Comparison of microtensile bond strength of universal adhesives utilizing various application methods: an in vitro study

Miss Suthatta Jeansuwannagorn

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Esthetic Restorative and Implant Dentistry
Common Course
Faculty of Dentistry
Chulalongkorn University
Academic Year 2018

การเปรียบเทียบความแข็งแรงในการยึดติดแบบดึงระดับจุลภาคของสารบอนดิงยูนิเวอซัลโดยการใช้วิธีต่างๆ: การศึกษาในห้องปฏิบัติการ

น.ส.สุทัตตา เจียรสุวรรณกร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม ไม่สังกัดภาควิชา/เทียบเท่า
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561

Thesis Title	Comparison of microtensile bond strength of universal a dhesives utilizing various application methods: an in vitro study
By	Miss Suthatta Jeansuwannagorn
Field of Study	Esthetic Restorative and Implant Dentistry
Thesis Advisor	Associate Professor SIRIVIMOL SRISAWASDI, Ph.D.

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Science

.... Dean of the Faculty of Dentistry
(Assistant Professor Suchit Poolthong, Ph.D.)

THESIS COMMITTEE

.... Chairman
(Associate Professor MANSUANG ARKSORNNUKIT, Ph.D.)

.... Thesis Advisor
(Associate Professor SIRIVIMOL SRISAWASDI, Ph.D.)

.... External Examiner
(Assistant Professor Pong Pongprueksa, Ph.D.)

สุทัตตา เจียรสุวรรณกร : การเปรียบเทียบความแข็งแรงในการยึดติดแบบดึงระดับจุลภาคของสารบอนดิงยูนิเวอซัลโดยการใช้วิธีต่างๆ: การศึกษาในห้องปฏิบัติการ. (Comparison of microtensile bond strength of universal adhesives utilizing various application methods: an in vitro study) อ.ที่ปรึกษาหลัก : รศ. ทญ. ดร.ศิริวิมล ศรีสวัสดิ์

จุดประสงค์: เพื่อศึกษาความแข็งแรงในการยึดติดแบบดึงระดับจุลภาคของสารบอนดิงแบบ 3 ขั้นตอน และสารบอนดิงยูนิเวอซัลร่วมกับวิธีการใช้กรดกัดแล้วล้างน้ำออก โดยใช้วิธีการทาสารบอนดิงในแบบต่าง ๆ แล้ววัดค่าแรงยึดติดหลังแช่น้ำ 24 ชั่วโมงและ 6 เดือน

วัสดุและวิธีการ: ฟันกรามซี่ที่สามจากมนุษย์ 72 ซี่ถูกตัดเพื่อเผยเนื้อฟัน โดยใช้สารบอนดิงระบบใช้กรดกัดแล้วล้างน้ำออกได้แก่ สารบอนดิง Optibond FL (OFL; Kerr, USA) ทาตามคำแนะนำของผู้ผลิต, สานบอนดิง Single Bond Universal (SU; 3M ESPE, USA), Clearfil Universal Bond Quick (CU; Kuraray Noritake Dental Inc., Japan), G-Premio BOND (GP; GC Corporation, Japan), Prime & Bond Universal (PB; Dentsply, Germany) ทาตามคำแนะนำของผู้ผลิตและใช้แบบทา 2 ชั้นจากนั้นนำเรซินคอมโพสิตมาก่อบนเนื้อฟันที่เตรียมไว้แล้วแบ่งออกเป็นแท่ง 1x1x8 มม.[3] เพื่อทดสอบค่าความแข็งแรงในการยึดติดแบบดึงระดับจุลภาค (μTBS) หลังจากแช่น้ำ 24 ชั่วโมง และ 6 เดือน และนำค่าที่ได้มาวิเคราะห์ทางสถิติด้วยการวิเคราะห์ความแปรปรวนสองทาง (p = 0.05) เพื่อดูความแตกต่างของชนิดของสารบอนดิงและความแตกต่างกันระหว่างการทาตามคำแนะนำของผู้ผลิตและใช้แบบทา 2 ครั้ง นอกจากนี้วิเคราะห์ข้อมูลทางสถิติด้วยการวิเคราะห์ความแปรปรวนทางเดียว ตามด้วยการทดสอบเปรียบเทียบรายคู่ (Tukey Post Hoc test) และเปรียบเทียบก่อนหลัง (Pair T-test)

ผลลัพธ์: การวิเคราะห์ความแปรปรวนสองทาง พบว่าค่าแรงยึดติดแบบดึงระดับจุลภาคของสารบอนดิงยูนิเวอซัลเมื่อทาแบบ 2 ชั้นไม่เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับการทาสารบอนดิงตามคำแนะนำของผู้ผลิตทั้งที่เวลาแช่น้ำ 24 ชั่วโมง (p = 0.652) และ 6 เดือน หลังแช่น้ำ (p = 0.173) ในทางกลับกันชนิดของสารบอนดิงมีผลต่อค่าความแข็งแรงในการยึดติดแบบดึงระดับจุลภาคอย่างมีนัยสำคัญทางสถิติทั้งที่เวลา 24 ชั่วโมง (p < 0.001) และ 6 เดือน หลังแช่น้ำ (p < 0.001) นอกจากนี้สารบอนดิง Optibond FL, Single Bond Universal Adhesive เมื่อทาแบบ 2 ชั้น, G-Premio BOND เมื่อทาตามคำแนะนำของผู้ผลิต และ เมื่อทาแบบ 2 ชั้น มีค่าความแข็งแรงในการยึดติดแบบดึงระดับจุลภาคลดลงอย่างมีนัยสำคัญทางสถิติหลังจากแช่น้ำ 6 เดือน

สรุป: การทาสารบอนดิงยูนิเวอซัลที่นำมาทดลอง แบบ 2 ชั้น ไม่ช่วยให้มีค่าความแข็งแรงในการยึดติดแบบดึงระดับจุลภาคเพิ่มขึ้นทั้งที่เวลาแช่น้ำ 24 ชั่วโมงและหลังแช่น้ำ 6 เดือน สารบอนดิง Single Bond Universal เมื่อทาตามคำแนะนำของผู้ผลิต, Clearfil Universal Bond Quick และ Prime&Bond Universal ทั้งเมื่อใช้ตามคำแนะนำของผู้ผลิตและ เมื่อทาแบบ 2 ชั้น ให้ค่าความแข็งแรงในการยึดติดต่อเนื้อฟันที่ไม่เปลี่ยนแปลงหลังจากแช่น้ำ 6 เดือนในขณะที่สารบอนดิง Optibond FL และ G-Premio Bond เมื่อใช้การทาทั้ง 2 แบบให้ค่าแรงยึดติดที่ลดลง หลังจากแช่น้ำ 6 เดือน

สาขาวิชา	ทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม	ลายมือชื่อนิสิต ....
ปีการศึกษา	2561	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

5875832332 : MAJOR ESTHETIC RESTORATIVE AND IMPLANT DENTISTRY
KEYWORD: Double application, Etch and Rinse, Microtensile bond strength, Three-step etch and rinse adhesive, Universal Adhesive, Water storage

Suthatta Jeansuwannagorn : Comparison of microtensile bond strength of universal adhesives utilizing various application methods: an in vitro study. Advisor: Assoc. Prof. SIRIVIMOL SRISAWASDI, Ph.D.

