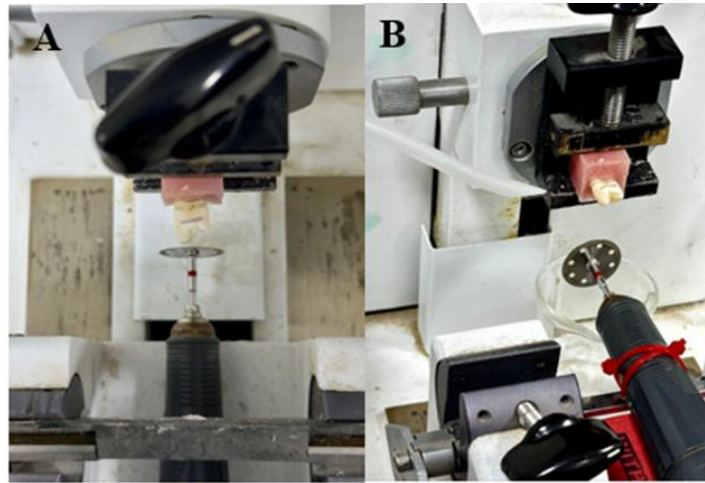
**Clinical significance:** this study tried to introduce a simplified technique to be used for improvement of the resin dentin interface for teeth. This is important in the conservative dentistry to reduce the amount of unnecessarily dentin removal and maintain normal tooth structure. In addition, it may help in the enhancement of the adhesive interface to improve its longevity.

the acrylic (Hameedi & Gholam., 2023). Using a dental surveyor, each tooth was aligned to ensure the long axis was parallel to the surveyor rod (Al-Obaidi and Jasim., 2023).

**Teeth Preparation:** The measurement of the distance from the pit to the mesial marginal ridge was taken using a periodontal probe to determine the ridge height. One millimeter added to this quantification and marked mesially on side of the tooth for future sectioning (Hameedi & Gholam., 2023). The handpiece was fixed to a specially modified microtom with metal screws, and a 20 ml syringe was stabilized for irrigation with distilled water during cutting using plastic locks. A metal gripe plate was used to control the level of cutting according to the marking on the tooth by adjusting the level of the metal and locking it using a screw lock, and the sample was descend by the

spinning wheel of the microtom on a diamond cutting disk 0.30mm thickness and

5000 revolution per minute (with continuous cooling by distilled water spray) (Figure 1).



**Figure 1.** Dentin sample preparation. A: occlusal view of modified microtom used for cutting sample. B: lateral view for the device showing the section procedure by rolling the spinning wheel so the sample descends on the diamond disk

To prepare a flat surface of dentin for study, the buccal and palatal cusps were cut 1 mm below the mesial pit using a diamond disk mounted on a straight handpiece under running water (Goncu et al., 2022). A 6X magnifying loupe was used to inspect the cut surface for any remaining enamel. The surface was then smoothed with 600-grit sandpaper attached to a flat plastic plane marked with a 10 cm length using white adhesive tape. The occlusal surface of each tooth was abraded against the moistened abrasive paper, repeating the grinding process four times per surface (Hameedi & Gholam, 2023; Al-Obaidi and Jasim., 2023). The abrasive paper was kept moistened to prevent dentin dryness and ensure wet bonding (Manihani et al., 2023).

**Sample Grouping:** The sample number was calculated utilizing G\*Power with the power of the study set at 80%, an

alpha error probability of 0.05 (two-sided), assuming an effect size of 0.7 (where small = 0.1, medium = 0.25, large = 0.4). Forty-eight samples were divided randomly into four main groups to test the  $\mu$ SBS of Scotchbond universal plus as follows:-

Group A (control group): (n=12) served as the control group with only adhesive application. In accordance with the manufacturer's instructions, the adhesive (scotchbond universal plus, 3M ESPE) was meticulously applied for duration of 20 seconds utilizing a disposable micro-brush. Following this, a 5-second air-drying period was implemented utilizing a triple syringe to facilitate solvent evaporation positioned 1 cm away. The light cure (woodpecker), with an intensity of 1200 mW/cm<sup>2</sup>, is positioned 1 mm away from the dentin with the help of digital caliper followed by a 40 second light-curing process (Figure 2).



**Figure 2.** A: Bonding application with micro brush B: Air drying C: measuring 1mm distance from dentin surface D: light cured for 20 sec.

Group B (Selective Dentin Etching Group): (n=12) Teeth in this group were etched with 37% phosphoric acid (Condac37, FGM, Brazil) for 3 seconds on the dentin, with the time being monitored using a digital stopwatch. Then, teeth were rinsed with water for 15 seconds, and excess water was

removed by air drying, leaving the surface visibly moist (Irmak et al., 2016). The adhesive agent was then applied following the manufacturer's instructions, similar to the procedure used in the control group A (Figure 3).

