Effect of remaining dentin thickness and primer application technique

on microtensile bond strength

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ผลของความหนาของเนื้อฟันและเทคนิคการทาไพรเมอร์ต่อความแข็งแรงดึงระดับจุลภาค

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Thesis Title   Effect of remaining dentin thickness and primer application

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By   Miss Paphawee Somrit

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ปภาวี สมฤทธิ์ : ผลของความหนาของเนื้อฟันและเทคนิคการทาไพรเมอร์ต่อความแข็งแรงดึงระดับ
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การศึกษานี้มีวัตถุประสงค์เพื่อเปรียบเทียบผลของความหนาของเนื้อฟันและเทคนิคการใช้ไพร
เมอร์ ต่อความแข็งแรงดึงระดับจุลภาค โดยผิวเนื้อฟันที่ได้จากฟันฟันกรามซี่ที่สามของมนุษย์จำนวน 112 ซี่
จะถูกแบ่งกลุ่มโดยการสุ่มจำนวน 16 กลุ่ม ตามความหนาของเนื้อฟันที่เหลืออยู่ 2 ความหนา เทคนิคการทา
ไพร์เมอร์ 2 เทคนิค โดยมีสารยึดติดทางทันตกรรมที่ใช้ในการศึกษานี้จำนวน 3 ระบบ ได้แก่ 1. ระบบเอทช์
แอนด์ริสส์ แบบสามขั้นตอน (ออปติบอนด์ เอฟแอล(Optibond FL; OFL)) 2. ระบบเซลฟ์เอทช์ สองขั้นตอน
(เคลียร์ฟิล เอสอี บอนด์ (Clearfil SE Bond; CSE)) 3. ยูนิเวอร์แซลแอดฮีซีฟ (ซิงเกิลบอนด์ ยูนิเวอร์แซล แอดฮี
ซีฟ (Single Bond Universal; SB)) โดยที่ ยูนิเวอร์แซลแอดฮีซีฟจะใช้งานทั้งระบบเอทช์แอนด์ริสส์และระบบ
เซลฟ์เอทช์ ทาสารยึดติดทางทันตกรรมตามกลุ่มทดสอบ บูรณะด้วยคอมโพสิตและเก็บชิ้นงานเป็นเวลา 6
เดือน ภายใต้เครื่องจำลองสภาวะแรงดันน้ำภายในท่อเนื้อฟันก่อนการทดสอบแรงดึงระดับจุลภาค วิเคราะห์
ผลการทดลองด้วยการวิเคราะห์ความแปรปรวนแบบจำแนกสามทาง ทางเดียวและการทดสอบที ที่ระดับ
ความเชื่อมั่นร้อยละ 95 ตรวจสอบรูปแบบการแตกหักด้วยกล้องจุลทรรศน์แบบสเตอริโอ และตรวจสอบ
พื้นผิวที่แตกด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดตามลำดับ พบปฏิสัมพันธ์กันใน 3 ปัจจัย คือ
ความสัมพันธ์ระหว่างความหนาของเนื้อฟัน เทคนิคการทาไพรเมอร์และชนิดของสารยึดติดทางทันตกรรม
ความหนาของเนื้อฟันและเทคนิคการทาไพรเมอร์ไม่มีผลต่อแรงดึงระดับจุลภาคในกลุ่มยูนิเวอร์แซลแอดฮีซีฟ
ที่ทาด้วยระบบเอทช์แอนด์รินส์ ในขณะที่ส่งผลต่อออปติบอนด์ เอฟแอล เคลียร์ฟิล เอสอี บอนด์ และซิงเกิล
บอนด์ ยูนิเวอร์แซล แอดฮีซีฟที่ใช้ระบบเซลฟ์เอทช์ ค่าความแข็งแรงดึงระดับจุลภาคในระบบเอทช์แอนด์ริสส์
ที่ยึดในเนื้อฟันระดับตื้นมีค่าสูงกว่าในเนื้อฟันระดับลึก ในทางกลับกันสารยึดติดระบบเซลฟ์เอทช์ได้รับอิทธิพล
จากระดับความหนาของเนื้อฟันและเทคนิคการทาไพร์เมอร์ ยูนิเวอร์แซลแอดฮีซีฟสามารถใช้ตามคำแนะนำ
ของบริษัทผู้ผลิตได้ทั้งในการยึดติดกับเนื้อฟันระดับตื้นและระดับลึก

สาขาวิชา	ทันตกรรมหัตถการ	ลายมือชื่อนิสิต ....
ปีการศึกษา	2564	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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KEYWORD: Microtensile bond strength, primer application technique, remaining dentin thickness, simulated pulp pressure

Paphawee Somrit : Effect of remaining dentin thickness and primer application technique on microtensile bond strength. Advisor: Assoc. Prof. SIRIVIMOL SRISAWASDI, D.D.S., M.S., Ph.D.

To evaluate the effect of primer application techniques, type of adhesives and remaining dentin thicknesses on microtensile bond strength (μTBS) of 4 different adhesive systems, 112 Flat occlusal surfaces of sound third molar were randomly allocated into 16 groups based on 2 remaining dentin thicknesses (RDT), 2 application techniques and 3 adhesive systems, e.g., Three step etch-and-rinse (Optibond FL; OFL), Two step self-etch (Clearfil SE Bond; CSE), and Universal adhesive (Single Bond Universal; SB). SB was applied in either etch-and-rinse (ER) or self-etch (SE) mode. Simulated pulpal pressure was performed during bonding procedure and 6-month water storage (37 $^{\circ}$C). After resin composite buildup and water storage, stick-shaped specimens from each tooth underwent μTBS testing. Statistical analysis was performed with three-way ANOVA test and Tukey Post Hoc test. The fractured specimens were evaluated for mode of failure using a stereomicroscope. The fracture sufaces of each group were also observed using SEM. The mean μTBS values were significantly affected by RDT, application technique, and types of adhesive. Neither RDT nor primer application technique affected μTBS of SB in ER mode whereas application technique affected both conventional and universal self-etch adhesive. RDT also influenced μTBS of OFL. The RDT and application technique differently affects the μTBS of dentin bonding which is product-related. Etch-and-rinse systems had higher bond strength to superficial dentin than to deep dentin whereas self-etch systems were more sensitive to both RDT and application technique. The universal adhesive should be use following the manufacturer's recommendations when apply on either superficial or deep dentin.

Field of Study: Operative Dentistry   Student's Signature ....

Academic Year: 2021   Advisor's Signature ....

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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 multicomponent 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).

Dentin is a heterogeneous substrate comprising of dentinal tubules surrounded

by inter- and intratubular 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 3-fold from the dentino-enamel junction

to the pulp in coronal dentin. The 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 timing (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 (μTBS) 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 μTBS of various

adhesives. Thus, the objective of this study was to evaluate the effect of primer application

techniques and remaining dentin thicknesses on the μTBS of conventional and universal

adhesives under simulated pulpal pressure. The bonded teeth were stored under pulpal pressure for 6 months before the µTBS tests. The null hypotheses were: (1) there was no significant difference in µTBS to dentin when using 2 different primer application techniques, (2) there was no significant difference in µTBS to dentin when using different types of adhesives, and (3) there was no significant difference in µTBS to different dentin thicknesses.

Research Question

Do primer application technique, types of adhesive, and remaining dentin thickness have the effect on microtensile bond strength of contemporary adhesive under simulated pulp pressure?

Research Objective

The aim of this study was to evaluate the effect of primer application technique, types of adhesive, and remaining dentin thickness on microtensile bond strength of contemporary adhesives under simulated pulp pressure

Type of research

Experimental study

Proposed benefits

This study clarified the effect of primer application technique, types of adhesive, and different remaining dentin thicknesses on microtensile bond strength under simulated pulpal pressure situation, in vitro. The results provided clinicians consideration about application technique when using dental adhesives in various cavity depths. Moreover, the information obtained may change the clinical guideline to enhance dentin bond durability.

Limitations

This experimental design limited to in vitro simulated environment. The result might not be inferred to the real clinical situation, although the researcher tried to control the confounding factors and simulated closely to the clinical situation. This study investigated
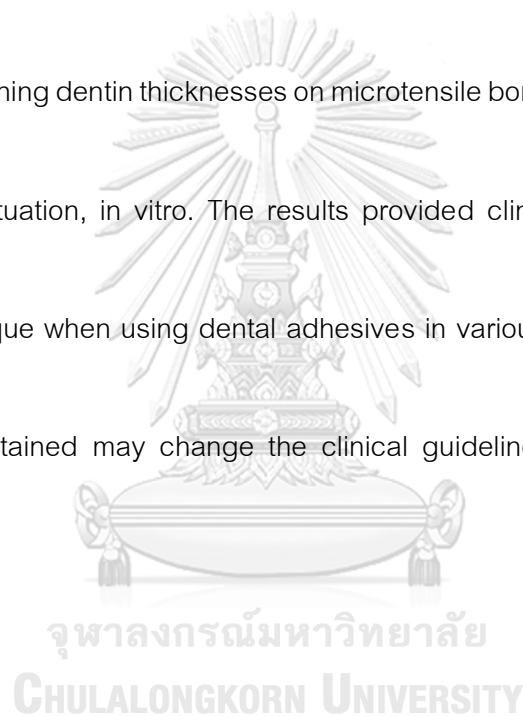
three adhesive systems from three manufacturers, thus, the results from this study may

not be inferred to other adhesive systems.