Purpose: To investigate microtensile bond strength (μTBS) of a conventional three-step etch and rinse, and universal adhesive systems with etch and rinse mode utilizing various application methods at 24 hour and after 6 month water-storage.

Material and methods: Seventy-two extracted human third molars were cut to expose a flat dentin surface. Optibond FL (OFL; Kerr, USA), Single Bond Universal (SU; 3M ESPE, USA), Clearfil Universal Bond Quick (CU; Kuraray Noritake Dental Inc., Japan), G-Premio BOND (GP; GC Corporation, Japan), Prime&Bond Universal (PB; Dentsply, Germany) were used in etch and rinse mode following the manufacturers' instruction and double application method. Subsequently, composite resins were constructed on prepared dentin and then sectioned into 1x1x8 mm^3 stick to be tested after 24 hours and 6 months of water-storage by microtensile bond strength (μTBS) testing. The data of universal adhesives were analyzed with two-way ANOVA (p = 0.05). Additionally, the μTBS values were obtained and analyzed with one-way ANOVA followed by a Tukey Post Hoc test and a Pair T-test.

Results: The μTBS of universal adhesives were not significantly increased when using double application method compared to manufactures' instruction at 24 hr (p = 0.652) and after 6 month water storage (p = 0.173). In contrast, kind of adhesives had statistically significant influence on μTBS at 24 hr (p < 0.001) and after 6 month water storage (p < 0.001). Optibond FL, Single Bond Universal Adhesive when applied in double application methods, G-Premio BOND when following the manufacturer's instruction, and appling in double application method had statistically significantly decreased μTBS after 6 month water-storage.

Conclusions: Double application of universal adhesives did not improve the bond strength of tested universal adhesives at 24 hours and after 6 months of water storage. Single Bond Universal used following manufacturer's instruction, Clearfil Universal Bond Quick and Prime&Bond Universal used both following manufacturer's instruction and modified double application method, had stable dentin microtensile bond strength, while Optibond FL and G-Premio Bond used both techniques had deceased bond strength after 6 months of water storage.

Field of Study:	Esthetic Restorative and Implant Dentistry	Student's Signature ....
Academic Year:	2018	Advisor's Signature ....

ACKNOWLEDGEMENTS

Throughout the writing of this dissertation I have received a great deal of support and assistance.I would first like to thank my thesis advisor, Associate Professor Sirivimol Srisawasdi, Ph.D. for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my dissertation.

I am profoundly grateful to the member of supervisory committee, Associate Professor Atiphan Pimkhaokham, Ph.D., Associate Professor Mansuang Arksornnukit, Ph.D. and Assistant Professor Pong Pongprueksa, Ph.D. for their insightful comments and encouragement, but also for the hard questions which incented me to widen my research from various perspectives.

I would also like to acknowledge Associate Professor Chanchai Hosawuan for suggestions and his helps all about statistical analyses.

My research would have been impossible without the aid and support of all staff members at Esthetic Restorative and Implant Dentistry Clinic and Dental Research Center of Chulalongkorn University for providing the facilities required for this thesis.

Last but not the least,I would like to thank my colleagues, who were of great support in deliberating over our problems and findings, as well as providing happy distraction to rest my mind outside of my research.

Finally, I must express my very profound gratitude to my parents for providing me with unfailing support and continuous encouragement throughout my years of study, and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

Suthatta Jeansuwannagorn

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I INTRODUCTION

Backgrounds and Rationale

To replace hard tissues with tooth-colored restorative materials, such as direct resin-based composites, a successful bonding of adhesive system is necessary (1). Establishing an effective bond between enamel/dentin and composite resins has remained challenge in restorative dentistry (2, 3).

Etch and rinse adhesive system that has been widely used nowadays, is able to totally remove the smear layer of dental substrates via the action of phosphoric acid (4-6). Thus, this system has been shown to provide high quality adhesion to both enamel and dentin (7, 8). Despite the advantage mentioned above, this system, along with a technique sensitivity, requires a long application time and several application steps, which are troublesome for the clinicians.

Hence the disadvantages of etch and rinse adhesive system, self-etch adhesive system was developed. In order to reduce a technique sensitivity, this system utilizes non-rinsed acidic monomers to maintain smear layer as a substrate for bonding (4, 7). However, the long-term bonding durability produced by one-step self-etch adhesives is inferior to the three-step etch and rinse adhesive (5, 9, 10) . Furthermore, many studies have also reported a significant bond strength reduction, and an extensive interfacial nanoleakage within the adhesive/dentin interface for this system (9, 11) . Therefore, current adhesive technology tends to simplify bonding procedures by reducing application steps, shortening clinical application time and decreasing technique sensitivity (12).

The new family of dental adhesive known as ‘‘universal adhesive’’ or ‘‘multi-mode adhesive’’ has been introduced. It was designed to bond to tooth structures via either the etch and rinse technique or the self-etch technique using the same single bottle of adhesive solution (13-15) . Studies have shown that the use of universal adhesives via etch and rinse technique did not have a negative impact on dentin bonding quality, when compared to the self-etch technique (16) . A reduction in enamel bonding efficacy was also observed with the use of universal adhesives in self-etch mode (14, 17, 18) . Moreover, the application of an etching step prior to universal adhesives significantly improved their dentin penetration pattern when compared to non-etching step prior to universal adhesive (15). An infiltration of adhesive into the dentin and a thickness of the adhesive layer were directly correlated to the adhesive's chemical characteristics influenced by the application mode. (13, 17, 19),

Different clinical approaches have been proposed to improve monomer infiltration such as the use of an additional layer of hydrophobic resin agent, an enhancement of a solvent evaporation(20) , prolonged curing-time intervals(19) , and multiple-layer application (21, 22) . The multiple-layer application has been shown to remove residual water and increase extent of resin impregnation into collagen, thereby, improving resin-infiltration and crosslinking of the adhesive comonomers within the hybrid layer(23) .