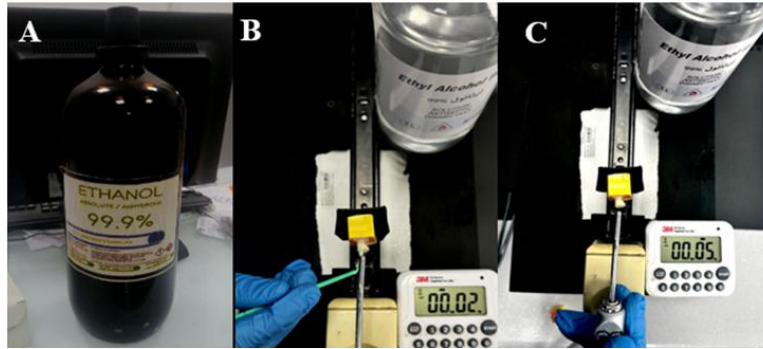


**Figure 3.** A: dentin etching for 3sec. B: rinsing dentin for 15 sec. C: air drying for dentin

Group C (ethanol wet bonding group): (n=12) All samples of this group were wetted with 100% ethanol for 1min only using rubbing action with micro-brush and

without any etching procedure (You X et al., 2022). Teeth were then blot dried for 5 sec, then adhesive bonding agent was applied, air

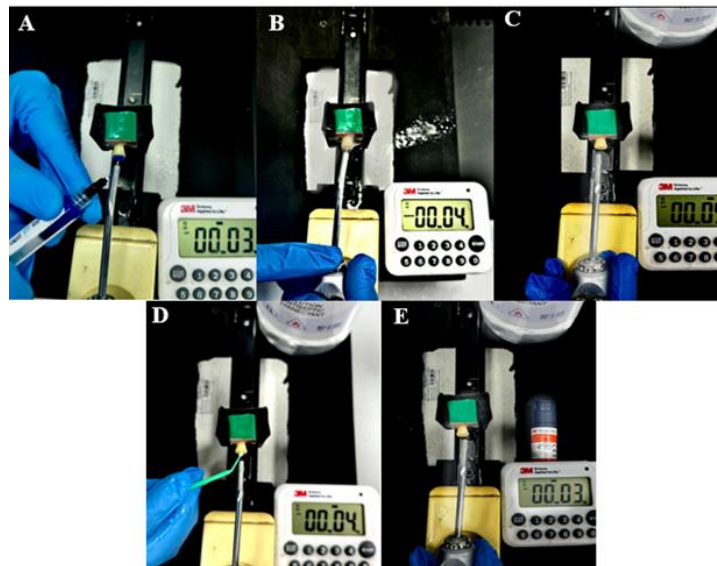
dried and cured as for control group (Figure 4).



**Figure 4.** A: Ethanol. B: Dentin surface was wetted with ethanol 100% for 60 sec using microbrush. C: dentin was blot dried for 5 sec.

Group D (mixed group): (n=12) Samples of this group were first etched for 3 sec with 37% phosphoric acid then rinsed with water for 15 sec, excess water were removed by air drying leaving the surface visibly moist

then secondly rewetted with 100% ethanol for 60 sec and then dried for 5 sec and the adhesive agent was be applied, air dried for 5 sec and cured for 20 sec.



**Figure 5.** A: dentin etching for 3sec. B: rinsing dentin for 15 sec. C: air drying of dentin surface. D: Dentin surface was wetted with ethanol 100% for 60 sec. E: dentin was blot dried for 5 sec and ready for bonding.

A specialized Teflon apparatus was designed to standardize the procedure for applying composite onto the dentin surface. The apparatus includes several key components: a cylindrical translucent Teflon structure to hold the acrylic block securely, a removable yellow Teflon cover made of two semi-circular segments (each 3mm thick), which attaches to the cylinder using a screw, and a Teflon bar with a central screw to fix the acrylic block against the Teflon cover, controlling the tooth's vertical position. Four screws around the cylinder stabilize the acrylic block and guide the dentin's horizontal position towards a 1mm diameter hole in the Teflon cover, allowing precise placement of the composite material.

**Application of composite:** A metal endodontic plugger tool was used to apply composite (Filtek Z350 XT) in a one increment through the designated hole in the Teflon mold cover. Excess composite was removed prior to light curing for 40 seconds. The light-curing device was placed directly in contact with the celluloid strip for 40 seconds (Al-Obaidi & Jasim, 2023).

**Storage and Aging Procedure:** The samples were placed in distilled water and incubated at 37°C for 24 hours to allow for post-polymerization (Goncu et al., 2022).

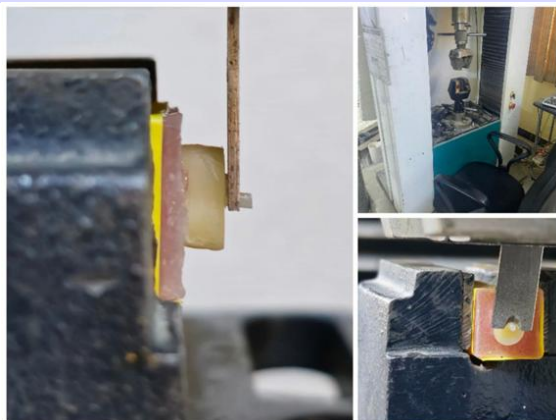
All samples were subjected to 500 cycles of thermocycling, with temperature fluctuations between 5°C and 55°C were set according to the ISO 11405 (ISO, 1994) (Khoroushi & Rafiei., 2013). Each cycle consisted of a dwell time of 30 seconds at each temperature extreme and a transfer time of 5 seconds.

