



# Primer application technique and remaining dentin thickness affected microtensile bond strength of contemporary dentin adhesives under simulated pulp pressure

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## Abstract

**Objectives** To evaluate the effect of application techniques, type of adhesives and remaining dentin thicknesses on microtensile bond strength ( $\mu$ TBS) of 3 adhesive systems.

**Materials and methods** 112 flat occlusal dentinal surfaces of third molar were randomly allocated into 16 groups based on 2 remaining dentin thicknesses (RDT), 2 application techniques, and 3 adhesive systems (Optibond FL, OFL; Clearfil SE Bond, CSE; and Single Bond Universal, SB); SB was applied in either etch-and-rinse (ER) or self-etch (SE) mode. Simulated pulpal pressure was performed during restorative procedure and water storage. The stick-shaped specimens from each tooth underwent  $\mu$ TBS testing. The data were evaluated using a paired *t* test and ANOVA followed by a post hoc test. The fractured specimens were evaluated for mode of failure using a stereomicroscope.

**Results** The mean  $\mu$ TBS values were significantly affected by RDT, application technique, and types of adhesives. Neither RDT nor application technique affected  $\mu$ TBS of SB in ER mode, whereas application technique affected both conventional and universal self-etch adhesives. RDT also influenced  $\mu$ TBS of OFL.

**Conclusions** RDT and application technique differently affected the  $\mu$ TBS of dentin bonding which was product-related. Etch-and-rinse systems had higher bond strength to superficial than to deep dentin, whereas self-etch systems were more sensitive to both RDT and application technique.

**Clinical relevance** The universal adhesive is less sensitive to intrinsic wetness and can be used according to manufacturer's instructions.

**Keywords** Microtensile bond strength · Primer application technique · Remaining dentin thickness · Simulated pulp pressure

## Introduction

Over time, dental adhesive systems have been developed to achieve high clinical success with much more simplification. The contemporary dental adhesive systems can currently be classified according to their strategies to interact with tooth substrate into etch-and-rinse and self-etch [1–3]. The multi-component etch-and-rinse adhesives, comprising of separate phosphoric acid, completely removed both smear layer and

superficial mineral, whereas self-etch adhesives simultaneously modified smear layer and superficial mineral using acidic monomer and provided resin infiltration into tooth substrate [2]. To reduce clinical step and technical sensitivity, newly developed dental adhesives have been introduced as a universal adhesive, which has been claimed to be simpler yet more versatile, since it could be used as either two-step etch-and-rinse or one-step self-etch according to the dentist's preference [2, 4]. However, previous version of simplified dental adhesives exhibited significantly higher water permeability and subsequently lowered microtensile bond strength after 5-year simulating pulpal pressure aging compared to multistep adhesives [5]. The universal adhesive also showed highly permeable to water in the resin-dentin interface after thermocycling [6], resulting in lower microtensile bond strength [7].

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Dentin is a heterogeneous substrate comprising of dentinal tubules surrounded by inter- and peri-tubular dentin. The relative ratio of these structures varies upon the dentin levels. The number of tubular densities increases when the dentin depth increases. The dentinal tubule density increases more than threefold from dentino-enamel junction to pulp in coronal dentin. Tubular diameter is also greater in the deep dentin closed to pulpal chamber [8]. This means inter-tubular dentin in deep dentin area is lesser than that in the superficial dentin. This difference can highly influence the mechanical properties and bonding efficacy. However, the remaining dentin thickness (RDT) presented a controversial effect on bond strength in several studies [9–12], probably due to difference of tested adhesive systems. Additionally, the intrinsic wetness of vital dentin was enhanced by outward seepage of dentinal fluid under physiologic hydrostatic pulpal pressure [13]. Such moist dentin may attenuate mechanical properties of resin bonding, eventually compromising bond efficacy [7, 9, 14, 15].

To achieve high quality of bonding to dentin, several strategies were proposed, for examples, the application technique [7], prolonged application times [5, 6], and the recently proposed technique, selective dentin etching for 3 s [16, 17]. Cardoso et al. demonstrated that longer adhesive application times increased dentin-resin microtensile bond strength ( $\mu$ TBS) of two-step etch-and-rinse resin adhesives in water/ethanol- and acetone-based systems [18]. Subjected samples to 3-year artificial aging, the resin-dentin interfaces formed using longer adhesive application times were more stable over time [19]. Chowdhury et al. [20] demonstrated that double primer application of a universal adhesive during dentin bonding increased its bond strength.

Altogether, these raise the question of whether different dentin thicknesses and double application techniques under simulated pulp pressure affect  $\mu$ TBS of various adhesives. Thus, the objective of this study was to evaluate the effect of primer application techniques and remaining dentin thicknesses on the  $\mu$ TBS of conventional and universal adhesives under simulated pulpal pressure. The bonded teeth were stored under pulpal pressure for 6 months before the  $\mu$ TBS tests. The null hypotheses were (1) there was no significant difference in  $\mu$ TBS to dentin when using 2 different primer application techniques, (2) there was no significant difference in  $\mu$ TBS to dentin when using different types of adhesives, and (3) there was no significant difference in  $\mu$ TBS to different dentin thicknesses.