Conceptual framework

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┌──────────────────────────┐      ┌─────────────────────┐    ┌─────────────────────────┐      ┌──────────────────────┐
│ Morphology of roof of pulp│─────▶│ Remaining dentin    │    │ Primer application      │◀─────│ Application motions   │
│         chamber          │      │ thickness           │    │ technique               │      └──────────────────────┘
└──────────────────────────┘      │  - Superficial dentin│    │  - Bonding steps        │      ┌──────────────────────┐
                                  │    (3±0.1 mm)       │    │    recommended by       │◀─────│ Solvent evaporation   │
┌──────────────────────────┐      │  - Deep dentin      │    │    manufacturer         │      │ technique            │
│       Age of tooth       │─────▶│    (1±0.1 mm)       │    │  - Double primer        │      └──────────────────────┘
└──────────────────────────┘      └─────────────────────┘    │    application technique│      ┌──────────────────────┐
                                                             │    recommended by       │◀─────│ Applicators used     │
                                                             │    manufacturer         │      └──────────────────────┘
                                                             └─────────────────────────┘      ┌──────────────────────┐
                                                                                              │ Bonding area         │◀
                                                                                              └──────────────────────┘
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Remaining dentin thickness
- Superficial dentin (3±0.1 mm)
- Deep dentin (1±0.1 mm)

Primer application technique
- Bonding steps recommended by manufacturer
- Double primer application technique recommended by manufacturer

Application motions

Solvent evaporation technique

Applicators used

Bonding area

Bond under simulated pulpal pressure

Water storage for 6 months

Microtensile bond strength test

Hypothesis

Null hypothesis

$H0_1$: There was no significant difference in µTBS to dentin when using 2 different primer

application techniques.

$H0_2$: There was no significant difference in µTBS to dentin when using different types of

adhesives.

$H0_3$: There was no significant difference in µTBS in different dentin thicknesses.

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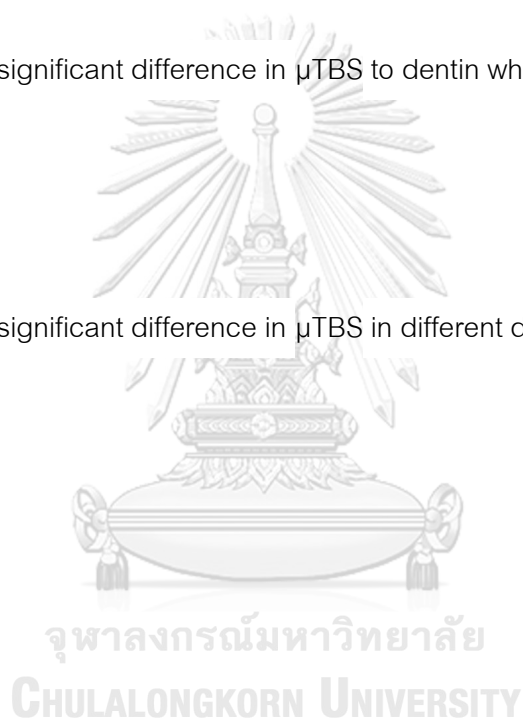
CHAPTER II

Literature review

Scope

I.   Dentin basic structure and composition

II.   Factors affecting dentin bonding

-   Depth, location, and tubule orientation of dentin

-   Internal/external moisture

-   Primer application

III. Laboratory studies related to microtensile bond strength

-   Simulated pulp pressure

-   Artificial aging

-   Microtensile bond strength

I. Dentin basic structure and composition

Dentin was the most abundant structural component of the human tooth.

Physically, dentin had an elastic quality that was the key for the proper function. Dentin

also contributed essential support to the highly mineralized and brittle enamel to resist

occlusal and masticatory forces without fracturing. Moreover, dentin was not only a

mechanical barrier but also the first vital tissue to meet external irritation, which associates

in the dentin–pulp complex to help its protecting reactions. Though dentin was usually

regarded as a whole entity, various depths of dentin have unique qualitative properties.

(21)
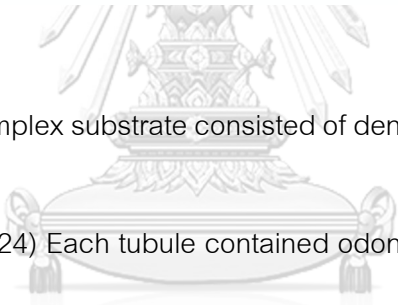
Mature dentin was made up of almost 70% inorganic material, 20% organic

material, and 10% water.(22) (Table 1) The inorganic component of dentin consisted of

substituted hydroxyapatite. The organic phase consisted of about 90% collagen (mainly

type I with small amounts of types III and V) with fractional inclusions of various non-

collagenous matrix proteins and lipids. Type I collagen acted as a scaffold containing a

large proportion of the mineral holes and pores of fibrils. The non-collagenous matrix

proteins regulated mineral deposition and act as inhibitors, promoters, and stabilizers for

dentinogenesis process.(23) They correlate with protease enzymes such as matrix

metalloproteinases (MMPs) and Cathepsins. (21)

Table 1 Basic composition of mineralized dentin (22)

	Inorganic	Organic	Water
% by weight	70	18	12
% by volume	30–50	30–50	20

Dentin was a complex substrate consisted of dentinal tubules, intertubular dentin,

and peritubular dentin. (24) Each tubule contained odontoblastic process and lined with

a layer of peritubular dentin, which was highly mineralized than surrounding intertubular

dentin. (25) Close to the pulp, tubules represented a larger portion of dentin volume and

had the greatest potential to wet the cut dentin surface immediately. The density of tubules

varied not only from the pulp to the dentin–enamel junction (DEJ), but also between

coronal and radicular dentin. The highest density of tubules was related to the inner third

of dentin associated with cusps. Hence The lowest density of tubules was related to the

outer third of cervical dentin. The density of dentinal tubules was increased more than

three times form the DEJ to the pulp in the coronal dentin. The surface area of dentin was

larger at DEJ and CEJ than its pulp cavity side. (Figure 2) Since odontoblast form dentin,

progressing inward to the pulp. The number of tubules increased from 15,000 to 20,000

/mm^2 at DEJ to 45,000 to 65,000 /mm^2 at the pulp. (Table 2) Moreover, dentin tubules were

penetrated with dentinal fluid which present in intertubular dentin area (3) The associated

density of tubule was highest near the pulp and cusps so that deep dentin was decreased

in the number of intertubular dentin. Moreover, size of the tubules also varied from the

DEJ to the pulp surface. In coronal dentin, the average diameter of tubules at the DEJ was

0.5-0.9 μm but increased to 2-3 μm near the pulp.

Distance from pulp (mm)	Number of tubules / cm²	Radius of tubules (µm)		Percentage of surface area					
				Tubules		Peritubular		Intertubular	
		B	A	B	A	B	A*	B	A
Pulp	4.5 × 10⁶	1.25	1.5	22.1	33.8	66.3	–	11.6	66.2
0.1–0.5	4.3 × 10⁶	0.95	1.5	12.2	30.4	36.6	–	51.2	69.6
0.6–1.0	3.8 × 10⁶	0.80	1.5	7.6	26.9	22.9	–	69.4	73.1
1.1–1.5	3.5 × 10⁶	0.60	1.5	4.0	24.7	11.9	–	84.2	75.3
1.6–2.0	3.0 × 10⁶	0.55	1.5	2.9	21.2	8.5	–	88.6	78.7
2.1–2.5	2.3 × 10⁶	0.45	1.5	1.5	16.2	4.4	–	94.2	83.9
2.6–3.0	2.0 × 10⁶	0.40	1.5	1.0	14.1	3.0	–	96.0	85.9
3.1–3.5	1.9 × 10⁶	0.40	1.5	1.0	13.4	2.9	–	96.2	86.6

Adapted from Nakabayashi & Pashley, 1998 (11).
* Assumes the more mineralized peritubular dentin is completely dissolved by the etchant.

Table 2 Changes in the area occupied by tubules, peritubular dentin, and inter tubular dentin before (B) and after acid etching (A), as a functional of location. (22)

Regarding dentin's structure, numerous studies revealed that dentin bond strength was affected by the remaining dentin thickness (RDT). The dentin bond strength significantly decreased when pulp chamber was neared.(26-28) Several authors have advanced some reasons for this finding, as followed:

(1) Intertubular dentin was the area available for micromechanical retention through hybridization. Hybridization decreased when the diameter and the number of dentinal tubules increased when closer to the pulp. (26, 29)

(2) The dentin permeability increased when using acidic condition completely

removed the smear layer. (27, 30)

(3) The pulpal pressure and intrinsic wetness increased. (31)

(4) The calcium concentration of deep dentin was less than superficial

dentin.(28)

II. Factors affecting dentin bonding

Depth, location, and tubule orientation of dentin

Because of the regional variance of dentin morphology related to tubule density,

the moisture of dentin also varied according to its features (11) and permeability when

smear layer and smear plugs are removed. The intrinsic water content of dentin was

higher when close to pulp and significantly lower toward the DEJ. So, moisture has been

considered as a factor that affected dentin bonding and resulted in lower bond strengths

in deep dentin compared to superficial dentin. (32) Nevertheless, lower bond strengths in

deeper dentin associated with the reduced quality of intertubular dentin. (32, 33) Using

the microtensile bond strength testing method, Yoshiyama et al. demonstrated regional

variance in coronal, cervical, and radicular dentin bond strength.(34) Bond strengths

tended to be lower in the apical third of the root and at the cervical margins of a cavity.

The orientation of the dentinal tubules had been reported that influence the

morphology of the hybrid layer produced by etch-and-rinse adhesives. Hybrid layers were

thicker, and resin tags were longer when bonding to dentin with a perpendicular tubule

orientation. On the contrary, thin hybrid layer formation and absence of resin tags were

reported for dentin with a paralleled tubule to the bonded area. (35) Tubule direction effect

on bond strength, however, remained inconclusive as it appeared to vary according to

adhesive used and testing method, as well as being subjected to confounding factors

such as dentin depth and location. (36, 37)

Internal/external moisture

Resin degradation was the result of a chemical reaction caused by a water

molecule that replaces the ester bond, which connect the methacrylate group in the

polymer chain (38) and monomer with the ester function group.(39) Apart from water,

esterase enzyme founded in saliva or bacteria byproduct could also produce this activity.