**Micro-Shear Bond Strength Test and Failure Mode Analysis:** The  $\mu$ SBS test was conducted using a computer-controlled universal testing machine (Laryee, China) operating at a crosshead speed of 0.5 mm/min until failure occurred (Goncu et al., 2022). The specimens were secured in the testing device's jaw and positioned horizontally. A custom-made notched end chisel (with a 1.1 mm central notch radius) mounted on the testing machine, equipped with a 5-kN load cell, was used to apply a shear load to each specimen. The load was applied parallel to the bonded interface at a crosshead speed of 0.5 mm/min until failure was observed (Figure 6). The micro-shear bond strengths were calculated by dividing the maximum force at the point of fracture (measured in Newtons, N) by the bonded area (measured in square millimeters, mm<sup>2</sup>), yielding bond strength values expressed in megapascals (MPa).

$$\text{Shear Bond Strength} = (\text{newton}) / \text{Surface (mm}^2)$$

$$\text{Surface area} = \pi r^2$$

Where: r = radius  $\pi = 3.14$



**Figure 6.** Universal testing machine with sample in lower jaw of machine

After shear testing, the deboned samples were stained with methylene blue dye for five minutes to enhance contrast between the composite and tooth substrate. The dentin surface was then examined using a 6X magnifying dental loupe to identify the mode of failure. Three failure modes were categorized:

Type I: Adhesive failure

Type II: Cohesive failure within dentin or composite.

Type III: Mixed failure

**Field Emission Scanning Electron Microscopy (FESEM) preparations and measuring resin tag length and thickness of the hybrid layer:**

One composite-bonded sample from each group was sectioned horizontally 1.5 mm below the occlusal surface to obtain a 1.5 mm dentin disk bonded with composite. The dentin disk was then sectioned axially from both sides until 1 mm of bonded composite remained. Shear force was applied using a NO.15 blade to obtain a clear cut of the composite. After storing the

specimens in distilled water for 24 hours to prevent dehydration, they were dehydrated in a graded ethanol series. Following cleaning, the samples were sputtered with gold nanoparticles. Finally, the adhesive layer was analyzed using an INSPECT F50 SEM at 30 kV. The average length of resin tags in each specific area (the field of view at 2000 magnification) has been measured employing digital image analysis software (Image J, USA). All visible tags in each sample with 2000 magnification were measured by drawing measuring lines that followed the direction of the resin tags, starting from the base of the hybrid layer and extending to the tips of the tags. Ultimately, the mean thickness of the hybrid layer was determined by calculating the average from the fields of view for each sample.

**Statistical Analysis:** Data were analyzed using SPSS version 26. To ensure normality and homogeneity of variance the Shapiro-Wilks test was used. One-way ANOVA is used to determine whether there are

statistically significant differences between the means of groups while Tukey test were used to determine which specific group means are significantly different from each other. The significance level was set at  $p < 0.05$ . In the aforementioned tests, a p-value equal to or greater than 0.05 was considered statistically non-significant (NS), and a p-value less than 0.05 was considered significant (S).

**Results:**

The  $\mu$ SBS data were analyzed to ensure normality and homogeneity of variance. The results indicated a normal distribution, and the data were homogeneous, the descriptive statistics, including the mean, standard deviation, standard error and minimum and maximum values of the micro shear bond strength in MPa for all groups, are shown in (Table 1 & Table 2).

**Table 1.** Descriptive statistics for micro shear bond strength (MPa) for all groups

Descriptive								
Groups	No.	Mean	SD. Deviation	SD. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
Control	12	16.5000	2.06706	0.59671	15.1867	17.8133	12.00	19.00
SDE	12	47.5000	3.91965	1.13150	45.0096	49.9904	40.00	54.00
EWB	12	35.2500	4.91981	1.42023	32.1241	38.3759	28.00	44.00
Mix	12	57.1667	7.17107	2.07011	52.6104	61.7229	48.00	73.00
Total	48	39.1042	16.05740	2.31769	34.4416	43.7667	12.00	73.00

No= number of teeth used, mean= mean of the results, SD= standard deviation, Min= minimum result, Max= maximum result.

**Table 2.** Tests of Normality

Groups	Statistic	Sig.
G1 (Control)	0.929	0.372 (NS)*
G2 (SDE)	0.987	0.999 (NS)
G3 (EWB)	0.958	0.750 (NS)
G4 (Mix)	0.937	0.456 (NS)

(NS)\*: Non-Significant difference

A One-way ANOVA test was performed to compare the significance between the different groups, using a significance level of 0.05. The results

indicated a statistically significant difference among the groups ( $p < 0.01$ ), as presented in (Table 3).

**Table 3.** One-Way ANOVA test.

ANOVA					
Groups	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11070.563	3	3690.188	154.944	0.000 (HS)
Within Groups	1047.917	44	23.816		
Total	12118.479	47			

\*HS: Highly Significant difference

Further comparisons among groups were done using the Tukey HSD test at a level of significance of 0.05 to see where the

significant difference occurred, as shown in (Table 4).