## Materials and methods

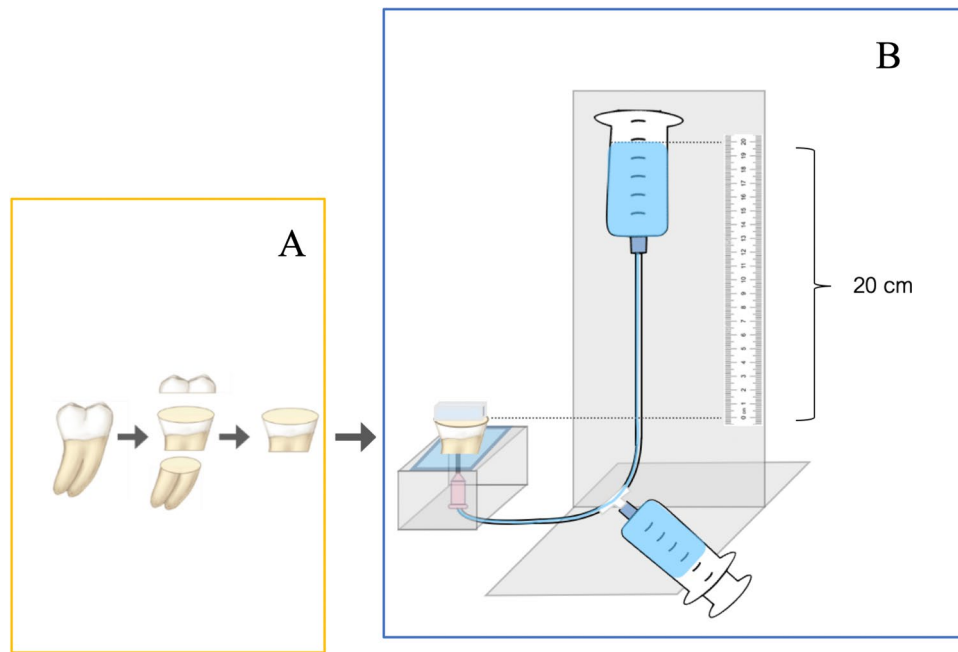
### Specimen preparation

The research proposal was approved by the Human Research Ethics Committee of the Faculty of Dentistry,

Chulalongkorn University (HREC-DCU 2020–042). 112 sound human third molars extracted from 16–40-year-old patients with informed consent were used in the study. Blood and adherent tissues were gently removed from extracted teeth under running water. Teeth were stored in a 1% aqueous solution of Chloramine-T for at least 1 week at room temperature. All teeth were used within 6 months after extraction. The desired RDT were obtained as following methods. Roots were sectioned at two-millimeter below the cemento-enamel junction perpendicular to the long axis. Then, occlusal crowns were parallelly cut at the specified level to obtain the desired dentin thicknesses using a low-speed diamond saw (IsoMet 1000, Buehler; Lake Bluff, IL, USA). Pulp tissue was gently removed using forceps. The RDT was measured by inserting a pincer-type caliper into pulp chamber and recorded vertically at the center of the tested interface to the roof of pulp chamber. The measurement was made in different areas of tested interface to confirm that the roof of pulp chamber was wide and plane. Any samples that pulp horns were involved in bonded area were excluded. Dentin surfaces were abraded with a 150-grit silicon carbide paper under water cooling to reach the desired RDT which were categorized into 2 groups;  $1 \pm 0.1$  mm. as a deep dentin, and  $3 \pm 0.1$  mm. as a superficial dentin. Smear layer was removed using a 10% citric acid for 1 min [21]. Standardized smear layer was created using a 600-grit silicon carbide paper (TOA, Thailand) with a polishing machine (Nano 2000, Pace technologies, USA) at 200 RPM for 60 s [22].

### Simulated pulpal pressure device

A simulated pulp pressure device was assembled and attached to the crown segment as mentioned in previous study [23]. Briefly, the crown segment was fixed to acrylic plates using cyanoacrylate glue (Model Repair II Blue, Dentsply, Japan), and an 18-gauge (0.13 cm) stainless steel tube was inserted through a hole in the middle of the plate. An intravenous tube was connected to the pulp chamber, and a hydraulic pressure device was filled with distilled water to generate a pressure of 20 cm H<sub>2</sub>O, as shown in Fig. 1. Fluid infusion by this model was presented during bonding and restoring, whereas modified method proposed by Feitosa and others [23] was performed during aging processes. Samples were secured to the inside of the cylindrical receptacle's lid by laying it obliquely into the wax on the lid, without obstructing the pulpal chamber opening as shown in Fig. 2. The cylindrical container was filled with sterile distilled water to reach 20-cm level, and the container was closed with samples attached to the lid. Then, the container was turned upside down to submit the samples to 20 cm H<sub>2</sub>O pulpal pressure.