Water penetration into resin was one of the factors that activated resin degradation. Hydrolytic degradation happened only in the presence of water. The chemical reaction could break covalent bonds between polymers causing loss of resin mass.(40) The resin monomers in a polymer could be arranged in many different ways. Polymers could be linear, branched, or cross-linked. Linear polymers were made up of one long continuous chain, without any excess appendages, causing water penetrates easily. (15) In contrast, dense polymer, i.e., branched or cross-linked polymers, lead to a higher density of polymer, so having a small gap between polymers and therefore resulting in less water absorption. (41)

Hashimoto et al., 2003, (42) demonstrated that the failure within the hybrid layer in immediate bonding procedure, the dentin side of a fracture surface, revealed the presence of collagen fibers. Furthermore, after 1-year water storage, it was increasing in the number of collagen fibers according to the resin degradation in interfibrillar space. The dissolution of the resin in interfibrillar space is caused by the dissolution of unpolymerized resin and hydrolysis reaction of polyHEMA.

Hydroxyethyl methacrylate: HEMA, which was an important part of the bonding system, was a small size hydrophilic monomer that could be dissolved in water, alcohol, and acetone. (43) HEMA was a solvent and helped to unite the hydrophobic and hydrophobic elements. There was also hydrophilic property promoting monomer diffusion into dentin, gaining wettability of adhesive (adhesion-promoting monomer). (44) So HEMA could penetrate deeper than other monomers with larger molecules, such as Bisphenol A diglycidyl methacrylate (Bis-GMA), and acted as a cross-linking monomer, resulting in hybrid layers with different monomers at different depths. (42)

In a self-etch bonding system, acidic monomers could demineralize tooth surfaces, create a chemical bond, and activate polymer reactions with other monomers. In general, monomers had two specific compositions: functional group and polymerizable group. Acidic monomer function requires water for its ionization. On the other hand, having water contained within one bottle could cause monomer degradation. Acidic methacrylate monomer which had ester bond pruned to hydrolysis reaction, especially in high temperature. As a result, the acidic monomer became acidic molecules that could

not polymerization. Instead, it continuously dissolved mineralized dentin regardless the

end of polymerization, causing leakages at the bottom of hybrid layer. It supported

evidence from the previous observation. (45)

For the etch-and-rinse system, nanoleakage resulted from incomplete penetration

of resin monomer that is unable to encapsulate demineralized collagen. There was

nanoleakage located at the bottom of hybrid layers. (46) There were distinctive causes of

nanoleakage among etch-and-rinse and self-etching systems. Typical morphological

evidence of degradations was provided by collagen hydrolysis of etch-and-rinse adhesive

systems, resin elution from the hybrid layers of all systems, and hydrolytic degradation at

the border between the dentin/adhesive interface of adhesive layer. (47) As mentioned

above, exposure to water was a factor known to degrade tooth resin composite bonds.

Nanoleakage, or the ingress of oral fluids through nanometer sized channels along

collagen fibrils within hybrid layer, was considered harmful to bond integrity. (48-50)

Primer application

Etch-and-rinse adhesive systems were characterized by an initial etching step,

followed by a rinsing procedure resulting in complete removal of smear layer and smear

plugs. (51) Dentin adhesion was more complicated than enamel adhesion due to dentin

composition. At the same time, acid-etching promotes dentin demineralization over a

depth of 5-8 μm, thereby exposing collagen fibrils that were free of mineral content.(52)

Following the etching step was the primer application. Primer played an essential

role in dentin bonding. Dentin acid etching step not only dissolved the mineral content of

dentin but also reduced its surface energy. Adhesive had a low surface tension, and

etched dentin must have high surface-free energy.(53) Primer in the three-step etch-and-

rinse system was meant to increase the surface energy. When the primer was applied to

etch dentin, it penetrated into interfibrillar space of intertubular dentin. The primer

contained a specific monomer with hydrophilic property, such as HEMA, dissolved in

organic solvents like ethanol, acetone, and water. While HEMA was efficient for improving

the wettability and promoting the re-expansion of the collagen network. The solvents could

displace water in the interfibrillar space of dentin and prepare the collagen network for

the following adhesive resin infiltration.(54)

In the bonding step, a solvent-free adhesive resin (hydrophobic resin) was applied

on the primed surface, leading to infiltration of hydrophobic monomers not only into the

interfibrillar spaces of the collagen network to create the hybrid layer but also into dentin

tubules to create resin tags resulting in micromechanical retention for the composite

restoration(55). Simplified adhesives have been developed that combine primer and

adhesive resin into one solution. These simplified adhesives presented a reducing

capacity to infiltrate the demineralized dentin substrate, thereby producing suboptimal

hybridization when compared to their three-step etch and rinse system. (56) Furthermore,

the hydrophilic property of such adhesives rendered them prone to water sorption and

consequently more susceptible to the effects of hydrolytic degradation. Solvent present

in these adhesives was also more difficult to evaporate, frequently remaining entrapped

within the adhesive layer after polymerization. (57)

The absence of separate etching step characterized self-etch adhesives to create the pathways for resin infiltration. Self-etch adhesives were also divided into two-step and one-step self-etch adhesives. (58, 59) The acidic primers were responsible for dissolving the smear layer and partly demineralizing the underlying dentin. This demineralization was self-limiting due to the acidity of monomers that were gradually buffered by the mineral content of dentin. (60)

Nowadays, self-etch adhesives were categorized as mild (pH $\approx$ 2) and ultra-mild (pH >2.5). (59) The pH value of acidic monomer was considered the main parameter determining how molecules interact with mineralized tissue. In general, these self-etching adhesive monomers were bifunctional molecules containing at least the following components: first, a polymerizable group (P), which could react both with the other monomers of the adhesive and the restorative material by copolymerization, second, an acid adhesive group (AD) capable of both etching the dental hard tissues and interacting with the tooth substance, and, finally, a spacer group (R) designed to influence, e.g. the solubility, flexibility and wetting properties of the adhesive monomer. (38)

Chemical interaction was obtained through specific functional monomers, such as 10 methacryloyloxydecyldihydrogen phosphate or 10-MDP, 4-methacryloxyethyl trimellitic acid or 4-MET, and phenyl-P. The ionic bond formation of these functional monomers carboxylic/phosphate groups to calcium ions of HAp was first demonstrated by Yoshida et al. in 2004. (61) The success of this material has been attributed to its functional monomer, MDP, which was capable of chemically bonding to hydroxyapatite, and to the stability of its filled, solvent-free bonding resin. (58, 62)
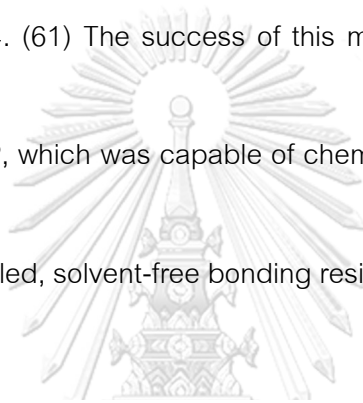
Cardoso et al., 2003 stated that increased application times could increase the dentin-resin microtensile bond strength of two-step etch-and-rinse resin adhesive in both water/ethanol and acetone based systems. (18) Similarly, Reis et al. compared immediate and 3-year bond strength to determine the effects of prolonged application time on the durability of resin-dentin bonds. Resin-dentin interfaces formed under prolonged application times were more stable over time. (19) In 2013, Ahmed et al. found dramatic difference in the microtensile bond strength of mild self-etch and strong self-etch adhesives when prolonged primer application time was used. Extending the primer

application time in strong self-etch primers increased dentin bond strength, which differed

from mild self-etch primers that extended time did not influence the bond strength. (63)

Duarte et al., 2006, argued that different etching times did not significantly increase silver

uptake within the hybrid layer. (64)

II. Laboratory studies related to microtensile bond strength

Simulated pulp pressure

Penetrated water through the hybrid and the adhesive layer could occur from hydrostatic pulpal pressure. (65, 66) Simulating physiological pulpal pressure in vitro has become a reliable assay to evaluate the behavior of dentin biomaterial bonding. Water sorption was enhanced, which plasticizes the polymer chains (67) , resulting in degradation of the bonding area, thus contributing to durability of resin-based materials. The impact of pulpal pressure on dentin bonding and durability was remarkable in some studies that measured physiological pulpal pressure in vivo. (68, 69) Wynn et al (70), stated that a direct relationship between pulpal pressure and arterial blood pressure, was important when treating patients with hypertension. Nevertheless, local anesthesia readily reduced pulpal blood pressure. (71, 72) Ciucchi et al (68) showed that normal human physiological pulpal pressure corresponded with a hydrostatic pressure of 8 to 22 cmH_2O. Most studies usually used simulated pulpal pressure with 15 to 20 cmH_2O. In vitro, this

procedure was performed with a water column connected to a plexiglass or acrylic plate,

through which an 18-gauge (0.13 cm) stainless steel tube was inserted. (73)

Simulated pulpal pressure played an essential role in adhesive dentistry

development and in vitro studies of dental bonding agents, resin composites, and resin

cements. This clinical variable revealed dentin sealing and restoration durability

difficulties and limitations during and after bonding. It promoted water penetration,

polymer degradation, and droplet disposition in the resin/dentin interface with a positive

physiological hydrostatic pressure through dentinal tubules. (73)

Aging Process

Nowadays, various techniques for aging specimens before bond strength testing

have been proposed.(74) Samples was stored in boiling water for 8 hours, soaking in citric

acid, storing in water with room temperature for a period of time, and thermocycling.

Among these techniques, the most popular were water storage and thermocycling

technique.(75) Aging by water storage, the specimens mostly stored in pure water at 37

^{o}C were utilized. The time can be modified from several months up to 4-5 years, or longer.

(76) The most used artificial aging technique is long-term water storage. Most studies

reported a significant decrease in bond strengths, even after relatively short storage

periods. In a study conducted by Armstrong et al. (2001), it was shown that the adhesive

failure deteriorated overtime in microtensile testing. It caused adhesive failures with

significantly different failure modes between one-month and six-month water storage. (77)

According to the ISO TR 11450 standard (1994), long-term tests after 6 month

storage in the water at 37°C could cause a significant decrease in bond strength. This

technique caused degradation of the interface from hydrolysis process. Moreover, water

could infiltrate into the polymer matrix leading to swelling and breaking down of ester bond

of the polymer chains.