**Table 4.** Post-hoc Tukey's HSD

Multiple Comparisons						
Dependent Variable: Groups						
Tukey HSD						
(I) Adhesive Technique	(J) Adhesive Technique	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	SDE	-31.00000*	1.99233	0.000(S)	-36.3195-	-25.6805-
	EWB	-18.75000*	1.99233	0.000(S)	-24.0695-	-13.4305-
	Mix	-40.66667*	1.99233	0.000(S)	-45.9862-	-35.3471-
SDE	Control	31.00000*	1.99233	0.000(S)	25.6805	36.3195
	EWB	12.25000*	1.99233	0.000(S)	6.9305	17.5695
	Mix	-9.66667*	1.99233	0.000(S)	-14.9862-	-4.3471-
EWB	Control	18.75000*	1.99233	0.000(S)	13.4305	24.0695
	SDE	-12.25000*	1.99233	0.000(S)	-17.5695-	-6.9305-
	Mix	-21.91667*	1.99233	0.000(S)	-27.2362-	-16.5971-

Mix	Control	40.66667*	1.99233	0.000(S)	35.3471	45.9862
	SDE	9.66667*	1.99233	0.000(S)	4.3471	14.9862
	EWB	21.91667*	1.99233	0.000(S)	16.5971	27.2362

\*. The mean difference is significant at the 0.05 level.

S\*: Significant difference

A statistically highly significant difference was observed between the control group and the other groups, as well as among the experimental groups, when using Scotchbond Universal Plus adhesive.

**Mode of failure**

After examining the samples stained with Methylene-blue dye for five minutes and observing them under dental loupe with 6x magnification following micro-shear testing, it was found that the failures fell into three types:

1. Adhesive failure that occurred between composite and dentin.

2. Cohesive failure that involved failure within composite.
3. Mixed failure that involved failure within composite and adhesive layer.

In this in vitro study the mode of bond failure was variant among the different study groups. The most common failure pattern was mixed with less incidence of adhesive failure and a few samples undergo cohesive failure within composite. However, no sample showed any failure within dentin as shown in Table (5).

**Table 5:** Mode of failure for all groups

Group	Mode of failure			
	Adhesive (%)	Cohesive (%)	Mix (%)	Total
<b>G1 (Control)</b>	8 (66.6%)	0	4 (33.3%)	12
<b>G2 (SDE)</b>	3 (25%)	2 (16.6%)	7 (58.3%)	12
<b>G3 (EWB)</b>	4 (33.3%)	0	8 (66.6%)	12
<b>G4 (Mix)</b>	2 (16.6%)	3 (25%)	7 (58.3%)	12
<b>Total</b>	17 (35.4%)	5 (10.4%)	26 (54.1%)	48

\*G: group, SDE: selective dentin etch, EWB: ethanol bonding technique,

\*Mix group:( SDE&EWB), Mix failure: (adhesive & cohesive failure).

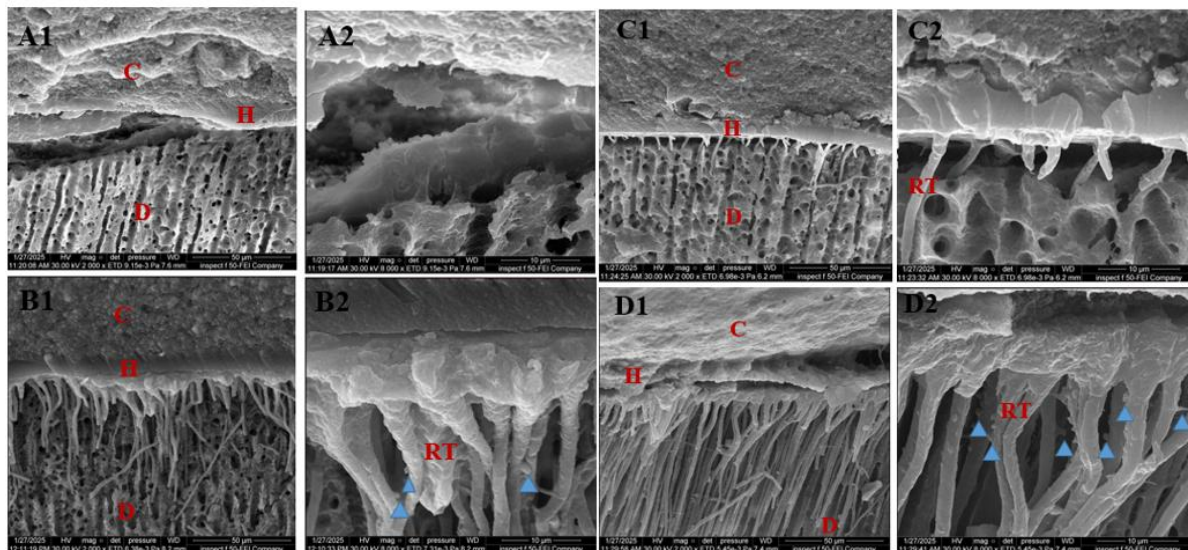
**FESEM observations:**

The FESEM images showing the dentin-composite interface for the control and other