Longevity of the adhesives has also been taken in consideration in order to justify the proper bond strength. A decrease in bonding effectiveness has been thought to be caused by the degradation of interface components due to hydrolytic degradation (24). Previous study showed that bond strength to dentin after 6 months was material-dependent since aging in artificial saliva reduced the bond strength of

Scotchbond Universal compared to immediate values (12). In contrast, Munoz et al. demonstrated that universal adhesives that contained MDP, after 6 months of water storage, showed a stable bond strength when compared to 24-hour, same as in the two-step self-etch adhesive (25).

Although there were several studies of enamel and dentin bonding performance using universal adhesives (15, 25-28), only limited information was available on the dentin bonding quality of universal adhesives using etch and rinse mode, and used in double application methods to achieve more monomers infiltration (12, 29, 30). Moreover, there had been no studies that investigated the bond strength of universal adhesive utilizing etch and rinse mode and a conventional three-step etch and rinse adhesive after 6-month water storage. Therefore, the purpose of this study was to investigate the bond strength of the universal adhesives utilizing etch and rinse mode in various application methods and the three-step etch and rinse adhesive using microtensile bond strength (μTBS) testing at 24 hours and after 6 months water-storage. The null hypotheses were that there was no significant difference in μTBS among universal adhesive systems utilizing etch and rinse mode and conventional three-step etch and rinse adhesive and at 24-hour and after 6-months water storage, and the application methods of universal adhesives had no effect on μTBS.

Research Questions

1. How the universal adhesive systems utilizing etch and rinse mode and the three-step etch and rinse adhesive perform at 24 hours and after 6 months water-storage using microtensile bond strength measurement?
2. Did various application methods of universal adhesive systems utilizing etch and rinse mode had effect on microtensile bond strength?
3. Did the microtensile bond strength of adhesive-dentin bond of tested adhesive systems was affected by water storage for 6 months?

Research Objectives

To investigate the bond strength of the universal adhesives utilizing etch and rinse mode in various application methods and the three-step etch and rinse adhesive using microtensile bond strength testing at 24 hours and after 6 months water-storage.

Hypothesis

Null hypotheses

1) There was no significant difference in the microtensile bond strengths among universal adhesive systems utilizing etch and rinse mode and a conventional three-step etch and rinse adhesive at 24 hours and after 6 months water-storage.

2) The various application methods of universal adhesives utilizing etch and rinse mode had no effect on dentin microtensile bond strength.

3) The microtensile bond strength of adhesive-dentin bond of tested adhesive systems was not affected by water storage for 6 months.

Alternative hypotheses

1) There was at least one significant difference in the microtensile bond strength among universal adhesive systems utilizing etch and rinse mode and a conventional three-step etch and rinse adhesive at 24 hours and after 6 months water-storage.

2) The various application methods of universal adhesives utilizing etch and rinse mode had significant effect on microtensile bond strength.

3) The microtensile bond strength of adhesive-dentin bond of tested adhesive systems was affected by water storage for 6 months.

Conceptual Framework

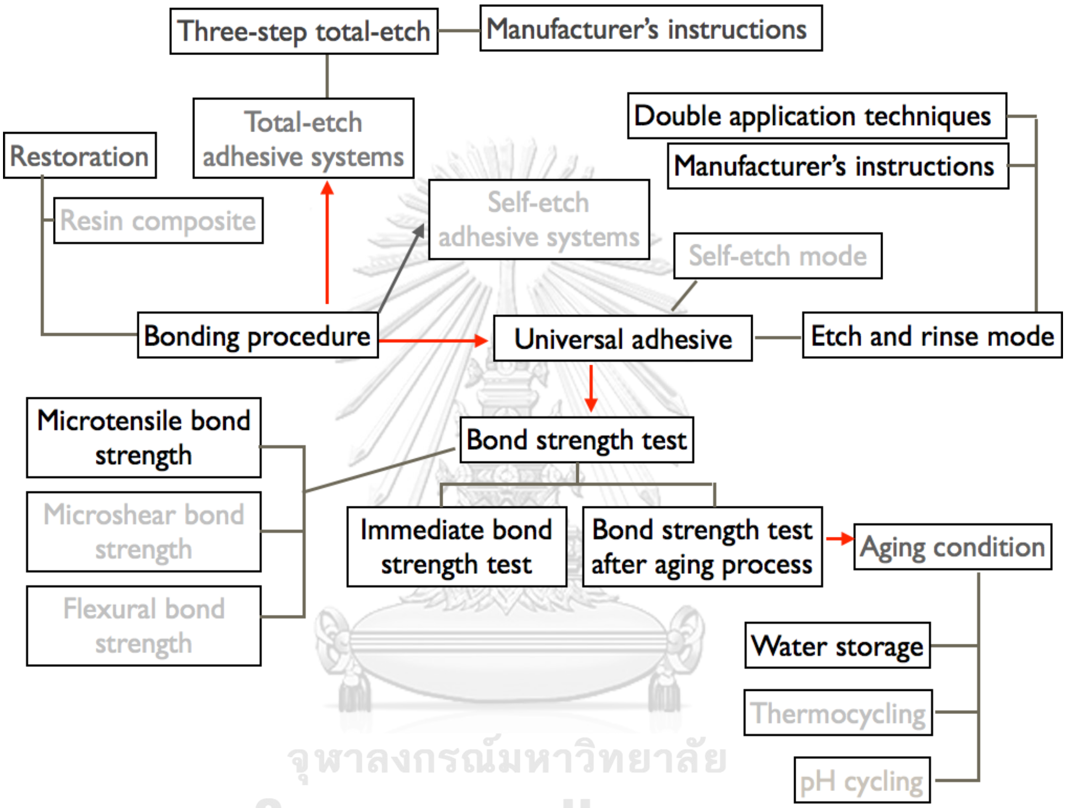


Figure 1 Diagram of Conceptual Framework

Key word

Double application, Etch and Rinse, Microtensile bond strength, Three-step etch and rinse adhesive, Universal Adhesive, Water storage

Expected Benefit of the Study

Outcome of the present study may provide useful information concerning methods to improve bonding performance for the universal adhesive systems.

CHAPTER II REVIEW OF THE LITERATURES

The literatures in these following topics have been reviews.