Bond strength test

There were many methods available for measuring the dentin-composite bond

strength, such as tensile bond strength test, flexural bond strength test, or shear bond

strength test. (78) The measurement of bond strength effected by the concentration of

flaws within or between materials, specimen size and geometry, materials properties, and

loading application method. Shear bond strength was one of the most common bond

tests.

The specimen preparation of the shear bond strength test was a common

laboratory technique. It was performed exclusively in specimens with relatively large

bonded areas; usually 3-6 mm in diameter (approximately 7-12 mm^2). (79) However, one

of the drawbacks of this method was the non-uniform stress distribution in the substrate.

(80) Mostly, the failure did not initiate at the weakest point of the specimens and usually

occurs in the material or tooth specimen.

Tensile bond strength test was performed perpendicular to the adhesive bond

interface, and the stress distribution was more uniform in the cross-sectional bonded area.

However, larger specimens seemed to contain more defects than smaller specimens.(81)

The smaller test specimens, the larger amount of bond strength was observed due to a

lower chance of the critical-sized defect, because the bond strength value at failure depended on both fracture strength and the presence of defects.

The microtensile bond strength test, introduced by Sanoin 1994, was developed to overcome the limitation of tensile and shear bond strength test. The greatest advantage of this technique was obtaining solely adhesive failures of materials (82) if the bonded surface area was about 1 mm^2. Multiple specimens could receive from a single tooth. If the cross-sectional area of each specimen was the same, one could calculate a mean and standard deviation of the bond strength of a material to a single tooth. (82) It was possible to evaluate quality of adhesion by comparing the tensile stress at failure for different bonding agents, assess the mode of failure and perhaps indicate the weakest link in the adhesive systems without their geometric design features.(80) Another significant advantage was that the bonded surface did not have to be flat. Small irregular surfaces could evaluate under clinical conditions. (79)

However, the limitations of microtensile bond strength test were labor-intensive,

technically demanding, and difficulty in measuring the bond strengths of less than 5 MPa.

The technique also required special equipment. Moreover, the specimens were rapidly

dehydrated because they were tiny. (79) Hence, the specimens should be prepared

carefully to minimize the defects.

CHAPTER III

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). One hundred

and twelve sound human third molars extracted from 16–40-year-old patients according

to ISO technical specification 11405, who had provided informed consent, free of caries

or cracks were used in this study. The extracted teeth were washed thoroughly under

running water, and all blood and adherent tissues were removed. The teeth were stored

in a 1% aqueous solution of Chloramine-T for at least 1 week at room temperature. Based

on ISO 3696, the collected teeth were used within six months after extraction. Roots were

cut at 2 mm below the cementoenamel junction using a low-speed diamond saw (IsoMet

1000, Buehler; Lake Bluff, IL, USA) with water-cooling. Crown was cut perpendicular to

the long axis of the tooth to obtain the specified dentin thicknesses (Figure 1). The RDT

was measured and recorded vertically from the center of the tested interface to pulp

chamber (Figure 2). Dentin was examined and categorized into 2 groups; deep when the

RDT was 1 ± 0.1 mm (D), and superficial 3 ± 0.1 mm (S). The RDTs were measured using

a digital caliper. Dentin surfaces were then abraded with a 150-grit silicon carbide paper

with water to reach the desired RDT. Smear layer on dentin surface of the abraded teeth

was removed using a 10% citric acid for 1 min. (83) Pulp chamber opening (pulpal horn

exposure) was blocked with wax. Finally, a standardized smear layer was created using

a 600–grit silicon carbide paper (TOA, Thailand) through running water for 60 sec with a

polishing machine (Nano 2000, Pace technologies, USA) at 200 RPM. A piece of vinyl

tape with a 5-mm diameter hole was firmly attached to demarcate the adhesive area of

dentin for bonding.

Simulated pulpal pressure device

As mentioned in the previous study, a simulated pulp pressure device was

assembled and attached to the crown segment. (73) Briefly, the crown segments were

moisture controlled by dropping water into pulp chamber for 5 minutes and then fixed to

acrylic plates using a cyanoacrylate glue (Model repair II Blue, Densply, 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 cmH$_2$O (Figure 3). The

fluid infusion was presented during bonding and restoring as well as storage processes.

Bonding procedure

All 112 teeth were categorized into two difference RDT and each RDT were

randomly allocated into 8 groups (n = 7 for each group) based on 2 independent

variables, i.e., types of adhesive systems and primer application techniques as shown in

Table 3. Chemical composition, lot number of material used in the study and application

techniques are presented in Table 4 and Table 5 respectively. A piece of adhesive tape

with a 5-mm diameter hole was firmly attached to define the adhesive area of the dentin

for bonding. Primer application technique was used following the manufacturer's

instructions; primer was applied one time for the single application technique and two

times for the double application technique. Resin composite (Harmonize^{TM}, Kerr, Orange,

CA, USA) was then used for restoration. A light-emitting diode (LED) light-curing unit

(Demi^{TM} LED light-curing system, Kerr, Orange, CA, USA) was used to cure three

incremental 2-mm resin composite layers with an intensity of no less than 600 mW/cm^2 for

40 s each layer. 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)

(Figure 4).

Table 3 Group Identity

Tooth	RDTs	Adhesives	Primer application techniques	Group code
	Superficial detin (RDT = 3 ± 0.1 mm)	OFL	Single	OPL-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
112 extracted third molars	Deep detin (RDT = 1 ± 0.1 mm)	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 4 The resin adhesives and resin composites and their application

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	2.0	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	3M ESPE, USA/ 5541216
Pre-etching agent		Main component		Manufacturer
Gel Etchant		37.5% phosphoric acid, silica thickener		Kerr; Orange, CA, USA
Resin composite		Main component		Manufacturer/ Lot No
Harmonize™ Shade A3D Resin composite		Resin matrix: bis-GMA, bis-EMA, TEGDMA Filler: zirconia/silica nanoparticles		Kerr; Orange, CA, USA/ 7478613

Abbreviations: Bis-GMA: bisphenol A diglycidyl ether dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; TEGDMA: triethylene glycoldimethacrylate; 10-MDP: 10-methacryoloyloxydecyl dihydrogen phosphate; UDMA: urethane dimethacrylate; GPDM: glycerol phosphate dimethacrylate; PAMM: Methacroyloxyethyl Phthalate; Bis-EMA: Ethoxylate biphenol A glycol diamethacrylate

Table 5 The resin adhesives and resin composite and their applications

Materials	Bonding Steps Recommended by Manufacturer	Bonding Steps of Double Primer Application Technique
CSE	Prime: Apply a layer of primer, wait 20 s, gently air dry	Prime: Apply a layer of primer, wait 20 s, repeat the step, gently air dry
	Bond: Apply bonding agent, remove excess with a light jet of air and light cure for 10 s	Bond: Apply bonding agent, remove excess with a light jet of air and light cure for 10 s
OFL	Etch: Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s	Etch: Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s
	Prime: Apply primer with light scrubbing motion for 15 s, gently air dry 5 s	Prime: Apply primer with light scrubbing motion for 15 s, repeat the step, gently air dry 5 s
	Bond: Apply a thin coat of bonding agent and light cure for 20 s	Bond: Apply a thin coat of bonding agent and light cure for 20 s
SB	*Etch-and-rinse mode*	*Etch-and-rinse mode*
	Etch: Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s	Etch: Apply etchant 15 s, rinse with water 15 s, gently air dry 3 s
	Bond: Apply adhesive and rub for 20 s, dry gently for about 5 s, light cure for 10 s	Bond: Apply adhesive and rub for 20 s, repeat the step, dry gently for about 5 s, light cure for 10 s
	Self-etch mode	*Self-etch mode*
	Bond: Apply adhesive and rub for 20 s, dry gently for about 5 s, light cure for 10 s	Bond: 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	

Abbreviations: CSE; Clearfil SE Bond : OFL; Optibond[tm] FL : SB; Single Bond[tm] Universal Adhesive

Aging process

After the restorative procedure, the specimens were fix to the inside of the cylindrical receptacle's lid by pushing it sideways into the wax on the lid, without obstructing the pulpal chamber opening, as shown in figure 6. Filled the cylindrical container with sterile distilled water to reach 20 cm and closed the container with samples attached to the lid. Turn the container upside down to submit the samples to 20 cmH$_2$O pulpal pressure. (73) 7 specimens in each group were submerged in water at 37 $^{\circ}$C in an incubator (Contherm 160M; Contherm Scientific Ltd., Lower hut, New Zealand) for 6 months under simulated pulpal pressure (Figure 6). Following the ISO 11450, water was changed every 7 days to avoid contamination. The specimens were tested for bond strength immediately after being removed from the water.

Microtensile Bond Strength Testing

After storage, 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 before being sectioned (82) occluso-gingivally across the bonded

interface. The resin-dentin sticks (1 mm^2 cross-section) (84, 85) were prepared with a low

speed cutting machine (IsoMet® 1000, Buchler, USA) using the non-trimming technique,

as shown in figure 7. The 3-5 central sticks from each tooth were used for the µTBS test.

The stick-shaped specimens were fixed to testing jig using 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 (Figure 8). The exact cross-sectional area of each tested sticks was

measured after failure using a digital caliper. The mean bond strength of the 4 sticks from

each   tooth   represented   the   µTBS   of   that   tooth   (73,   86),   generating

7 values per group.

Failure Mode Analysis

After μTBS test, the fractured surface of both dentin and composite sides were evaluated by a stereomicroscope at 45X magnifications (ML 9300®, MEJI, Japan), as shown in figure 9 and recorded as percentage of the followings (87):

- Type A: adhesive failure at the interface between resin composite, adhesive and hybrid layers

- Type M: mix failure, i.e., fracture occurred involving both the resin-composite interfaces and the neighboring substrates

- Type C: cohesive failure in resin composite

- Type D: cohesive failure in 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).