groups are presented in (Figures 7). In the control group (A1 & A2 images), a well-defined hybrid layer with a mean thickness

of 8.133  $\mu\text{m}$  is observed, but no resin tags are present. This is attributed to the self-etch mode used in the in vitro study, which did not etch the dentin but only fixed and infiltrated the smear layer, as shown in Figure 3-3 (B1 & B2). For the SDE group (B1 & B2 images), a well-defined hybrid layer without voids or gaps is present, with a mean thickness of 15.595  $\mu\text{m}$ . The resin tags appear as long, thick cylindrical shapes with short lateral branches, and the mean resin tag penetration into the dentin is 47.159  $\mu\text{m}$ , with a mean diameter of 3.209  $\mu\text{m}$ . In the ethanol group, the resin tags are short and

few in number, with a penetration depth of 7.374  $\mu\text{m}$  and a diameter of 1.968  $\mu\text{m}$ . The hybrid layer is well-defined, with a mean thickness of 10.413  $\mu\text{m}$ , as shown in Figure 3-4 (C1 & C2). The mixed group (Figure 3-4 D1 & D2) shows numerous long resin tags with multiple short lateral branches and a well-defined hybrid layer with a mean thickness of 17.162  $\mu\text{m}$ . The mean diameter of the resin tags is 3.379  $\mu\text{m}$ , and the mean depth of penetration into dentinal tubules is 102.783  $\mu\text{m}$ . Additionally, a few resin tags appear to be cut, which may indicate debonding during the cutting procedure.



**Figure 7.** FESEM images for control and SDE groups showing dentin composite interface (A1-A2, B1-B2). FESEM images for EWB and Mix groups showing dentin composite interface (C1-C2, D1-D2). The following abbreviations are used; C: composite resin, RT: resin tags, blue arrowheads for lateral tags. H: hybrid layer between two blue arrowheads. D: dentin Magnification power: 1=2000X, 2=8000X. Voltage: 30000 KV

**Discussion:**

In restorative dentistry, the bonding between dental composites and tooth

structure, particularly at the dentin-bond interface, is crucial for the durability of restorations (Pradeep et al., 2021). Acid etching exposes dentin collagen fibrils by

removing hydroxyapatite minerals, allowing resin monomers to form a hybrid layer by penetrating the collagen matrix and dentinal tubules (Van Meerbeek et al., 2020). The quality of this hybrid layer is vital for long-term bond stability, but the complexity of dentin, including fluid movement in tubules and intrapulpal pressure, complicates the bonding process (Pradeep et al., 2021).

Now, within modern dentistry, there's this trend towards minimal invasive dentistry, which aims to preserve as much of the tooth structure as possible, while at the same time attempting to increase the longevity of composite restorations. I think it's a big deal regarding research in adhesive dentistry that this whole issue of the strength of the bond degrading over time is involved here. Just things such as novel etching methods or ethanol prebonding can have an effect on this strength of the bond (Hardan et al., 2021).

In this study, thermocycling is used to determine how resilient the restorations are. It essentially simulates every one of those temperature changes due to eating hot food or drinking cold beverages, or even breathing. That's according to Gönülo et al. (2015). They cycled the temperatures 500 times, alternating between 5°C and 55°C, with a duration of 30 seconds at each temperature. This is in accordance with the International Standards Organization TR 11450 (Salah & Sleibi., 2023).

According to Pejcak et al. (2018): "Water, respectively, distilled water or artificial saliva, was sealed. Instead, they opted to do a micro-shear instead of a micro-tensile. That appears to avoid damaging the bond at the point of processing, allowing good results without needing to cut

anything. Regarding the loading portion, this research observed a notched end. It's meant to be more accurate than past methods, such as wire-loop, where there's alignment issues or where something will bend or deform (Sabri & Mahdee., 2024; Jessop., 2013). That one is a bit more complex, but they clearly made a valid selection there. Once again, the topic of interest revolves around bond strength. It appears they wanted to improve adhesive dentin bond strength while adding stability using a new type of selective dentin etching technique, along with ethanol bonding, along with universal adhesives. From what's known, no research has combined these concepts before to follow this aim, making this research original by itself. Self-etching technique is selected for this research to serve as its control since this closely represents real-world settings, where self-etched bonding has provided better results over time than an etch-and-rinse procedure (Van Meerbeek et al., 2022).