Etch and rinse adhesive system

Three-step etch and rinse adhesive

Self-etch adhesive

Universal adhesive

Aging process

Microtensile bond strength and Mode of failure

Etch and rinse adhesive system

Etch and rinse adhesive system was established by applying 35-37.5 % phosphoric acid to enamel and dentin to increase infiltration of resin monomers (31). Acid-etching completely demineralized surface of the intertubular dentin to create nanometer-sized porosities within the underlying collagen fibrillar matrix (32). Collagen fibrils were nearly completely denuded of hydroxyapatite. The microretentive network was formed and ready for micromechanical interlocking of monomers applied successively by the primer and adhesive resin (33). The network created at interfacial structure is referred to as a “hybrid layer” or “resin-dentin interdiffusion zone” (34, 35). Additionally, resin tags sealed the unplugged dentin tubules generating more retention through hybridization of the tubule orifice wall (33).

The role of dentine endogenous matrix metalloproteinases (MMPs) has also been involved in the stability of the hybrid layer over time. Direct evidences of increased MMP-2 and -9 activities following adhesive application were found, with higher levels of activities reported for etch-and-rinse compared to self-etch adhesives. These findings are probably correlated to the fact that the etching step of the etch and rinse adhesives exposed more dentine matrix than the use of self-etch adhesives. (12)

Three-step etch and rinse adhesive

Three-step etch and rinse adhesive commonly performs at a superior level compared to other adhesives (33, 36-38). Moreover, the bonding integrity of three-step etch and rinse adhesive was better maintained (32, 39, 40). Therefore, three-step etch and rinse adhesives are the most favorable and reliable for long-term usage (2, 7) and are also typically considered by some as the 'gold standard' among adhesives (33, 41). After the conditioning step, adhesion-promoting monomers are applied in two application steps to penetrate the exposed collagen network, so called priming step. The priming step in three-step etch and rinse adhesives ensures sufficient wetting of the exposed collagen fibrils and removes remaining water, thereby preparing dentin for adhesive resin infiltration. A primer solution is a mixture of specific monomers with hydrophilic properties dissolved in organic solvents. HEMA is a monomer that is very frequently added to these primer solutions, due to its low molecular weight and hydrophilic nature. HEMA promotes resin infiltration and re-expansion of the collagen network, thereby improving the bond strength of adhesive. However, the higher number of application steps, the higher the risk of making manipulation errors (41).

Most published information regarding three-step etch and rinse adhesives have been evaluated for manipulation technique of materials in strict accordance with manufacturers' instructions. However, errors or variations in application protocols occur frequently in daily practice. These modified application procedures may affect dentin bonding quality such as poor marginal adaptation and dramatically lower bond strengths (42-45).

Clinical evaluation at 13 years, two three-step etch and rinse adhesives (Optibond FL and Permaquick) showed a highly acceptable clinical performance. Optibond FL showed the highest success rate (88%). The most obvious sign of bond degradation in this 13-year clinical trial was marginal deterioration. A small number of restorations using Optibond FL needed to be repaired or replaced due to the presence of clinically unacceptable severe marginal defects (3%) and deep marginal discoloration (6%) (46).

Self-etch adhesive

The bonding mechanism of "self-etch" adhesives to dentin is also based on hybridization with the difference that mostly only submicron hybrid layers are formed and resin-tag formation is less pronounced (7). The 10-MDP of MDP-containing adhesives has been shown to readily adhere to hydroxyapatite and form nanolayering structures. These nanolayering structures appeared very stable, as confirmed by low dissolution rate in water. This may improve sustainability of these products (47, 48).

In an attempt to improve bonding effectiveness of the self-etch adhesives, the selective etching of enamel margins has been recommended prior to the application of self-etch adhesives (27, 28). In addition, active application or agitation could be used as a simple and rapid application technique. Agitation provided a consistent etching effect and enhanced interaction of acidic monomers with etched dental substrate (49). This procedure also increased the moieties kinetics and allowed for better monomer diffusion (50).

Universal adhesive

They are called multi-mode or universal adhesives due to their versatile instructions for use. The chemical interaction is a crucial characteristic of universal adhesives to enhance durability of dentin–resin interfaces. The *in vitro* performance of universal adhesives has been reported as material-dependent due to the complexity of their chemical composition (13, 17). As explained elsewhere, all simplified adhesives behave as permeable membranes (i.e. two-step etch and rinse or one-step self-etch adhesives). As universal adhesives are one-step self-etch adhesives in nature, they behaved in the same fashion (17, 51). If the exposed collagen has not been fully encapsulated by the polymerized adhesive monomers, demineralized collagen fibrils were vulnerable to time-dependent hydrolytic degradation by water, leaving voids within the hybrid layer or demineralized nano-channels (52). As for one-step self-etch adhesives, coating universal adhesives with an extra layer of a hydrophobic resin improved their immediate (51, 53) and long-term bond strengths, increased degree of conversion, and consequently lowering nanoleakage. Furthermore, universal adhesive infiltration was enhanced if active application was used (51).

In 2013, the results of previous study indicated that when the universal adhesives were tested using self-etch or etch and-rinse strategy on dentine, they were inferior to the respective controls (Clearfill SE Bond, a two-step self-etch or Adper Single Bond 2, a two-step etch and rinse) with respect to at least one of the properties tested, microtensile bond strength, nanoleakage and in situ degree of conversion (17).

Marchesi, et al. (2014) also showed that bond strength to dentine after 6 months was material-dependent since aging in artificial saliva reduced the bond strength of Scotchbond Universal (irrespective of the application mode) compared to immediate values (12).

Aging process

Many techniques for aging the specimens before testing the bond strength were proposed. Adhesive restorations are often situated in wet environments surrounded by saliva. Water is crucial in the deterioration of bonding interfaces (54). Therefore, many previous studies have immersed samples into distilled water to assess bonding durability (55). Intraoral temperature varies depending on eating, drinking and breathing habits. Rapid temperature changes inevitably affect the stability of adhesive restoration. Different in vitro artificial aging methods, such as water storage, thermocycling, NaOCl storage and pH cycling, may have different effects on the degradation of adhesive–dentine interfaces (56). However, storing in water and thermocycling technique are widely used for aging the specimens.