SEM Analysis

The parts of fractured specimens were paired, air-dried, and mounted on aluminum stubs, coated with gold (Figure 10), and evaluated at magnifications of 5,000x using a scanning electron microscope (SEM) (JSM-6610LV Scanning Electron Microscope JEOL, USA) at an acceleration voltage 20 kV to confirm mode of failure.

Statistical Analysis

All statistical procedures were performed using the Statistical Package for Social Sciences software, Version 25. 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 µTBS values were evaluated using a Paired t-test and ANOVA followed by a Post Hoc test. For all analyses, statistical significance was set at α =0.05

Figure 1 Specimen preparation(A) lateral view (B) top view shown flat dentin (C) bottom view shown pulp chamber

Figure 2 Remaining dentin thickness was confirmed by a digital vernier caliper
(SHAHE, Chaina).

Figure 3 Tooth preparation and simulated fluid flow through a sectioned crown using 20
cm distilled water pressure

Figure 4 Light-curing unit with > 600 mW/cm^2 intensity was checked with a Radiometer

(L.E.D. radiometer by Demetron, Kerr Corporation, Danbury, CT, USA)
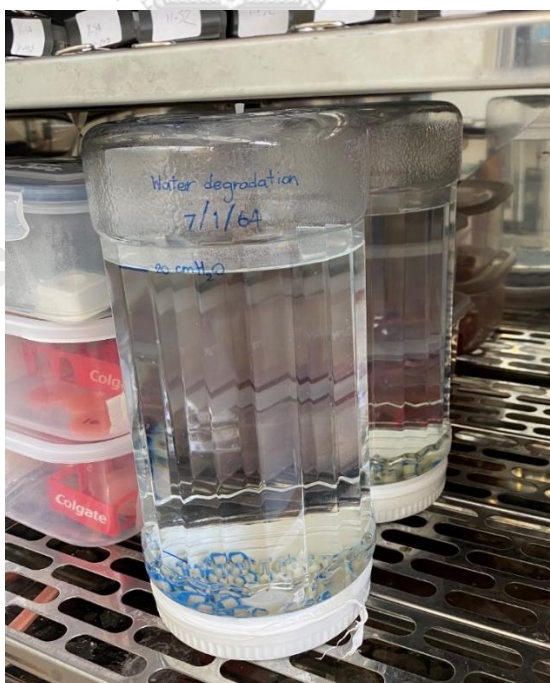
Figure 5 The samples were stored under 20 cmH$_2$O in an incubator at 37 $^{\circ}$c for 6

months

Figure 6 The resin-dentin stick used for microtensile test

(A)The resin-dentin stick of deep dentin

(B)The resin-dentin stick of deep dentin
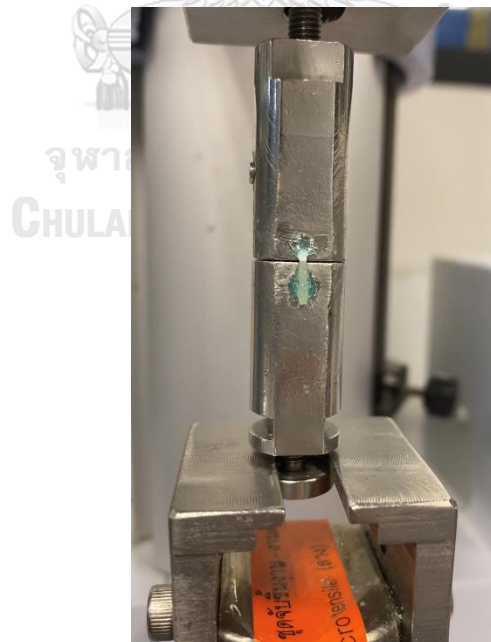
Figure 7 Microtensile bond strength was tested using a universal testing machine

(EZ-S Shimadzu, Japan)

Figure 8 Stereomicroscopre ML 9300 (MEIJI, Japan)
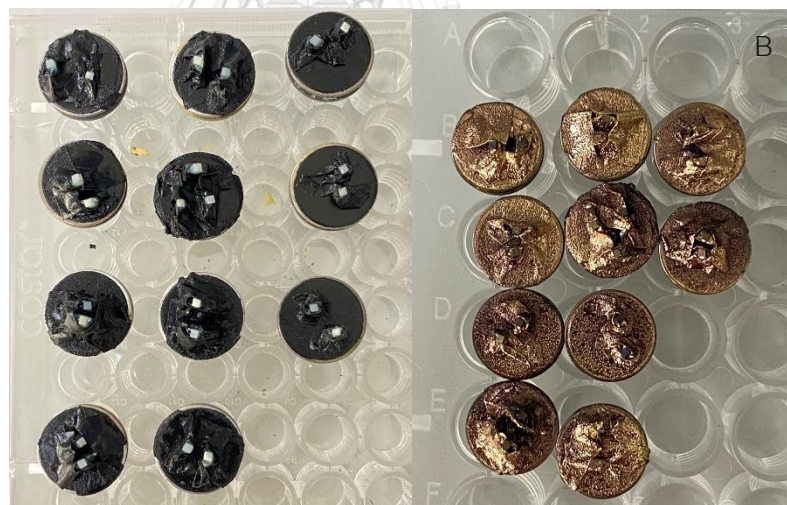
Figure 9 Sample preparation for SEM evaluation

(A) fractured specimens were mounted on aluminum stubs.

(B) fractured specimens were coated with gold.

CHAPTER IV

Results

Microtensile bond strength

Three-way ANOVA data, presented in Table 6, and demonstrated that dentin depth ($p < 0.001$) types of adhesives ($p < 0.001$), and the number of applications ($p =0.014$) statistically significantly impacted the µTBS. The interaction of dentin depth and types of adhesive ($p < 0.001$), depth and application ($p =0.038$) were also significant, except the number of application did not significantly interact with types of adhesive ($p = 0.145$). The interaction of these 3 factors was also significant ($p < 0.001$).

Table 6 Three-way ANOVA for depth, adhesive, number of application, and their interaction on µTBS.

Source	df	Sum of Squares	Mean Square	F	p
Depth (A)	1	1551.985	1551.985	26.120	< 0.001*
Adhesives (B)	3	3574.109	1191.370	20.051	< 0.001*
Application (C)	1	370.552	370.552	6.236	0.014*
A x B	3	1259.550	419.850	7.066	<0.001*
B x C	3	328.329	109.443	1.842	0.145
A x C	1	263.161	263.161	4.429	0.038*
A x B x C	3	1867.370	622.457	10.476	<0.001*
Error	96	5704.052	59.417		

*Significant at $p < 0.05$

Mean µTBS values and standard deviations (SD) are presented in Table 7.

Considering the number of primer application, in single application groups, overall µTBS

values of OFL and SBSE groups bonded to superficial dentin were significantly higher

than mean values obtained from deep dentin. Despite not significant difference, SBER

bonded to superficial dentin showed higher µTBS value (35.72 ± 9.49) than that bonded

to deep dentin (33.76 ± 6.76). Contrasting with other groups with single application, CSE

group bonded to superficial dentin (15.55 ± 4.56) showed no significant lower mean µTBS

value than that bonded to deep dentin (22.09 ± 7.75). With double application, while SBER

and SBSE groups with double application showed no statistically significant difference of

µTBS values between superficial and deep dentin, in OFL and CSE groups bonded to

showed superficial dentin statistically significantly higher mean µTBS values than deep

dentin.

Considering the depth of dentin, when using the single application technique, the

OFL and SBSE groups had a significantly higher µTBS value in superficial dentin

compared with deep dentin, however, there was no significant difference in µTBS values

between superficial dentin and deep dentin in the SBER and CSE groups. When a double

application was used, the OFL and CSE groups demonstrated a significantly higher μTBS

value in superficial dentin than deep dentin, however, there was no significant difference

in μTBS values between superficial dentin and deep dentin in the SBER and SBSE groups.

Table 7 Means ± SD of the microtensile bond strength values (MPa) in each group of 3 kinds of adhesive systems applied in 2 modes, 2 different remaining dentin thicknesses, and 2 different primer application techniques

Adhesive system	Optibond FL		Single Bond Universal (Etch&rinse mode)		Clearfil SE Bond		Single Bond Universal (Self-etch mode)	
Application Procedure	Manufacturer's Instruction	Double Primer Application	Manufacturer's Instruction	Double Primer Application	Manufacturer's Instruction	Double Primer Application	Manufacturer's Instruction	Double Primer Application
Superficial dentin	34.43 ± 4.47 [a,A]	29.55 ± 6.45 [a,A]	35.72 ± 9.49 [a,A]	31.82±14.30 [a,A]	15.55±4.56 [b,B]	22.51± 5.16 [a,A]	50.97±11.68 [c,A]	25.97±13.87 [a,A]
Deep dentin	18.11±5.17 [a,B]	18.46 ± 5.77 [a,B]	33.76±6.76 [b,A]	31.78±3.58 [b,A]	22.09 ±7.75 [a,B]	14.99 ± 2.47 [a,B]	20.67 ± 4.34 [a,B]	27.11±3.85 [b,A]

Means ± 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.