The control group reveals the lowest bond strength value with a mean  $\mu$ SBS of 16.50 MPa, which will be used for comparing the effect of selective dentin etching and ethanol concentration on universal adhesive bond strength after thermocycling. The results obtained in this study revealed a statistical difference in means for  $\mu$ SBS among control and other groups. The decreased bond strength in control groups after thermocycling can be attributed to several factors. First, it might be associated with matrix metalloproteinases in dentin, including collagenase (MMP-1, MMP-8), which degrade collagen in the presence of water. In addition, gelatinases (MMP-2, MMP-9) can degrade intact collagen molecules on their specific

cleavage sites, consequently degrading free collagen molecules slowly at the foot of the hybrid layer (Breschi et al., 2018). Second, it may result from water left trapped at the foot of the hybrid layer. These conditions often occur due to improper displacement of water from expanded collagen fibers by adhesives, consequently degrading bare collagen fibers (De Munck et al., 2010). Thirdly, the plasticizer effect experienced by the adhesive concerning water absorption by the hydrophilic resin constituents in the hybrid layer may also result in the hydrolytic degradation of the unreduced adhesive resin, thereby weakening the adhesive strength (Walter et al., 2013). Lastly, the degradation of the ester component in 10-MDP, as well as the other methacrylate resin monomers, may account for a marked decrease in adhesive strength (Kokubo et al., 2024).

Compared with the control group, the SDE group (B) showed significant mean values of  $\mu$ SBS with 47.50 MPa. This increase can be explained by the increased capability to eliminate smear layers and optimize the opening of the tubules, thus favoring the formation of resin tags and ensuring micromechanical retention for the adhesive resin. The establishment of a high bonding strength is known to be predominantly influenced by the micromechanical retention on the demineralized tooth surface (Kharouf et al., 2019). On the other hand, excessive acidity can increase the demineralization on the peritubular and intertubular dentin. Thus, this could increase the formation of bonding weakness instead of establishing an integral resin monomer penetration (Kharouf et al., 2019). As Van Meerbeek et al. stated, the conservation of the hydroxyapatite layer

surrounding the collagen is essentially important for maintaining the integrity against hydrolysis and early bonding failure. To maintain the existing chemical bonding step and retain calcium ions on the bonding surface, the functional monomer "10-methacryloxydecyl dihydrogen phosphate" or "10-MDP" can form a regular two-dimensional layer on the surface of the apatite and establish "stable calcium phosphate" ions on the bonding interface (Yoshihara et al., 2010; Zhang et al., 2013). Given that there is a need for three seconds of etching of the dentin before placing a universal adhesive, it underlines that SDE (Stape et al., 2018) is also very crucial. It also increases the bond strength by 30% because of enhanced bonding of micromechanisms and chemistry due to three seconds of etching in preference to the self-etching approach and because of disruptions in loosely bound globules of smear layer etched with H<sub>3</sub>PO<sub>4</sub>. On the other hand, excessive time of etching may trigger more MMP.

Similar results were reported by (Stape et al., 2018; Kharouf et al., 2019), and Hardan et al. (2021, 2022). Stape et al. found significant differences in micro tensile bond strength tests of composite to dentin, noting that 3-second phosphoric acid application improved both immediate and long-term resin–dentin bonding without overexposing demineralized collagen. In contrast, in 2019, Kharouf et al. recorded a decline in  $\mu$ TBS after SDE for 3 seconds, attributing it to the rubbing action that accompanies the application of phosphoric acid and may crush collagen fibers and hydroxyapatite crystals. Hardan et al. also did not find an advantage of this approach in 2022, when both HEMA and HEMA-free

universal adhesives were tested, with bond strength only reduced for the HEMA-free adhesives. This was explained by the presence of HEMA in preventing phase separation between hydrophobic components and water diffused from dentinal tubules within the bond interface.

Dentin etching for 3 seconds is advantageous for Scotchbond Universal Plus, since this adhesive is a polyalkenoic acid copolymer that chemically bonds to calcium in HAp, yielding higher stability of the bond between dentin and the adhesive. More than 50% of the carboxyl groups in the polyalkenoic acid copolymer bond to HAp, substituting phosphate ions on the dental substrate and forming ionic bonds with calcium. This enhances the bonding of Scotchbond Universal Plus to dentin (Hardan et al., 2022). These findings align with the results of this *in vitro* study, leading to the rejection of the null hypothesis that SDE has no effect on bond strength.

Aside from improving resin-dentin bond strength, selective dentin etching for 3 seconds had no negative impact on hybrid layer integrity after challenging the bonded interface with thermocycling or long-term aging, as observed through FESEM images. Nevertheless, there remains some debate regarding the etching time. Etching dentin for 3 seconds in the case of a complex cavity is difficult to apply clinically, as it is easy to exceed this time. Therefore, future studies should test etching agents with lower demineralization potential, which would allow for longer etching periods without causing collagen overexposure.

For EWB group, results show that group C which is EWB group has a statistically significant differences compared

with control group, and exhibited a mean  $\mu$ SBS value of 35.25 MPa. Compared with control group, EWB significantly increased the bonding strength of universal adhesive.