With aging by storing in water, specimens stored in pure water at 37°C were utilized. Storage time can be varied from a few months up to 4-5 years (57), or even longer. According to the ISO TR 11450 standard (2015), 6 month storage in water at 37°C can cause a significant decrease in bond strength. A decrease in bonding effectiveness is thought to be caused by the degradation of interface components via hydrolysis process. However, water can also infiltrate and decrease mechanical properties of polymer matrix by swelling and reducing frictional forces between polymer chains, a process known as 'plasticization' (24). To prevent bacterial contamination during the storage period, antibiotics (58), sodium azide (59), and chloramine (40, 60) can be added.

Microtensile bond strength and Mode of failure

The adhesion testing of adhesive to tooth structure can be measured by a great number of methods such as tensile bond strength test, flexural bond strength test, or shear bond strength test.

Tensile and shear bond strength tests were performed in specimens with relatively large bonded areas, usually 3-6 mm in diameter (~ 7-28 mm^2) (61), However, the validity of expressing bond strength has been questioned due to the heterogeneity of stress distribution at the bonded interface, influence by variability in specimen geometry, loading conditions and material properties. It has come to use of new methods using specimens with smaller bonded area approximately 1.6-1.8 mm^2. Using this smaller bonded area, failure of the specimens usually occurred at the adhesive interface. These new methods are called microtensile and microshear tests (62).

A microtensile bond strength (µTBS) methodology was introduced by Sano and others in 1994. These authors showed that microtensile bond strength was inversely related to the bonded surface area (58, 62, 63) and that, although much higher bond strengths were measured, most failures still occurred at the interface between tooth substrate and adhesive (7). Moreover, the advantages of microtensile bond test include easier sample collection, the ability to compare a variety of substrates and areas in the same tooth, and more uniform loading stress distribution over a smaller bonded area (61, 64, 65).

The bond strength in this study is evaluated by microtensile testing technique. Although microtensile testing was more sensitive technique than tensile and shear testing (66), this technique was more cost effective. As a large number of specimens can be produced with a reduced volume of material, it also provides a precise observation of the bonding between luting materials and clinically relevant substrates by allowing the selection of specimens free of bubbles and other defects and is therefore more accurate than shear and tensile tests (63, 67).

The specimens have to be cut into a number of slabs and further sectioned into a stick with approximate thickness of 0.5-1.5 mm. Each stick composed two substrates of tooth structure-resin composite, which were bonded together and could be tested at the interface (64). The shape of specimens can be prepared in either non-trimming bar-shape or trimmed with bur at the bonding site to create an hourglass profile, which will reduce the bonding area making the stress to be more concentrated at the bonding site (64). However, the non-trimming method of specimens preparation has proved able to measure bond strengths as low as 5 MPa, which was expected to be less traumatic than the methods where an hourglass profile is created with burs at the bonding interface (63, 68). Additionally, the non-trimmed, square microspecimens of 1 mm^2, can be resulted in a higher value, a lower CV, and a higher percentage of failures at the actual interface, as compared to the trimmed, circular microspecimens (69).

CHAPTER III MATERIALS AND METHODS

Research Design

This study was an in vitro experimental study, which compared microtensile bond strengths between a conventional three-step etch and rinse adhesive system and universal adhesives utilizing various application methods.

Research Methodology

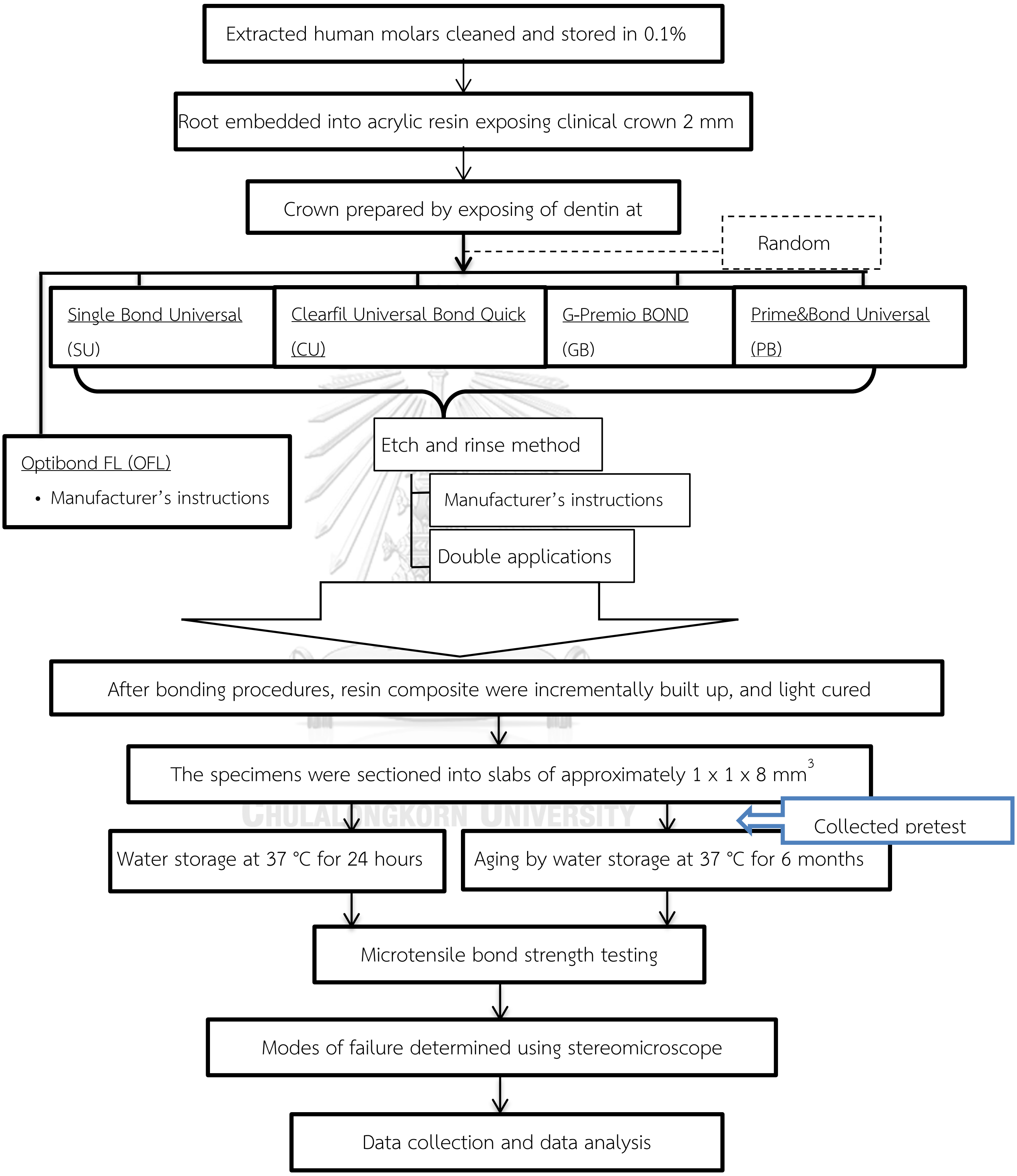


Figure 2 Diagram of study design

Sample size description

Sample size was calculated by using the formula for two independent groups shown below;

$$n = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2[\sigma_1^2 + \frac{\sigma_2^2}{r}]}{(\mu_1 - \mu_2)^2}$$

$$r = \frac{n_2}{n_1}$$

n is sample size estimation (per group).