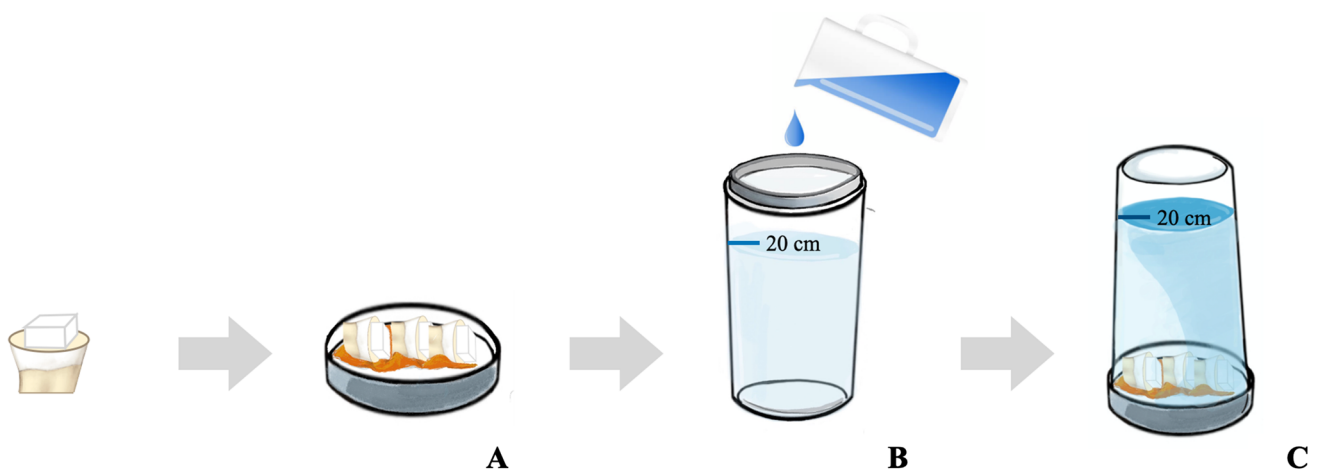
**Fig. 1** Schematic picture of the tooth preparation and simulated fluid flow through a sectioned crown using a 20 cm H<sub>2</sub>O. In brief, **A** to obtain flat occlusal dental surface with clean pulp chamber, roots were sectioned at two-millimeter below the cemento-enamel junction perpendicular to the long axis. Then, occlusal crowns were parallelly cut at the specified level to obtain the desired dentin thicknesses. **B** After gently removal of pulp tissue, the crown segment was fixed to

acrylic plates, and an 18-gauge (0.13 cm) stainless steel tube was inserted through a hole in the middle of the plate. An intravenous tube was connected to the pulp chamber, and a hydraulic pressure device was filled with distilled water to generate a pressure of 20 cm H<sub>2</sub>O. Fluid infusion was presented during bonding and restoring as well as storage processes

### Bonding and restoring procedures

All teeth were randomly allocated into 16 groups ( $n = 7$  for each group) based on 3 independent variables, i.e., primer application techniques, types of adhesive systems,

and RDTs as shown in Table 1. Chemical composition, lot number of materials used in the study and application techniques are presented in Table 2 and Table 3, respectively. Primer application technique was used following the manufacturer’s instructions; primer was applied one



**Fig. 2** Schematic picture of modified pulp pressure model using a 20 cm H<sub>2</sub>O for aging process. **A** Samples were secured to the inside of the cylindrical receptacle’s lid by laying them obliquely into the wax on the lid, avoiding obstructing the pulpal chamber opening.

**B** The cylindrical container was filled with sterile distilled water to reach 20 cm level, and the container was closed with samples attached to the lid. **C** The container was turned upside down submit the samples to a 20 cm H<sub>2</sub>O pulp pressure

time for the single application technique and two times for the double application technique. Resin composite (Harmonize™) was then used for restoration. A light-emitting diode (LED) light-curing unit (Demi™ LED light-curing system, Kerr, Orange, CA, USA) was used to cure three incremental 2-mm resin composite layers for 40 s each layer with an intensity of more than 600 mW/cm<sup>2</sup>. The LED light was calibrated at the start of each new group with Optilux Radiometer (L.E.D. radiometer by Demetron, Kerr Corporation, Danbury, CT, USA).

### Aging process

All restored samples were submerged in distilled water at 37 °C with the presence of modified simulated pulpal pressure device [23] and kept in an incubator (Contherm 160 M; Contherm Scientific Ltd., Lower Hut, New Zealand) for 6 months. Water was changed every 7 days. All samples were tested for bond strength immediately after being removed from water.




### Microtensile bond strength testing

After 6-month aging, the restored teeth were etched with a 37% phosphoric acid (Kerr Gel Etchant; Kerr, Orange, CA, USA) and filled with resin composite (Harmonize™; Kerr, USA) into the pulp chamber before being sectioned occluso-gingivally across the bonded interface. Resin-dentin sticks (1 mm<sup>2</sup> cross-section) were prepared with a low speed cutting machine (IsoMet® 1000, Buchler, USA) using the non-trimming technique [24, 25]. Stick-shaped specimens were fixed to the testing jig using a cyanoacrylate glue (Model Repair II Blue, Dentsply Sirona, Japan) and tested to failure under tension using a Universal testing machine (EZ-S, Shimadzu, Japan) with a 500-N load cell at a crosshead speed of 1.0 mm/min. The exact cross-sectional area of each tested sticks was measured after failure using a digital caliper. The mean bond strength of 4 sticks from each tooth represented the  $\mu$ TBS of that tooth [23, 26], generating 7 values per group.