Adhesive resin degradation and collagen fibril proteolysis, due to incomplete resin monomer penetration, are primary causes of limited bond durability. Ethanol replaces water in demineralized dentin, reducing hydrophilicity and enhancing the penetration of hydrophobic resin monomers, leading to stronger bonding and improved durability (Yi et al., 2019; Porto et al., 2018). Since ethanol has better solvent properties than water, it lowers the collagen fibrils' ability to form hydrogen bonds, which causes the demineralized fibrils to chemically dehydrate. By decreasing the amount of water extracted from the substrate, this creates a comparatively hydrophobic matrix that reduces hydrolysis at the interface (Comba et al., 2024). Hydrophilic resin monomers, such as HEMA, have better penetration ability in the area where ethanol exists around the collagen fibers in ethanol-saturated dentin based on the solubility parameter concept proposed by Hoy. Since the inhibition of collagen helps to package collagen and passivate the host MMPs (Venigalla et al., 2016), this approach makes dentin hydrophobic. In addition, ethanol saturation leads to the contraction of the diameter of collagen fibers of the dentin, making the spaces between fibers wider and hydrophobic. As such, the hydrophobic resin helps to improve the bonding ability (Ahn et al., 2015; Aggarwal et al., 2016). The ethanol-wet bonding (EWB) approach helps to improve the bonding ability of the hydrophilic total and self-etching adhesive

systems commonly used in dentistry (Wang et al., 2020).

The results of this study are consistent with the findings of (Hardan et al., 2021; You et al., 2022; Wang et al., 2020; Comba et al., 2024), but contrast with the study by (Ozsoy et al., 2015), which reported a decrease in bond strength, especially when testing caries-affected dentin. (Wang et al., 2020) also observed that a wetting time of 1 minute or less was not optimal, as it did not improve the 24-hour bonding strength and durability of Single Bond (SB) and Gluma Comfort Bond (GB). This brief wetting time may not allow sufficient dehydration, which could limit the curing effect of adhesives, while the positive impact of dehydration may not be enough to overcome the negative effects on curing. Moreover, ethanol has a rapid evaporation rate, which could lower wettability after application. But in the study of (Li et al., 2012) ethanol-wetted dentin was revealed to have a rapid loss of wettability in the first 20 seconds, making the application of the adhesive critical. For successful adhesive penetration, the process must be performed rapidly to ensure ethanol saturation. The loss of ethanol due to evaporation prior to the application of the adhesive could lower wettability, thus affecting the penetration of the adhesive (Wang et al., 2020). Another inhibiting factor of MMPs is presented by ethanol. It occupies the zinc active site of the catalytic domain of matrix metalloproteinases (MMPs) and the hydroxyl oxygen of alcohols; thus, it inhibits MMPs and increases the stability of the resin-dentin bond (Basting et al., 2023).

Ethanol acts as the solvent for both hydrophilic and hydrophobic resins in the adhesive system. It aids in the penetration of

resins as well as forming the protective layer on the exposed collagen fibrils. This can shield the collagen from the enzyme MMP, thus enhancing the strength of the adhesive joint (Abdulrasool et al., 2019). The results of this in vitro study align with this explanation, showing an increase in  $\mu$ SBS for the ethanol group compared to the control group.

For Mix group (SDE & EWB), the combined application group of SDE and EWB shows the highest bond strength mean value of  $\mu$ SBS which is about 57.16 MPa. So the mix group has resulted in highly significant increase in bonding strength compared with all other groups. Based on the findings of the current in vitro study, the initial null hypothesis was rejected, as dentin bond strength was considerably affected by selective dentin etching for 3 seconds and by ethanol pre-treatment.

The improvement in  $\mu$ SBS observed in the mix group is likely due to the synergistic effect between SDE and ethanol, which together create a more durable bond with a highly significant difference. This can be explained by the selective dentin etching for 3 seconds, as mentioned earlier, which leaves a partially demineralized substrate after rinsing and drying (Stape et al., 2018; Hardan et al., 2021). This approach helps preserve hydroxyapatite around the collagen fibrils, and allows a functional monomer such as 10-MDP, found in universal adhesives, to form stable calcium-phosphate complexes that self-assemble into a regular layered structure at the apatite surface. Furthermore, the etching process improves bonding by creating micromechanical retention since it eliminates the smear layer and penetrates around 1  $\mu$ m into the dentin, further enhancing the adhesive procedure.

Therefore, SDE provides both micromechanical and chemical adhesion.

Moreover, the impact of SDE will be enhanced by the application of 100% ethanol for 1 minute, which will substitute the water within the dentin, diminish the diameter of collagen fibrils, and augment the interfibrillar space, facilitating the infiltration of monomers into the collagen fibrils (Hardan et al., 2021; Abdulrasool & Al-Shamma, 2019). This will effectively seal the dentinal matrix, decrease the permeability of the dentin-resin interface and so enhancing bond endurance to the dentin substrate. Ethanol's inhibitory impact on MMP contributes to the prolonged durability of the adhesive interface, hence establishing a solid bond, as previously noted (Comba et al., 2024; Basting et al., 2023). Both procedures led to an enhancement of the  $\mu$ SBS of universal adhesive in dentin.

In this in vitro study, the observed failure modes were adhesive, mixed, and cohesive in composite, with no cohesive failure in dentin. Pure failure, with intact hybrid layers and dentin, yielded the highest shear bond strength, suggesting that the bond to the composite was stronger than that to the tooth. The present study was performed to show that in order for those hybrid layers and tags to form properly and hold things mechanically, as mentioned by Spencer and others back in 2020, adhesive resin monomers have to sink deep down into the dentin. FESEM images were taken for only one sample per group, as preparation is such a hassle, yet these still agreed with our results of micro-shear bond strength.