Z_α is the value of the standardized score cutting off $\alpha/2$ proportion of each tail of a standard normal distribution (for a two-tailed hypothesis test) (Z_α=1.96 for α = 0.05).

Z_β is the value of the standardized score cutting off the upper proportion (Z_β = 0.84 for β = 0.2 = 80% power).

μ is mean of microshear bond strength in each group.

σ is standard deviation of microshear bond strength in each group.

The highest number of specimen was calculated from values of microtensile bond strength of Clearfil Universal Bond Quick® when followed the manufacturer's instruction and when applied in double application methods as shown in the equation below:

$$n_1 = \frac{(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 [\sigma_1^2 + \frac{\sigma_2^2}{r}]}{(\mu_1 - \mu_2)^2}$$

n_1 = (1.96 + 0.84)2[161.04+314.35]/(42.35-44.15)2

n_1 = (7.84)[475.39] /(3.24)

n_1 = 1150.33

Eight numbers of specimens in each group were selected for this study. There were 9 experimental groups in this study so the total number of specimens was 72 specimens.

Table 1: Material, Manufacturer, and Component

Material, Manufacturer (batch number)	Components
OptiBond FL (Kerr, USA) Lot No. 6046873,6110569	Etchant: 37.5% phosphoric acid Primer: HEMA, GPDM, PAMM, ethanol, water, photoinitiator Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler (fumed SiO_2, barium aluminoborosilicat, Na_2SiF_6), coupling factor A174 (approximately 48 wt% filled) photoinitiator
Single Bond Universal Adhesive (3M ESPE, USA) Lot No. 651936	Adhesive: 10-MDP, Bis-GMA, phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol, water, initiators, silane-treated silica, silane
Clearfil Universal Bond Quick (Kuraray Noritake Dental Inc., Japan) Lot No. 7L0039	Adhesive: 10-MDP, Bis-GMA, HEMA, ethanol, hydrophilic amide monomers, colloidal silica, dl- camphorquinone, Sodium fluoride, silane coupling agent and water
G-Premio BOND (GC Corporation, Japan) Lot No. 1611211	Adhesive: 10-MDP, 4-MET, 10-methacryoyloxydecyl dihydrogen thiophosphate, methacrylate acid ester, distilled water, acetone, photo initiators, silica fine powder
Prime&Bond universal (Dentsply, Germany) Lot No. 1705000051	Adhesive: PENTA, 10-MDP, Bi- and multifunctional acrylate, Initiator, Stabilizer, Isopropanol, water
Premise (Kerr, USA) Lot No. 6093677	Filler: Prepolymerized filler (PPF), 30 to 50 μm, Barium glass, 0.4 μm, Silica filler 0.02 μm Resin: Ethoxylated bis-phenol-A-dimethacrylate, TEGDMA, Light-cure initiators and stabilizers

HEMA: Hydroxyethyl methacrylate; Bis-GMA: Bisphenol A-glycidyl methacrylate 2,2-bis [4-(2-hydroxy-3-methacryloxypropoxy) phenyl] propane; MDP; Methacryloyloxydecyl dihydrogen phosphate; TEGDMA: Triethyleneglycol dimethacrylate; UDMA: Urethane dimethacrylate; GDM: Glycerol-dimethacrylate; GPDM: Glycero-phosphate dimethacrylate; PAMM: Phthalic acid monoethyl methacrylate; 4-MET: 4 methacryloxy ethyltrimellitate anhydride; PENTA: Phosphoric acid modified acrylate resin;

Table 2: Instrument lists

Instrument	Manufacturer
Low-Speed Cutting Machine (Isomet® 1000)	Buehler, USA
Universal Testing Machine (EZ-S Shimadzu)	Shimadzu, Japan
Grinder-Polisher Machine (Automet® 250)	Buehler, USA
Diamond Wafering Blade	Buehler, USA
LED Light-Curing System: Demi™ Plus	Kerr, USA
Radiometer: Demetron	Kerr, USA
Stereomicroscope: Optical Oylmpus	Oylmpus, Japan
Scanning Electron Microscope (JSM-6610LV)	JEOL, USA

Material and method

This study was approved by the ethical committee of the Faculty of Dentistry, Chulalongkorn University, Thailand (approval number: HREC-DCU 2017-064). All experimental procedures were performed by the one investigator.

Preparation of dentin specimens

Seventy-two extracted human third molars collected from private clinic, free of debris and soft tissue, were stored in a 0.1% thymol solution at 4°C and used within

3 months of extraction. Teeth were analyzed at 4× magnification using a stereomicroscope (Olympus, Japan) and following selection criteria: no caries nor previous restorations, no cracks, and the presence of completely formed apexes. Teeth were mounted in a clear self-curing acrylic resin (Shofu, Kyoto, Japan) sized 22×18×12 mm^3 with the cemento-enamel junction (CEJ) exposed (Figure 3A). Occlusal one third of dental crowns were removed perpendicular to the long axis of each tooth using a water-cooled Isomet low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) to expose a flat mid-coronal dentin surface (Figure 3B). The dentin surfaces were treated by wet-sanding with 600-grit silicon carbide sandpaper at 100 rpms for 30 s (Automet® 230, Buehler, USA) to produce standard smear layer (70-72). Then they were washed thoroughly with distilled water and immediately dried with moisture-free air.

Bonding and restorative procedure

The specimens were randomly assigned into 9 treatment groups (n=8 per group, calculated from pilot study) treated by five adhesives including: Optibond FL®

(OFL), Single Bond Universal® (SU), Clearfil Universal Bond Quick® , G-Premio BOND® , Prime&Bond Universal® (PB). The application methods shown in Table 3, were followed following the manufacturers' instructions, except for groups that application technique were modified.