Failure mode analysis

The failure modes were classified by group, as shown in figure 11. Adhesive failure was the predominant mode of failure for both superficial dentin and deep dentin; however, the SBER-S1, SBER-S2, SBER-D1 and SBER-D2 groups demonstrated a tendency toward multiple modes of failure. The representative stereomicroscope photographs of failure mode were shown in figure 12.
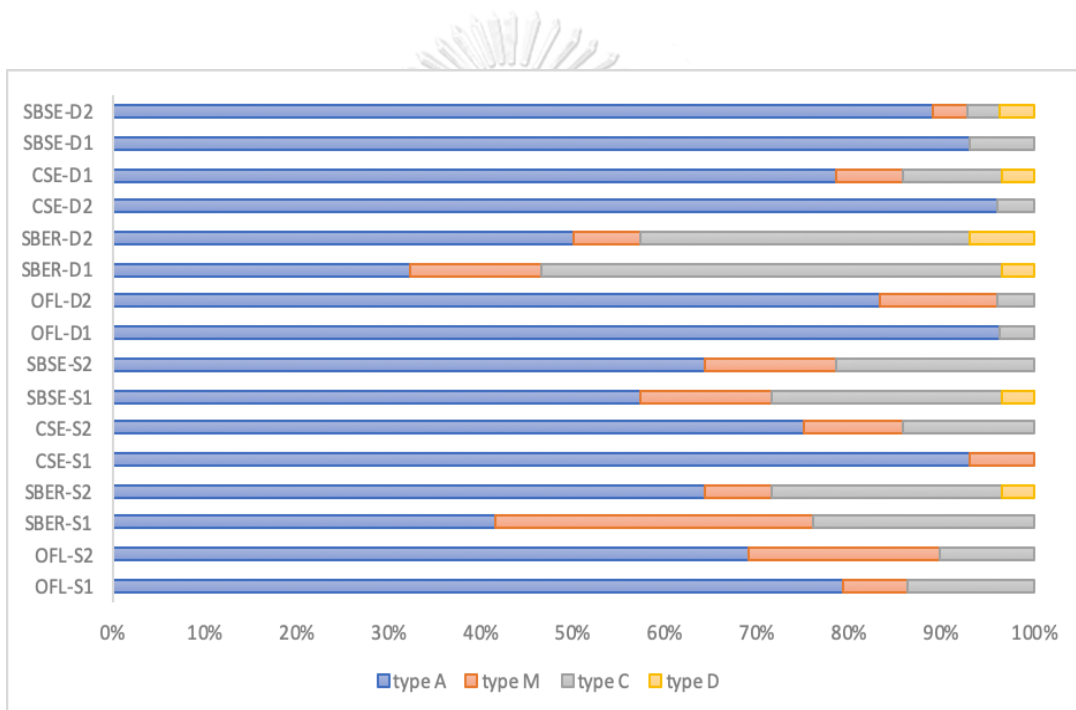
Figure 10 The percentages of the failure modes of the µTBS samples analyzed using a stereomicroscope
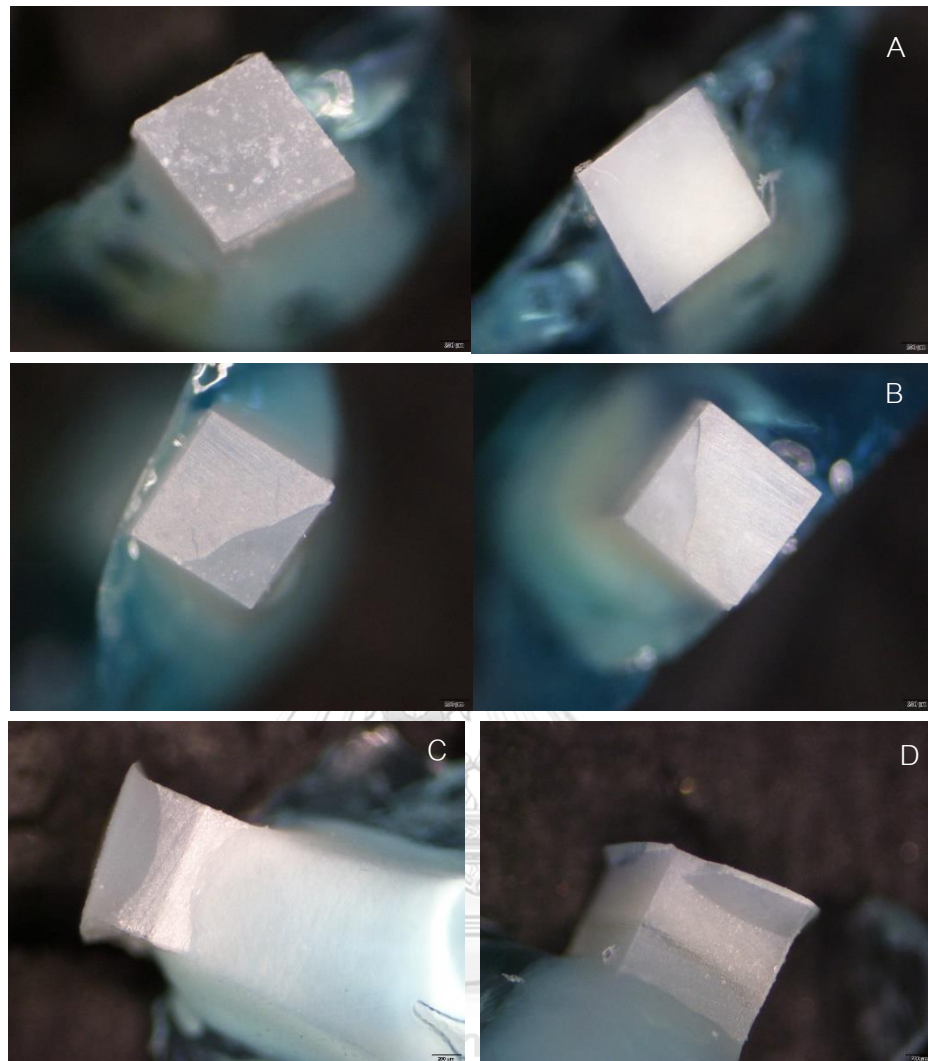
Figure 11 Representative stereomicroscope photographs at 45X magnification of pair of fracture samples

(A) The fracture occurred at the resin-dentin interface (adhesive failure).

(B) The fracture occurs involving both of resin-dentin interfaces and resin composite (mixed failure)

(C) The fracture occurred within the resin composite layer (cohesive failure of composite).

(D) The fracture occurred within the dentin (cohesive failure of dentin).

SEM analysis

A predominant adhesive failure was shown in figure 13. 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 (Figure 13A and 13B). In contrast to etch-and-rinse

sample, self-etch sample demonstrated occluded dentinal tubule presenting in most of

examined area (Figure 13C). The fractured surface of composite side showed scant resin