### Failure mode analysis

After  $\mu$ TBS test, the fractured surface of both dentin and composite sides were evaluated by a stereomicroscope at

**Table 1** Group Classification

Tooth	RDTs	Adhesives	Primer application techniques	Group code
 <p>112 extracted third molars</p>	 <p>Superficial dentin (RDT = 3 ± 0.1 mm)</p>	OFL	Single	OFL-S1
			Double	OFL-S2
		SBER	Single	SBER-S1
			Double	SBER-S2
		CSE	Single	CSE-S1
			Double	CSE-S2
	SBSE	Single	SBSE-S1	
		Double	SBSE-S2	
	 <p>Deep dentin (RDT = 1 ± 0.1 mm)</p>	OFL	Single	OFL-D1
			Double	OFL-D2
		SBER	Single	SBER-D1
			Double	SBER-D2
		CSE	Single	CSE-D1
			Double	CSE-D2
SBSE		Single	SBSE-D1	
		Double	SBSE-D2	

**Table 2** Chemical composition and batches of materials used

Code	Adhesive	Main component	pH	Manufacturer/Lot No
CSE	Clearfil SE Bond	Primer: 10-MDP, HEMA, Hydrophilic dimethacrylate, camphorquinone, water Adhesive: 10-MDP, bis-GMA, HEMA, hydrophobic dimethacrylates, camphorquinone, colloidal silica	1.9	Kuraray Noritake; Osaka, Japan/000059
OFL	Optibond FL	Primer: HEMA, GPDM, PAMM, ethanol, water, photoinitiator Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler, photoinitiator	1.8	Kerr; Orange, CA, USA/7480512
SB	Single Bond Universal Adhesive	Adhesive: 10-MDP, Vitrebond copolymer, HEMA, dimethacrylate resins, filler, silane, initiator, ethanol, water	2.7	3 M ESPE, USA/5541216
Gel Etchant		37.5% phosphoric acid, silica thickener		Kerr; Orange, CA, USA
Harmonize™		Resin matrix: bis-GMA, bis-EMA, TEGDMA		Kerr; Orange, CA, USA/7478613
Shade A3D Resin composite		Filler: zirconia/silica nanoparticles		

*Bis-GMA*, bisphenol A diglycidyl ether dimethacrylate; *HEMA*, 2-hydroxyethyl methacrylate; *TEGDMA*, triethylene glycol dimethacrylate; *10-MDP*, 10-methacryloyloxydecyl dihydrogen phosphate; *UDMA*, urethane dimethacrylate; *GPDM*, glycerol phosphate dimethacrylate; *PAMM*, phthalic acid monoethyl methacrylate; *Bis-EMA*, ethoxylated bisphenol A glycol dimethacrylate

**Table 3** Resin adhesives and resin composites and their application

MATERIALS	BONDING STEPS RECOMMENDED BY MANUFACTURER	BONDING STEPS OF DOUBLE PRIMER APPLICATION TECHNIQUE
CSE	<b>Prime:</b> Apply a layer of primer, wait 20 s, gently air dry <b>Bond:</b> Apply bonding agent, remove excess with a light jet of air and light cure for 10 s	<b>Prime:</b> Apply a layer of primer, wait 20 s, repeat the step, gently air dry <b>Bond:</b> Apply bonding agent, remove excess with a light jet of air and light cure for 10 s
OFL	<b>Etch:</b> Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s <b>Prime:</b> Apply primer with light scrubbing motion for 15 s, gently air dry 5 s <b>Bond:</b> Apply a thin coat of bonding agent and light cure for 20 s	<b>Etch:</b> Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s <b>Prime:</b> Apply primer with light scrubbing motion for 15 s, repeat the step, gently air dry 5 s <b>Bond:</b> Apply a thin coat of bonding agent and light cure for 20 s
SB	<i>Etch-and-rinse mode</i> <b>Etch:</b> Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s <b>Bond:</b> Apply adhesive and rub for 20 s, dry gently for about 5 s, light cure for 10 s <i>Self-etch mode</i> <b>Bond:</b> Apply adhesive and rub for 20 s, dry gently for about 5 s, light cure for 10 s	<i>Etch-and-rinse mode</i> <b>Etch:</b> Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s <b>Bond:</b> Apply adhesive and rub for 20 s, repeat the step, dry gently for about 5 s, light cure for 10 s <i>Self-etch mode</i> <b>Bond:</b> Apply adhesive and rub for 20 s, repeat the step, dry gently for about 5 s, light cure for 10 s
RESIN COMPOSITE	Apply in 2-mm increment and light cure for 40 s	

CSE, Clearfil SE Bond; OFL, Optibond FL; SB, Single Bond Universal Adhesive

45 × magnifications (ML 9300®, MEJI, Japan) and classified as the followings: adhesive failure, mixed failure, cohesive failure of resin composite, and cohesive failure of dentin. The recorded numbers of each mode were calculated based on all fractured sticks in each group and

shown as a percentage of each group. Additionally, the most two representative fractured ends from each group were further analyzed under a scanning electron microscope (SEM).