Table 3: Material and Application technique (19, 21-23, 73, 74)

Material	Etch and rinse application techniques	
	Manufacturer's instructions	Double application
OptiBond FL	Group 1 (OFL): 1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets. 1. Apply OptiBond FL primer, 5 microliters (µl), with a light scrubbing motion for 15 s. Gently air dry for 5 s. 2. Apply OptiBond FL adhesive, 5 µl, uniformly creating a thin coating for 15 s, then light curing for 10 s	
Single Bond Universal Adhesive	Group 2 (SU): 1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets. 2. Apply the adhesive to etched dentin, 5 µl, and rub it in for 20 s. 3. Gently air-dry the adhesive for approximately 5 s for the solvent to evaporate. 4. Light cure for 10 s.	Group 3 (SU2C): 1. Follow the Manufacturer's instructions in step 1-3 2. Repeat in step 2 and 3 3. Light cure for 10 s.
Clearfil Universal Bond Quick	Group 4 : 1. Apply 37.5% phosphoric acid for 10 s, then rinse thoroughly with water and dry with foam pellets. 2. Apply Clearfil Universal Bond Quick, 5 µl, and rub it in for 10 s. 3. Dry the cavity wall sufficiently by blowing mild air for more than 5 s until the adhesive shows no movement. 4. Light cure for 10 s.	Group 5 (CU2C): 1. Follow the Manufacturer's instructions in step 1-3 2. Repeat in step 2 and 3 3. Light cure for 10 s.
G-Premio BOND	Group 6 (: 1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets. 2. Apply adhesive, 5 µl, and leave undisturbed for 10 s after application. 3. Dry thoroughly for 5 s with oil free air under maximum air pressure 4. Light cure for 10 s.	Group 7 (GP2C): 1. Follow the Manufacturer's instructions in step 1-3 2. Repeat in step 2 and 3 3. Light cure for 10 s.
Prime&Bond Universal	Group 8 (PB): 1. Apply 37.5% phosphoric acid for 15 s, then rinse thoroughly with water and dry with foam pellets. 2. Adhesive applied to etch dentin, 5 µl, (do not desiccate) with rubbing action for 20 s. 3. Gentle stream of air applied over the liquid for at least 5 s. 4. Light cure for 10s	Group 9 (PB2C): 1. Follow the Manufacturer's instructions in step 1-3 2. Repeat in step 2 and 3 3. Light cure for 10 s.

To complete the bonding procedures, light-curing was applied with a light tip held perpendicularly and within 1 mm superior to the bonding surface using a Demi™ Plus (De/Kerr, Danbury, CT, USA) device with a light output intensity of 1,100 mW/cm^2. Light output was checked regularly using a radiometer (Demetron/Kerr, Danbury, CT, USA)

Following bonding procedures, in all groups, resin composite (Premise, shade XL1, body, Kerr, Orange, CA, USA) was incrementally built up to a height of 4 mm (2 mm thick in each layer) (Figure 3C). Each increment was light-cured for 40 s from the top, and for 20 s at other surfaces, with light tip held perpendicularly and within 1 mm superior to resin composite. After the completion of the resin composite built up, all specimens were stored in water at 37°C for 24 hours.

Preparation of µTBS Specimens

All specimens were sectioned into 1×1×8 mm^3 sticks using a low-speed diamond saw under water-cooling (Figure 3D). Eight sticks, using only the central dentin portion, were chosen and labeled (75). All sticks were examined using a stereomicroscope at a 25× magnification to verify crack-free structural, then intact stitcks and their exact dimensions were measured at a magnification of 40×. The sectioned sticks of each specimen were divided into 2 subgroups to store in distilled water at 37°C for 24 hours and 6 months.

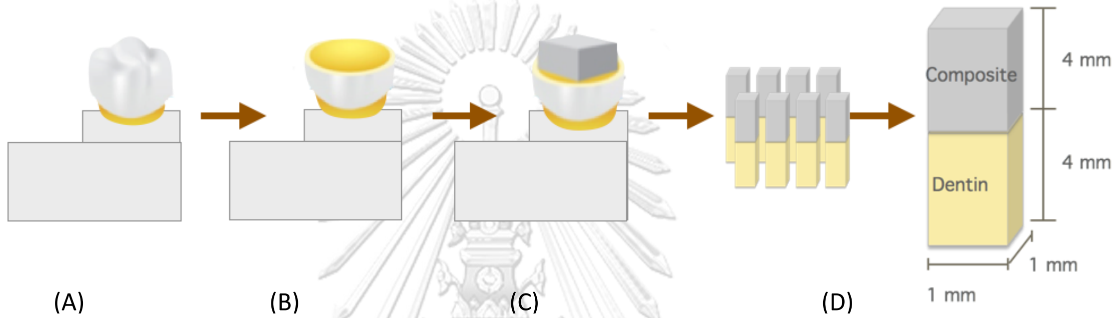


Figure 3 Preparation of Specimens

After storage, four sectioned sticks of each subgroups were attached to a Ciucchi's jig, using a cyanoacrylate glue (Model Repair II Blue, Sankin Kogyo, Otahara, Japan) (Figure 4), and subjected to a microtensile bond strength testing using a universal testing machine (EZ-S; Shimadzu Corporation, Kyoto, Japan) at a cross-head speed of 1 mm/min (76).

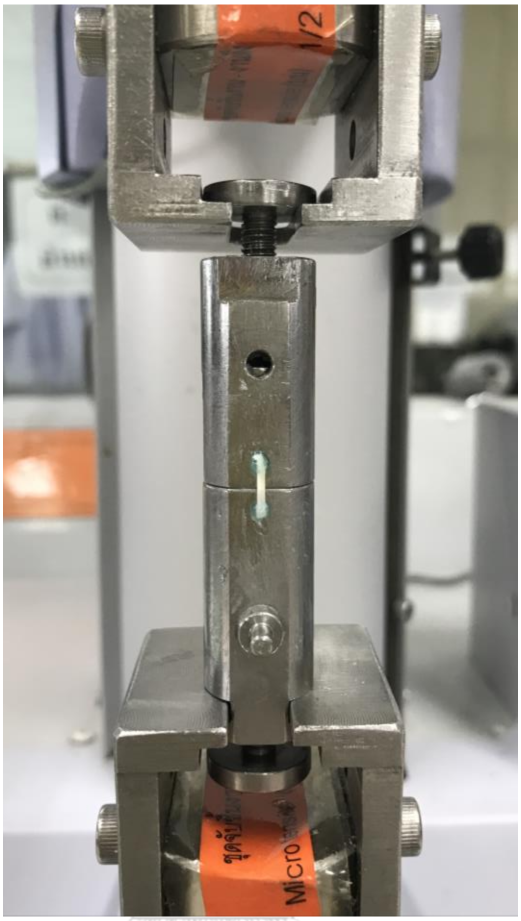


Figure 4 Microtensile bond strength testing

Statistical analysis

The μTBS values in MPa were recorded when fracture occurred, and analyzed statistically using an SPSS software 20.0 (Chicago, IL, USA). The normality of data was determined using a Shapiro-Wilk test. One-way analysis of variance (one-way ANOVA) and a Tukey Post Hoc multiple comparison test was used to determine the difference of adhesive systems and application methods. The storage period of each adhesive was analyzed with a Pair T test. Moreover, the µTBS means were analyzed with two-way measures ANOVA in term of Universal adhesive types and the different of application methods. The level of significance was determined as $p = 0.05$. The pretest failed sticks were excluded (77, 78).