In the control group, the adhesive could barely make it in, I guess because of

those hydrated proteoglycans blocking the way, kind of like what Fadhil and Al-Shamma pointed out in 2020. The hybrid layer was clear enough, around 8.133 micrometers thick, no resin tags, though. That probably comes from the self-etch mode not etching the dentin fully, or at least not as much as it sometimes should. The Mix group ended up with the best results, combining SDE and EWB. Their hybrid layer hit 17.162 micrometers, tags going super deep at 102.783 micrometers with a diameter of 3.379 micrometers. Plus, there were all these short lateral branches on the tags, from resin squeezing into tiny spots in the dentin tubules. Hao et al. (2022) and Santos et al. (2023) said those branches make the mechanical bond tougher and more durable.

For Selective Dentin Etching, it was better than control, hybrid layer thickness greater at 15.595 micrometers, resin tags well-formed and 47.159 micrometers deep, around 3.209 micrometers wide. Phosphoric acid increased demineralization, allowing resin to better invade the area. This definitely makes a difference here. Ethanol Wet Bonding, EWB, was intermediate. Hybrid layer thickness was 10.413 micrometers, tags were shorter at 7.374 micrometers deep and 1.968 micrometers in diameter. The ethanol was certainly beneficial to the resin to better adhere and spread in the area. This correlated well with the bond strength results. I may well be overcomplicating this result. Control was tagless, EWB was present but not terribly impressive, SDE was quite good even by itself. Blending both achieved even greater depths, and the FESEM was well-able to detect those branches within the Mix treatment. This kind of observation of the

observational branches there indicates the resin penetrated highly into the dentin layer, indicating better bonds overall within dental applications. This may indicate it would last longer, I suppose, though the Mix method incorporates both aspects of the others to beneficial effect. This experiment really highlights the need for the adhesive resin monomers to penetrate the dentin and create a hybrid layer and these resin tags that provide the mechanism of binding everything together like what Spencer et al. mentioned in their 2020 experiment. The FESEM images were taken only from one sample in each group since the process of making one is a bit complicated, and it verifies the results anyway coming from the micro-shear bond strength test.

The hybrid layer in the control group was relatively clear at 8.133 micrometers, without any resin tags at all. I believe it has to do with the self-etching mode, which does not etch the dentin enough at times like it should. Moving on now to the Selective Dentin Etching group, or SDE for short, there was improvement in the hybrid layer thickness at 15.595 micrometers, and then resin tags formed nicely at 47.159 in depth and 3.209 in width. This works because the phosphoric acid assists in the dentin demineralization process, making it easier for the resin infiltration. This appears to be a vast improvement in the product's design and functionality. The Ethanol Wet Bonding group, or EWB, was in the middle with the hybrid layer at 10.413 micrometers in depth and the resin tags at a mere 7.374 in depth and 1.968 in width. However, the method of ethanol became more effective in spreading and adhering well, which equated well with the bond strength results observed in the experiment.

In the Mix group, which combined SDE and EWB, there was the best result overall. The hybrid layer had the highest thickness at 17.162 micrometers, and the resin tags penetrated much deeper at 102.783 micrometers, having a diameter of 3.379 micrometers too. Then there were plenty of these small lateral extensions of the tag too, which occur when resin enters the smaller areas within the dentin tubules. Such extensions will improve the longevity and strength of the mechanical bond, explained Hao et al. in the research paper from 2022 and Santos et al. in the paper from 2023. Maybe, just maybe, the Mix method works because it combined the best of both worlds, which means the best results from each method combined can get an even better result by combining the two individual results. And just by looking at the results, the control had no tags, the EWB had some but not much, the SDE was good, but then combined, they got an even better penetration! The FESEM image revealed the presence of these extensions within the Mix group. Overall, seeing those lateral branches means the resin really got deep into the dentin, which should lead to better, more reliable bonds in dental materials. This could help make bonding last longer, I suppose. The study has several limitations: it relies on micro-shear bond strength and surface topography, which may not fully represent biological behavior. As an *in vitro* study, its findings may not apply to clinical situations. The short 3-second etching time and lack of dentin sensitivity assessment are concerns, and standardizing the smear layer with 600 SiC paper may not reflect clinical conditions. The 24-hour water storage period is shorter than typical long-term exposure, and the small sample size limits generalizability. Additionally, using a single

flat dentin surface and one adhesive system may not apply to different cavity shapes or adhesives.

### Conclusions:

Within the limitations of the present in vitro study, the following conclusions can be made: etching with 37% phosphoric acid for 3 s followed by ethanol wetting technology for 1 min before applying a universal adhesive can improve the bond strength, durability, and quality of scotchbond universal plus adhesive significantly.

### Supplementary Material

None.

### Author Contributions

Ahmed Ali: data curation, writing-original draft preparation. Haider Hasan Jasim: Conceptualization, methodology, writing-review and editing.

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### Data Availability Statement

Data are available from the authors upon reasonable request.

### Conflict of interest

The authors reported that they have no conflicts of interest.

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