**Table 4** Means  $\pm$  SD of the microtensile bond strength values (MPa) in each group and the percentage of fracture modes (AM/CC/CD) of the  $\mu$ TBS samples analyzed using a stereomicroscope

Adhesive system	OFL		SBER		CSE		SBSE	
	Application procedure	Manufacturer's Instruction	Double primer application	Manufacturer's instruction	Double primer application	Manufacturer's instruction	Double primer application	Manufacturer's instruction
Superficial dentin	34.43 $\pm$ 4.47 <sup>a,A</sup>	79/71/4/0	29.55 $\pm$ 6.45 <sup>a,A</sup>	35.72 $\pm$ 9.49 <sup>a,A</sup>	31.82 $\pm$ 14.30 <sup>a,A</sup>	15.55 $\pm$ 4.56 <sup>b,B</sup>	22.51 $\pm$ 5.16 <sup>a,A</sup>	50.97 $\pm$ 11.68 <sup>c-A</sup>
			69/21/10/0	41/35/24/0	64/7/25/4	93/7/0/0	75/11/14/0	57/14/25/4
Deep dentin	18.11 $\pm$ 5.17 <sup>a,B</sup>	96/0/4/0	18.46 $\pm$ 5.77 <sup>a,B</sup>	33.76 $\pm$ 6.76 <sup>b,A</sup>	31.78 $\pm$ 3.58 <sup>b,A</sup>	22.09 $\pm$ 7.75 <sup>a,B</sup>	14.99 $\pm$ 2.47 <sup>a,B</sup>	20.67 $\pm$ 4.34 <sup>a,B</sup>
			83/13/4/0	32/14/50/4	50/7/36/7	96/0/4/0	88/4/4/4	93/0/7/0

Means  $\pm$  SD in MPa. Means with the same lowercase letters in each row and means with the same capital letters in each column are not significantly different at  $p > 0.05$ . A, adhesive failure; M, mixed failure; CC, cohesive failure of resin composite; CD, cohesive failure of dentin

## SEM analysis

The parts of fractured specimens were paired, air-dried, and mounted on aluminum stubs, coated with gold, and evaluated at a magnification of 5,000 $\times$  using a scanning electron microscope (SEM) (JSM-6610LV Scanning Electron Microscope JEOL, USA) at an acceleration voltage 15 kV to confirm mode of failure.

## Statistical analysis

All statistical procedures were performed using SPSS software (IBM SPSS statistics V25.0, IBM; Armonk, NY, USA). The data were evaluated for a normal distribution using the Shapiro–Wilk test. A three-way ANOVA was used to analyze the factors and their interactions. The  $\mu$ TBS values were evaluated using the paired  $t$  test and one-way ANOVA followed by a Tukey's post hoc test. For all analyses, statistical significance level was set at  $p < 0.05$ .