tags comparing to etch-and-rinse sample (Figure 13D).
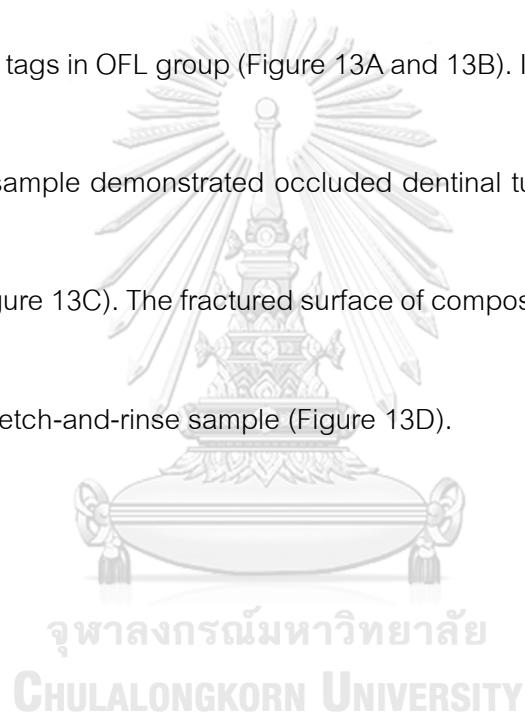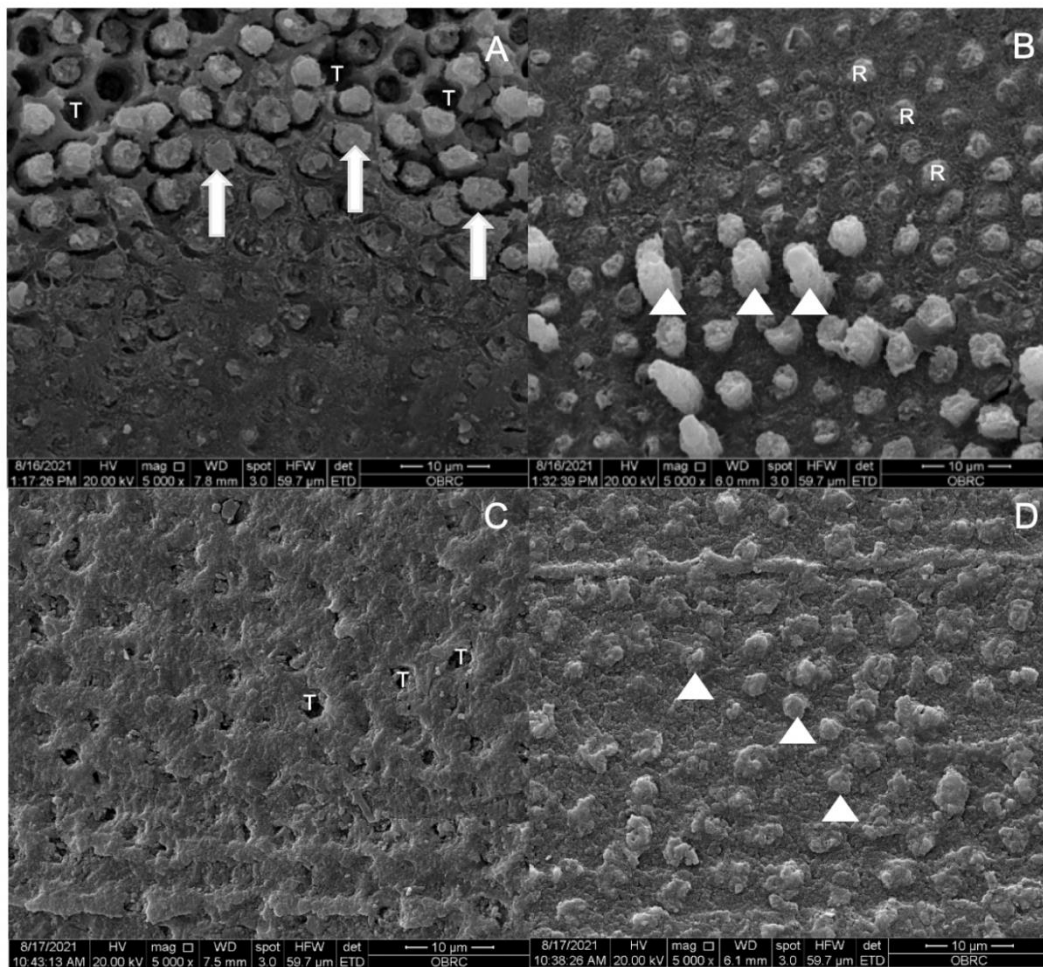
Figure 12 representative SEM photographs at 5,000X magnification of samples of samples in the OFL-D2 and SBSE-D1 group which represent adhesive failure. (A) Fracture surface of the dentin side revealed adhesive failure with open dentinal tubules (T) and dentinal tubules filled with resin tags (arrow), (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), and (D) Fracture surface of the composite side revealed adhesive failure with prominent resin tags (white arrowhead).
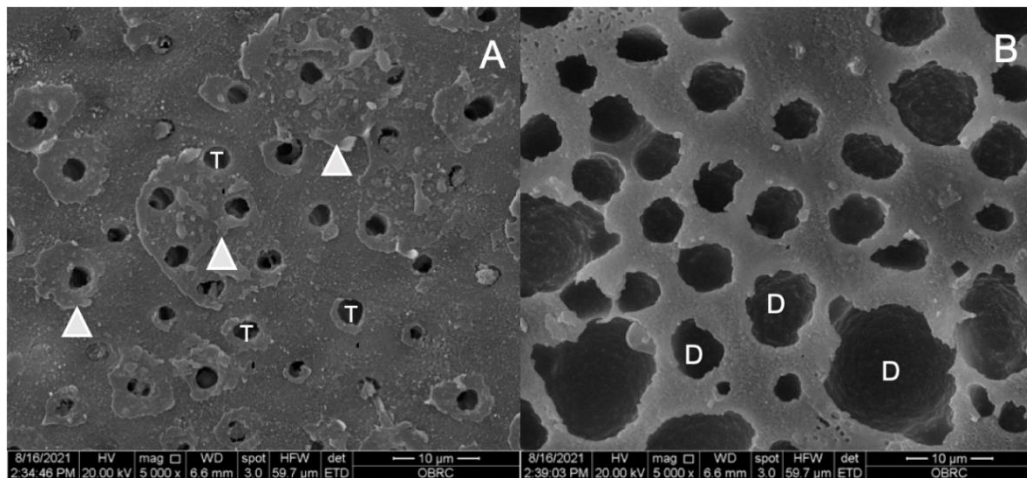
Figure 13 representative SEM images at 5,000X magnification of samples in the SBER-S2 group. (A) Fractured surface of the dentin side revealed adhesive failure with open dentinal tubules (T) and blemish of adhesive (white arrowhead). (B) Fractured surface of the resin composite side revealed adhesive failure with voids representing water droplets (D) within the bottom of resin composite side.
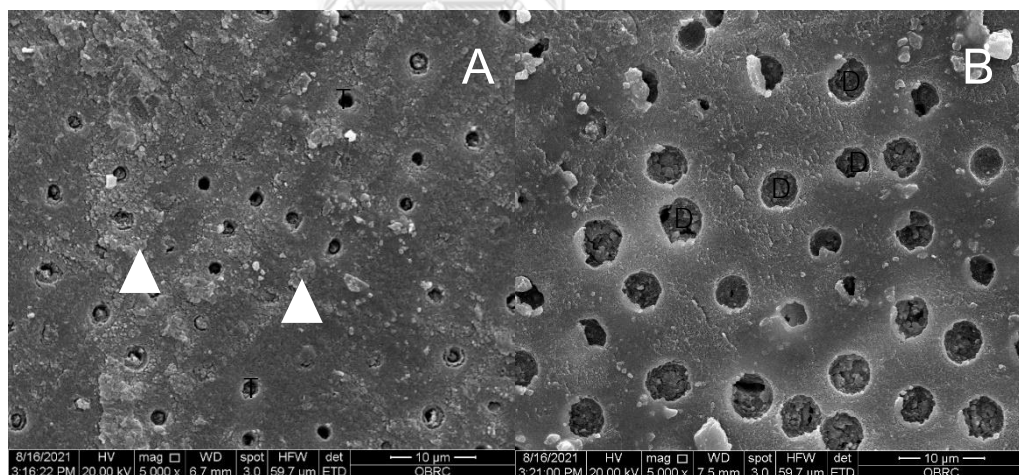
Figure 14 representative SEM images at 5,000X magnification of samples in the CSE-S1 group. (A) Fractured surface of the dentin side revealed adhesive failure with open dentinal tubules (T) and blemish of adhesive (white arrowhead). (B) Fractured surface of the resin composite side revealed adhesive failure with voids representing water droplets (D) within the bottom of resin composite side.

CHAPTER V

Discussion

The present study was designed to determine the effect of application technique, types of adhesives, and RDT on microtensile bond strength (µTBS) of conventional and simplified universal adhesive system under simulating 20 cmH$_2$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 mostly adhesive failure, which was desirable to demonstrate the true bond strength between two substrates (79).

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 (88) or amount of primer, as in this study, seemed unable to increase the bond strength of 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, application technique impacted their behaviors in this study.

Considering the adhesive systems, 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 (89). 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 (90). However, a recent study reported inconsistent double application in

increasing the µTBS of this adhesive in either mode (91) 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 to interact with greater quantity of inter-tubular dentin in superficial dentin (92).

This technique increased amount of solvent, though. Clearfil SE was a water-based

adhesive. Water from double application may hinder ability to evaporate both intrinsic

wetness from simulated pulpal pressure and extrinsic water from solvent itself, which

could be seen in SEM as shown in figure 15. It might be residual solvent in adhesive layer.

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 (93). 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 (89, 94, 95). 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).

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

presented oppositely. Hydrophilic resin adhesive could infiltrate and polymerize in such

moist condition (96) 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 bond 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 figure 14.

Such defects in adhesive layer may attribute to water sorption and harm the bond efficacy

in a long-term of clinical service. (15)

According to the results of this study, the bonding performance of superficial

dentin generally presented higher than deep dentin. Dentin permeability was lower when

treated with mild acidic primer in self-etch adhesive system (65, 97). 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 (98). and in Clearfil SE 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 (89), resulting in lower bond strength to deep

dentin than superficial dentin when using a single application. Etching step in etch-and-

rinse system, either conventional or universal, completely removed all smear layer, smear

plug, and demineralized dentin up to 5 µm (99) resulting in increased outward flow of

dentinal fluid. In deep dentin, a greater number of tubules and a higher fluid flow rate

(100) 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 (101), 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 (102). These combined factors

attributed to different result of OFL from previous studies (101, 103) which evaluated one

factor without aging. In this study, one confounding factor of the substrate was using

human third molars, which were selected from patients aged 16-40 according to ISO

11405. Still, it varies in some conditions, such as eruption patterns, opposed tooth,

morphology, and even the age of the tooth. These variations might affect each tooth's

mean value of microtensile bond strength, which could explain why some groups have a

large standard deviation. Therefore, further study should specify eruption patterns,

opposed tooth, and control the average tooth age for each group.

In terms of the failure modes, adhesive failure was desirable to indicate the real

bond strength between two substrates.(79) Overall, adhesive failure mode was

predominantly observed in all groups except SBER-D1, which exhibited a cohesive failure

in a resin composite. The result might due to the geometry of microtensile specimen that

stick-shaped specimen with a 1 mm x 1 mm cross-sectional area. The most stress

concentration did not perform at the bonded interface, but it distributed in materials

between the glue attachment.(104) This reason was possible to explain that a large

standard deviation of SB group in superficial dentin. This study was designed only to

simulate one circumstance that provides pulpal pressure from bonding step through six-

month period while hydrolysis degradation not only occurred in bonding interface but also

in resin composite. It was well known that long-term water storage could cause bond

degradation.(77) The penetration of water into resin was one factor that can activate resin

degradation. Hydrolytic degradation happened in the presence of water and led to a

chemical reaction capable of breaking the covalent bonds between polymers causing

loss of resin mass.(39) The degradation was determined by the effects of water storage.

Alshali et al. (2015) assessed sorption and solubility of resin composite after one-year

storage, and they found that BisGMA-based systems were more hydrophilic properties,

water absorptive and solute than BisEMA-, UDMA-BisEMA-based resin.(105) There is

consorted with Harmonize®, BisGMA-base resin. Accordingly, it was possible to explain

that cohesive failure of resin composite occurred due to water degradation.

Our results indicated that universal adhesive was less sensitive to intrinsic

wetness. Therefore, we suggest that manufacturer's instructions can be followed when all

tested adhesive systems are used. However, many new bonding materials, nowadays,

have been available on the market, only three adhesive systems from three manufacturers

were investigated in the study, thus, the results from this study may not be inferred to other

adhesive systems. Further studies involving other compositions of adhesive systems is

recommended. Besides, the application technique should be further investigated in order

to improve bond efficacy under other fluid perfusion and different storage times. Even

though in vitro microtensile bond strength could not completely imply the clinical

performance of these adhesives, our results can be informative data for future studies and

to urge clinician to be aware of these factors.