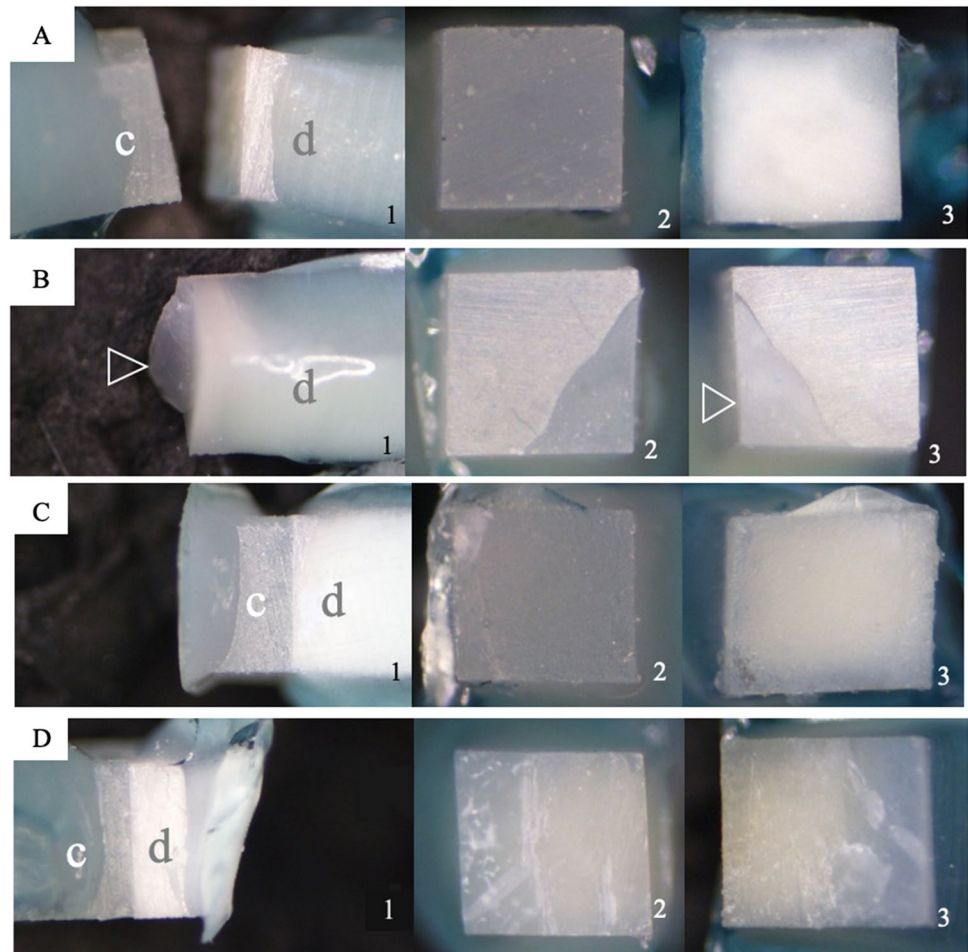
## Results

### Microtensile bond strength

Three-way ANOVA demonstrated that primer application technique ( $p = 0.014$ ), types of adhesives ( $p < 0.001$ ), RDTs ( $p < 0.001$ ), and interaction of these 3 factors ( $p < 0.001$ ) statistically significantly impacted the  $\mu$ TBS. The interaction of RDT with either application technique ( $p = 0.038$ ) or types of adhesives ( $p < 0.001$ ) was also significant, while types of adhesives did not significantly interact with primer application technique ( $p = 0.145$ ).

Mean  $\mu$ TBS values and standard deviations (SD) are presented in Table 4. With either single or double application, OFL bonded to superficial dentin showed higher mean  $\mu$ TBS values than that bonded to deep dentin, while SBER showed no statistically significant difference of  $\mu$ TBS values between superficial and deep dentin. Conversely, application technique influenced  $\mu$ TBS value of CSE and SBSE. With single application, despite no statistical difference, CSE bonded to superficial dentin showed lower mean  $\mu$ TBS value than that bonded to deep dentin, whereas with double application, CSE bonded to superficial dentin showed significantly higher mean  $\mu$ TBS values than deep dentin. SBSE bonded to superficial dentin with single application were significantly higher than mean values obtained from deep dentin, while with double application,  $\mu$ TBS values of SBSE showed no significant difference between bonded to superficial and deep dentin. In spite of lower  $\mu$ TBS values than bonded to superficial dentin, SBER showed the highest mean  $\mu$ TBS value comparing to other groups.

**Fig. 3** This picture shows stereomicroscope photographs at 45× magnification of fractured samples. (1) Lateral view, (2) top view of composite side, (3) top view of dentin side. **A** Adhesive failure: Fractured surface of composite side (c) is completely detached from dentin side (d). **B** Mixed failure: Fractured surface of composite side (c) is partially detached from dentin side (d) and some fractured composite (transparent arrowhead) adhered to dentin side (d). **C** Cohesive failure of composite: The lateral view of fractured stick shows the fracture is in composite side (c). **D** Cohesive failure of dentin: The lateral view of fractured stick shows the fracture is in dentin side (d)



### Failure mode analysis

The failure modes were classified by group (Table 4). Mostly, adhesive failure was the predominant mode for both superficial and deep dentin; however, SBER groups demonstrated a tendency toward multiple modes of failure. The representative stereomicroscope photographs of failure mode were shown in Fig. 3.

### SEM analysis

A predominant adhesive failure was shown in Fig. 4. The fractured surfaces of the dentin side revealed a combination of vacant dentinal tubules and resin-tag occupied dentinal tubules, whereas the fractured surfaces of the composite side showed prominent and fractured resin tags in OFL group (Fig. 4A and B). In contrast to etch-and-rinse sample, self-etch sample demonstrated occluded dentinal tubule presenting in most of examined area (Fig. 4C). The fractured surface of composite side showed scant resin tags comparing to etch-and-rinse sample (Fig. 4D).

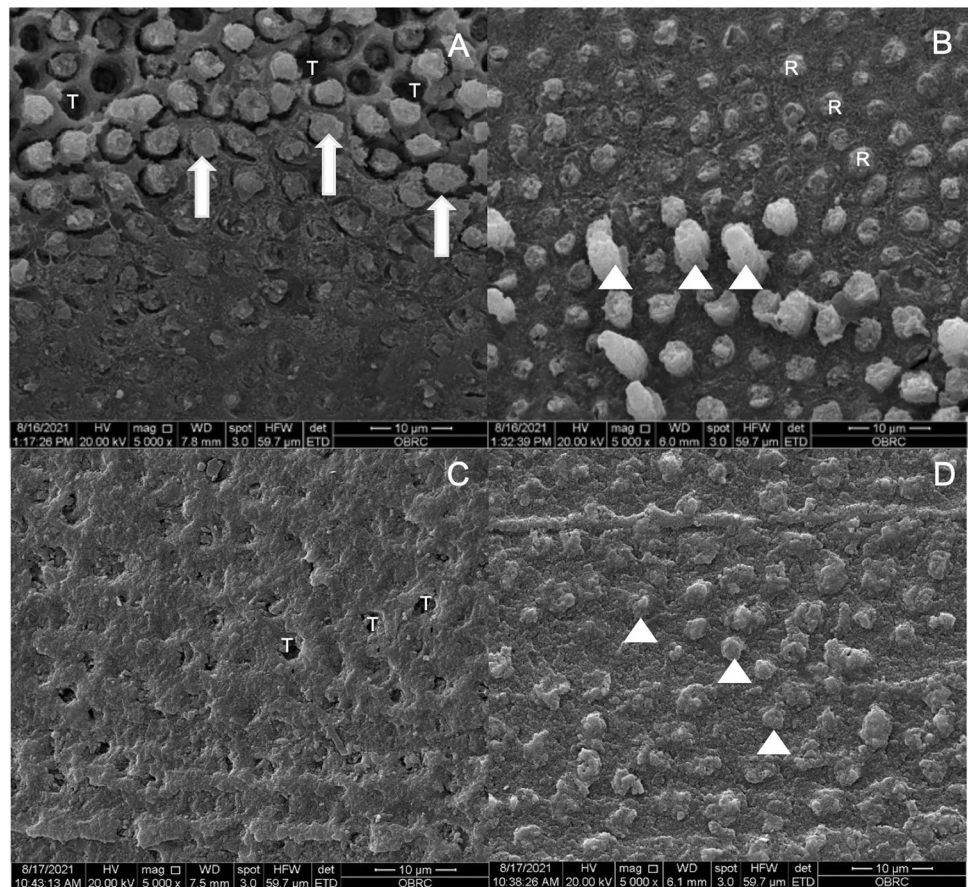
### Discussion

The present study was designed to determine the effect of application technique, types of adhesives, and RDT on microtensile bond strength ( $\mu$ TBS) of conventional and simplified universal adhesive systems under simulating 20 cm H<sub>2</sub>O pulp pressure. The results showed that each type of adhesive system revealed different behaviors influenced by remaining dentin thickness and application technique. Therefore, all null hypotheses were rejected. Moreover, failure mode in the present study was a predominantly adhesive failure, which was desirable to demonstrate the true bond strength between two substrates [27].

It is generally accepted in the literature that intrinsic wetness from intrapulpal pressure attenuated bond efficacy of various adhesive systems [15, 28]. Therefore, bonding procedure and storage in the present study was performed in the presence of simulated pulpal pressure intended to mimic the clinical reality that positive pulpal pressure created slow seepage of fluid to dentin surface [29].

Etching step in etch-and-rinse system, either conventional or universal, completely removed all smear layer, smear plug,

**Fig. 4** Representative SEM photographs at 5,000× magnification of samples in the OFL-D2 and SBSE-D1 group. **A** Fracture surface of the dentin side revealed adhesive failure with open dentinal tubules (T) and dentinal tubules filled with resin tags (arrow). **B** Fracture surface of the composite side revealed adhesive failure with prominent (white arrowhead) and fractured resin tags. **C** Fracture surface of the dentin side revealed adhesive failure with open dentinal tubules (T). **D** Fracture surface of the composite side revealed adhesive failure with scant resin tags (white arrowhead)

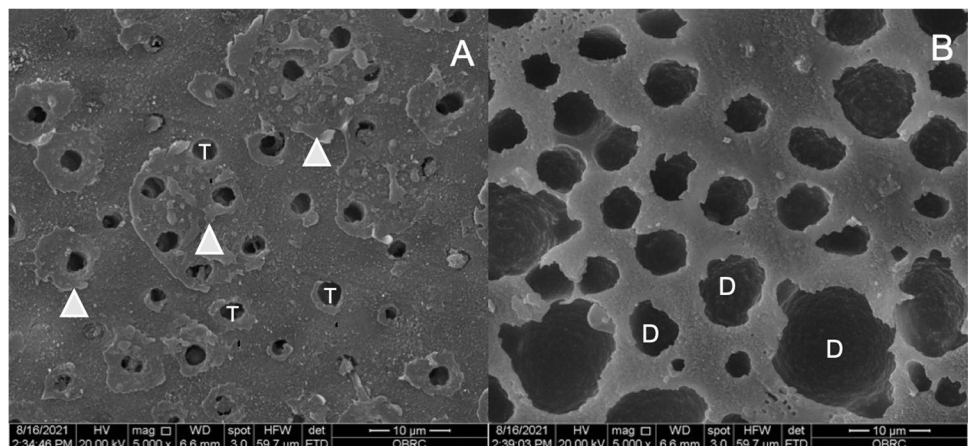


and demineralized dentin up to 5  $\mu\text{m}$  [30] resulting in increased outward flow of dentinal fluid. In deep dentin, a greater number of tubules and a higher fluid flow rate [31] resulted in higher fluid perfusion during bond and storage when compared to superficial dentin. Such fluid perfusion from simulated pulpal pressure hampered the ability of solvent to remove all the wetness during bonding step [32], subsequently leaving behind fluid remnants at the bottom of hybrid layer which attenuated infiltration and polymerization of hydrophobic resin in

conventional etch-and-rinse adhesive system (OFL). Moreover, additional water storage and simulated pulpal pressure increased dentin perfusion that gradually caused hydrolytic and enzymatic degradation over time, decreasing the bond strength values in long-term storage [33]. These combined factors attributed to different result of OFL from previous studies [28, 32] which evaluated one factor without aging.

Dealing with similar wetness, simplified universal adhesive in etch-and-rinse mode presented oppositely.