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APPENDIX

Appendix A. Remaining dentin thickness of superficial dentin (mm)

Superficial dentin

	1	2	3	4	5	6	7	Mean	SD
SBSE-S1	2.99	2.93	2.93	3.09	2.95	2.94	2.9	2.96	0.06
SBSE-S2	2.95	2.97	3.01	3.05	3.07	3.03	2.93	3.00	0.05
SBER-S1	3.08	3.06	3.09	3.07	3.01	3.08	3.04	3.06	0.03
SBER-S2	2.95	3.03	3.04	3.04	2.93	2.97	3	2.99	0.05
CSE-S1	2.93	3	2.94	3.07	2.97	2.98	2.96	2.98	0.05
CSE-S2	3.05	3.07	3	3.03	3.02	3.06	3.05	3.04	0.02
OFL-S1	3.03	2.95	3.04	2.9	2.9	3.07	3	2.98	0.07
OFL-S2	2.98	3.05	2.99	2.92	2.95	3.08	2.93	2.98	0.06

Appendix B. Remaining dentin thickness of deep dentin (mm)

Deep dentin

	1	2	3	4	5	6	7	Mean	SD
SBSE-D1	1.1	1.07	0.99	1	1.06	1.08	1.04	1.05	0.04
SBSE-D2	1.05	1.02	1.03	1.08	1.04	1.08	1.1	1.06	0.03
SBER-D1	1.1	1.06	1.09	1.08	1.1	0.97	0.9	1.04	0.08
SBER-D2	1.04	1.05	1.01	1.06	1.09	0.97	1.1	1.05	0.05
CSE-D1	1.07	1.01	1.1	1.09	1.08	1.1	1.08	1.08	0.03
CSE-D2	1.08	0.99	1.09	0.99	1	1.06	0.98	1.03	0.05
OFL-D1	0.95	1.07	1.06	1	0.94	0.98	0.94	0.99	0.05
OFL-D2	0.97	1.02	1.09	0.96	0.99	0.99	0.91	0.99	0.02

Appendix C. Microtensile bond strength of OFL-S1 group

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T1	30.93	43.07	21.16	33.37		32.13

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T2	51.81	50.10	33.96	40.16		44.01
T3	15.68	39.47	26.36	45.57		31.77
T4	39.57	29.11	43.16	22.85		33.67
T5	33.30	35.50	43.26	31.19		35.81
T6	37.74	27.22	27.22	36.30		32.12
T7	24.84	41.75	43.85	24.39	22.84	31.53

Mean Group	34.43
SD	4.47
Predominance Failure Mode	Adhesive failure

Appendix D. Microtensile bond strength of OFL-S2 group

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T1	20.03	13.56	25.91	4.15		15.91
T2	37.73	24.45	34.60	26.60		30.85
T3	37.65	31.95	49.77	37.25	27.32	36.79
T4	34.26	26.87	29.33	35.09		31.39
T5	23.20	33.58	37.01	26.06		29.96
T6	25.96	38.87	31.82	23.65		30.07
T7	20.17	42.16	20.65	44.53		31.88

Mean Group	29.55
SD	6.45
Predominance Failure Mode	Adhesive failure

Appendix E. Microtensile bond strength of SBER-S1 group

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T1	66.79	58.48	42.84	57.89		56.50

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T2	44.51	22.79	20.96	27.58		28.96
T3	42.70	40.11	26.05	34.08		35.74
T4	33.78	43.95	37.01	27.31	21.54	32.72
T5	25.73	37.74	27.72	30.49		30.42
T6	22.50	40.96	25.69	33.65		30.70
T7	44.23	23.87	31.99	39.85		34.99

Mean Group	35.72
SD	9.45
Predominance Failure Mode	Adhesive failure

Appendix F. Microtensile bond strength of SBER-S2 group

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T1	44.82	41.73	30.14	26.86		35.89
T2	30.66	23.56	28.59	52.06		33.72
T3	7.50	4.40	11.05	6.88		7.46
T4	11.20	31.70	14.11	13.23		17.56
T5	55.45	30.58	53.65	53.42		48.27
T6	42.98	22.08	41.41	45.22		37.92
T7	52.69	55.17	39.36	24.20	36.47	41.94

Mean Group	31.82
SD	14.3
Predominance Failure Mode	Adhesive failure

Appendix G. Microtensile bond strength of CSE-S1 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	12.01	12.84	23.11	19.70	16.91
T2	16.83	14.20	16.50	21.74	17.31

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T3	10.36	9.11	13.54	7.13	10.03
T4	35.30	9.85	37.94	11.09	23.54
T5	20.84	19.00	15.31	10.24	16.35
T6	11.73	10.75	10.94	10.09	10.88
T7	11.91	15.52	20.59	7.29	13.83

Mean Group	15.55
SD	4.56
Predominance Failure Mode	Adhesive failure

Appendix H. Microtensile bond strength of CSE-S2 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	45.74	18.14	12.68	30.22	26.69
T2	36.55	23.99	18.99	19.32	24.71
T3	17.83	15.15	24.61	27.55	21.28
T4	25.84	25.22	16.94	9.94	19.48
T5	32.81	28.13	30.61	32.00	30.89
T6	9.40	20.35	17.66	18.24	16.41
T7	22.34	13.43	22.13	14.66	18.14

Mean Group	22.51
SD	5.16
Predominance Failure Mode	Adhesive failure

Appendix I. Microtensile bond strength of SBSE-S1 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	42.93	57.82	53.51	49.52	50.94
T2	61.69	52.50	60.31	47.52	55.51

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T3	55.07	54.44	64.32	70.37	61.05
T4	29.99	77.34	65.54	96.69	67.39
T5	61.54	35.24	39.52	38.30	43.65
T6	35.38	28.86	35.89	29.21	32.33
T7	55.10	28.30	60.57	39.58	45.89

Mean Group	50.97
SD	11.68
Predominance Failure Mode	Adhesive failure

Appendix J. Microtensile bond strength of SBSE-S2 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	34.99	31.08	25.28	42.10	33.36
T2	62.65	46.97	27.31	75.31	53.06
T3	18.13	18.07	19.54	29.12	21.22
T4	19.09	25.18	20.60	28.25	23.28
T5	27.57	12.94	16.40	29.84	21.69
T6	6.82	5.22	12.55	12.07	9.17
T7	24.86	7.75	13.69	33.73	20.01

Mean Group	31.82
SD	14.3
Predominance Failure Mode	Adhesive failure

Appendix K. Microtensile bond strength of OFL-D1 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	10.41	25.13	21.89	-	19.14
T2	11.35	25.94	19.99	-	19.09

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T3	24.32	4.14	7.02	17.53	13.25
T4	33.85	17.9	15.06	8.88	18.92
T5	7.94	13.17	39.51	-	20.20
T6	9.65	12.76	7.68	-	10.03
T7	15.56	6.51	41.01	41.44	26.13

Mean Group	18.11
SD	5.17
Predominance Failure Mode	Adhesive failure

Appendix L. Microtensile bond strength of OFL-D2 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	24.67	12.92	20.66	-	19.42
T2	11.26	16.01	16.38	-	14.55
T3	10.78	26.32	16.76	-	17.95
T4	14.4	8.49	33.99	21.85	19.68
T5	41.44	17.64	31.35	-	30.14
T6	22.88	13.38	7	-	14.42
T7	16.43	12.23	18.77	4.77	13.05

Mean Group	18.46
SD	5.77
Predominance Failure Mode	Adhesive failure

Appendix M. Microtensile bond strength of SBER-D1 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	32.88	22.94	16.83	36.21	27.22
T2	24.49	26.49	35.03	20.53	26.63

					Mean Tooth
T3	51.08	45.79	36.83	33.82	41.88
T4	39.32	25.93	45.56	50.95	40.44
T5	38.55	30.87	50.72	31.63	37.94
T6	18.81	37.84	28.39	21.76	26.7
T7	37.3	28.07	34.59	41.97	35.48

Mean Group	33.76
SD	6.76
Predominance Failure Mode	Cohesive failure of composite

Appendix N. Microtensile bond strength of SBER-D2 group

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T1	25.65	47.28	22.66	18.54	-	28.53
T2	43.71	14	33.97	26.13	-	29.45
T3	23.11	27.95	35.7	41.23	21.7	29.94
T4	36.27	37.91	31.39	33.89	-	34.86
T5	40.89	34.07	37.68	37.34	-	37.5
T6	28.39	39.82	22.12	23.33	-	28.42
T7	36.42	30.73	27.03	40.79	-	33.74

Mean Group	31.77
SD	3.58
Predominance Failure Mode	Adhesive failure

Appendix O. Microtensile bond strength of CSE-D1 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	30.27	9.92	17.2	16.8	18.55
T2	10.49	8.3	18.43	15.38	13.15

	Stick1	Stick2	Stick3	Stick4		Mean Tooth
T3	25.27	37.06	35.77	39.78		34.47
T4	21.31	30.59	19.91	36.03		26.96
T5	16.72	26.56	34.67	10.57		22.13
T6	12.93	7.04	17.5	16.15		13.41
T7	47.89	35.39	3.3	17.35		25.98

	Mean Group	22.09
	SD	7.75
	Predominance Failure Mode	Adhesive failure

Appendix P. Microtensile bond strength of CSE-D2 group

	Stick1	Stick2	Stick3	Stick4	Stick5	Mean Tooth
T1	2.85	27.24	5.43	26.93	8.28	14.15
T2	13.92	18.46	18.66	17.01	-	17.01
T3	12.07	13.09	11.93	12.36	-	12.36
T4	25.15	27.21	8.98	16.34	-	19.42
T5	7.73	18.11	13.14	-	-	12.99
T6	8.9	13.58	19.1	-	-	13.86
T7	10.27	15.08	16.8	18.4	-	15.14

	Mean Group	14.99
	SD	2.47
	Predominance Failure Mode	Adhesive failure

Appendix Q. Microtensile bond strength of SBSE-D1 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	22.58	12.94	15.02	33.1	20.9

					Mean Tooth
T2	19.28	18.79	30.64	2562	23.58
T3	15.5	14.46	14.63	29.66	18.55
T4	18.43	15.09	26.02	18.84	19.59
T5	27.84	10.07	34.26	40.97	28.29
T6	18.21	10.46	14.92	13.74	14.33
T7	23.3	22.67	14.93	20.08	20.25

Mean Group	20.67
SD	4.34
Predominance Failure Mode	Adhesive failure

Appendix R. Microtensile bond strength of SBSE-D2 group

	Stick1	Stick2	Stick3	Stick4	Mean Tooth
T1	14.34	27.58	21.95	26.36	22.55
T2	33.49	28.77	19.26	19.38	25.23
T3	28.95	24.5	31.13	25.32	27.48
T4	32.62	19.6	29.27	26.26	26.94
T5	28.52	36.52	36.45	-	33.83
T6	24.03	32.81	35.53	27.51	29.97
T7	23.06	33.39	16.48	22.19	23.78

Mean Group	27.11
SD	3.85
Predominance Failure Mode	Adhesive failure

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