**Fig. 5** Representative SEM images at 5,000× magnification of samples in the SBER-S2 group. **A** Fractured surface of the dentin side revealed adhesive failure with open dentinal tubules (T) and blemish of adhesive (white arrowhead). **B** Fractured surface of the resin composite side revealed adhesive failure with voids representing water droplets (D) within the bottom of resin composite side





Hydrophilic resin adhesive could infiltrate and polymerize in such moist condition [34] of deep dentin resulting in similar bond strength to superficial dentin. Our results revealed that universal adhesive in etch-and-rinse mode, having scarce chemical bond due to completely demineralized dentin, provided sufficient bond strength with respect to only micromechanical bonding despite intrinsic wetness during bonding or storage. However, the simulated pulp pressure together with osmotic pressure initiated by hydrophilic character created water droplets within adhesive layer resulting in nanoleakage in this adhesive [3, 6], which could be seen in SEM as shown in Fig. 5. Such defects in adhesive layer may attribute to water sorption and harm the bond efficacy in a long-term of clinical service.

In the present study, application technique did not affect both conventional and universal etch-and-rinse adhesives. Since double application was believed to increase the chemical interaction of acidic monomer to dentin, this technique could not increase the bond strength of adhesive that depends mainly upon micromechanical bonding. Increase either time of application [35] or amount of primer, as in this study, seemed unable to increase the bond strength of universal adhesive in etch-and-rinse mode. On the other hand, mild self-etch adhesive systems, both conventional and universal, provide both mechanical and chemical bonds by the functional monomers. Therefore, tooth structure and application technique impacted their behaviors in this study. Considering the remaining dentin thickness, dentin permeability was lower when treated with mild acidic primer in self-etch adhesive system [36, 37]. Partially demineralized dentin and remnants of modified smear layer decreased dentin perfusion, resulting in a reduction of water to interfere with polymerization of resin adhesive. This attributed to the findings by Choi et al. [38]. and in Clearfil SE Bond in our study. However, together with simulated pulp pressure, hydrophilic characteristics of Single Bond Universal in self-etch mode may draw fluid through permeated dentin. Such fluid may reduce the concentration of acidic monomer, preventing it from effectively chemically interacting with smear layer and dentin [39], resulting in lower bond strength to deep dentin than superficial dentin when using a single application.

Not only intrinsic wetness but extrinsic wetness from either bonding composition or bonding procedure also influence behavior of self-etch adhesive systems. The functional monomer, 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), is one factor that responsible for the bond strength. 10-MDP is the most widely used functional monomer that provides high efficacy and durability to dentin bonding because of its stable ionic bond to the calcium in hydroxyapatite (Hap) presented in nanolayer [2]. The more intense of nanolayer is, the higher bond strength it provides. Such nanolayer was shown to be 10-MDP

concentration-dependent [39]. Double application may provide high concentration of MDP leading to more intense of nanolayer, subsequently increasing bond strength of Single Bond Universal in self-etch mode to deep dentin. Our result supported Fujiwara et al., who found that double application of a universal adhesive increased shear bond strength and shear fatigue strength [40]. However, a recent study reported inconsistent double application in increasing the  $\mu$ TBS of this adhesive in either mode [41] probably resulting from performing bonding procedure without water infusion, differently from our study. In contrast to universal adhesive, double application increased the functional monomer of Clearfil SE Bond to interact with greater quantity of inter-tubular dentin in superficial dentin [42]. This technique increased amount of solvent, though. Clearfil SE Bond was a water-based adhesive. Water from double application may hinder ability to evaporate both intrinsic wetness from simulated pulp pressure and extrinsic water from solvent itself.

In addition to different solvents, different functional monomers might boost the bond strength up. A polyalkenoic acid copolymer in Single Bond Universal adhesive served the carboxyl group to bond with hydroxyapatite [43]. Moreover, application motion may also affect bond efficacy of self-etch adhesive system. Rubbing action kept the acidic monomer freshly when closely contacting with dentin by disrupting the smear layer, resulting in increased bond strength [39, 44, 45]. The difference in both ingredients and application motions between the two adhesives might explain why a higher bond strength was achieved in Single Bond Universal in self-etch mode (SBSE group) compared to Clearfil SE Bond.

Our results indicated that the universal adhesive was less sensitive to intrinsic wetness. Therefore, the manufacturer's instructions can be followed when all tested adhesive systems are used. Nowadays, many adhesive systems are clinically available. Moreover, in the present study, only one circumstance which provided simulated pulp pressure at the beginning of the bonding step through the 6-month storage period was utilized. Hence, further studies involving other compositions of adhesive systems and other application techniques under fluid perfusion and different storage periods are recommended. Even though in vitro microtensile bond strength could not completely imply the clinical performance of these adhesives, our research can be informative for future studies and urge clinicians to be aware of these factors.

## Conclusion

Within the limitation of the present study, primer application techniques and remaining dentin thicknesses differently affected the  $\mu$ TBS of dentin bonding which was

product-related. Overall, etch-and-rinse systems had higher bond strength to superficial dentin than that bonded to deep dentin, whereas self-etch systems were more sensitive to both remaining dentin thickness and application technique. The results suggested that universal adhesive should be used following the manufacturer's recommendations when applied to either superficial or deep dentin.

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## Declarations

**Ethics approval** Extracted caries-free human third molars were collected following a protocol by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2020–042).

**Consent to participate** The present study did not involve human participant.

**Conflict of interest** The authors declare no competing interests.

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