Modes of failure

After debonding, the specimens were examined, under a stereomicroscope (Olympus, Japan) at a magnification of 40×, to verify failure type. Failure modes were classified as shown in Table 4.

Table 4: Mode of failure

Mode of failure	
Co-De	Cohesive failure exclusively within dentin (>75% of the failure is within the dentin)
Co-Re	Cohesive failure exclusively within resin composite (>75% of the failure is within the resin composite)
Ad/Mixed	Adhesive failure at the resin/dentin interface (>75% of failure between resin/dentin interface included cohesive failure of the neighboring substrates)

Scanning electron microscopy (SEM)

One stick from 24 hour and one stick from 6 month water storage were sequentially polished with 600-grit, 800-grit, 1000-grit, 1200-grit SiC papers and diamond pastes, then ultrasonicated in distilled water for 10 min. Following the above procedures, the sticks were then treated with 5N HCl for 30 s, washed with distilled water, soaked in 5% NaOCl for 5 min, and rinsed again with distilled water one last time. The sticks were placed in 90% alcohol and left in chamber containing silica gel for 24 hours to eliminate water (9, 30, 79). After 24 hours, the sticks were mounted on aluminium stubs and sputter-coated with 20 nm layer of gold-palladium. The adhesive/dentine interfaces morphology were examined at x1000 magnifications using a Scanning Electron Microscope (JSM-6610LV, Japan) at 15 kV.

CHAPTER IV RESULTS

The μTBS values of all experimental groups were normal distributed ($p > 0.05$). The μTBS of universal adhesives were not significantly increased when using double application method compared to manufactures' instruction at 24 hr ($p = 0.652$) and after 6 month water storage ($p = 0.173$) as shown in Table 5 and 6. Thus, the modified application method showed no effect on improvement of μTBS. In contrast, kind of adhesives had statistically significant influence on μTBS at 24 hr ($p < 0.001$) and after 6 month water storage ($p < 0.001$) (Table 5 and 6).

Table 5: Two-way ANOVA revealed the significant effects of kind of adhesives and application technique and the interaction factor ANOVA, Analysis of variance; tested universal adhesive at 24-hour water storage.

Source of variation	df	Sum of squares	Mean square	*F*	*P*
Adhesive factor	3	6486.52	2162.172	19.171	<.001
Coat factor	1	23.224	23.224	.206	.652
Interaction	3	24.297	8.099	.072	.975

Table 6: Two-way ANOVA revealed the significant effects of kind of adhesives application technique and the interaction factor ANOVA, Analysis of variance; tested universal adhesive after 6 months of water storage.

Source of variation	df	Sum of squares	Mean square	*F*	*P*
Adhesive factor	3	6635.24	2211.75	18.812	<.001
Coat factor	1	224.32	224.32	1.908	.173
Interaction	3	57.63	19.21	.163	.921

The μTBS (means ± standard deviations) of 24-hour and 6-month water storage specimens of all experimental groups were shown in Table 7.

Table 7: Comparison of μTBS values between 24 hour and 6 month water storage (means ± standard deviations (MPa)) of the different experimental groups (n= 8).

Group	Water storage	
	24-hr	**6-month**
OFL	42.09 ± 7.17 $^{A, B, 1}$	21.24 ± 4.17 $^{b, 2}$
SU	52.55 ± 12.34 $^{A, 1}$	44.80 ± 17.43 $^{a, 1}$
SU2C	55.01 ± 8.01 $^{A, 1}$	48.22 ± 3.21 $^{a, 2}$
CU	41.80 ± 11.29 $^{A, B, 1}$	32.51 ± 14.17 $^{a, b, 1}$
CU2C	43.15 ± 14.74 $^{A, 1}$	39.44 ± 8.59 $^{a, 1}$
GP	25.95 ± 5.26 $^{B, C, 1}$	18.07 ± 6.74 $^{b, 2}$
GP2C	25.13 ± 4.47 $^{C, 1}$	20.54 ± 4.32 $^{b, 2}$
PB	41.04 ± 13.30 $^{A, B, C, 1}$	40.00 ± 11.19 $^{a, 1}$
PB2C	42.87 ± 10.80 $^{A, 1}$	42.16 ± 12.76 $^{a, 1}$

* Similar superscripts capital letters indicate no significant differences between groups at 24-hr (left columns), similar superscript lowercase letters indicate no significant differences between groups after 6 months of water storage (right columns), and similar superscript numbers indicate no significant differences between storage times within each group within each group (rows) according to Tukey's (HSD) test ($p > 0.05$).

At 24-hour, Single Bond Universal Adhesive when used in double application method had the highest μTBS. While, G-Premio BOND used in double application had the lowest bond strength (Table 7). After 6-month water storage, the highest bond strength was found in Single Bond Universal Adhesive when used in double application. While, the lowest value was found in G-Premio BOND following manufacture's instruction. Single Bond Universal Adhesive and Prime&bond universal adhesive following manufactures' instruction and double application method, and Clearfil universal bond quick following double application technic had statistically significant higher μTBS than Optibond FL adhesive and G-Premio BOND using manufactures' instruction and modified double as shown in Table 7.

Comparison of μTBS values between 24 hour and 6 month water storage of the experimental groups was presented in Table 7. Considering the length of storage time, the bond strength of adhesives utilizing etch and rinse mode were statistically significantly decreased after 6 month water-storage specimens comparing to those of 24 hour specimens in the following treatment groups: Optibond FL, Single Bond Universal Adhesive when applied in double application methods, G-Premio BOND when following the manufacturer's instruction, and appling in double application method.

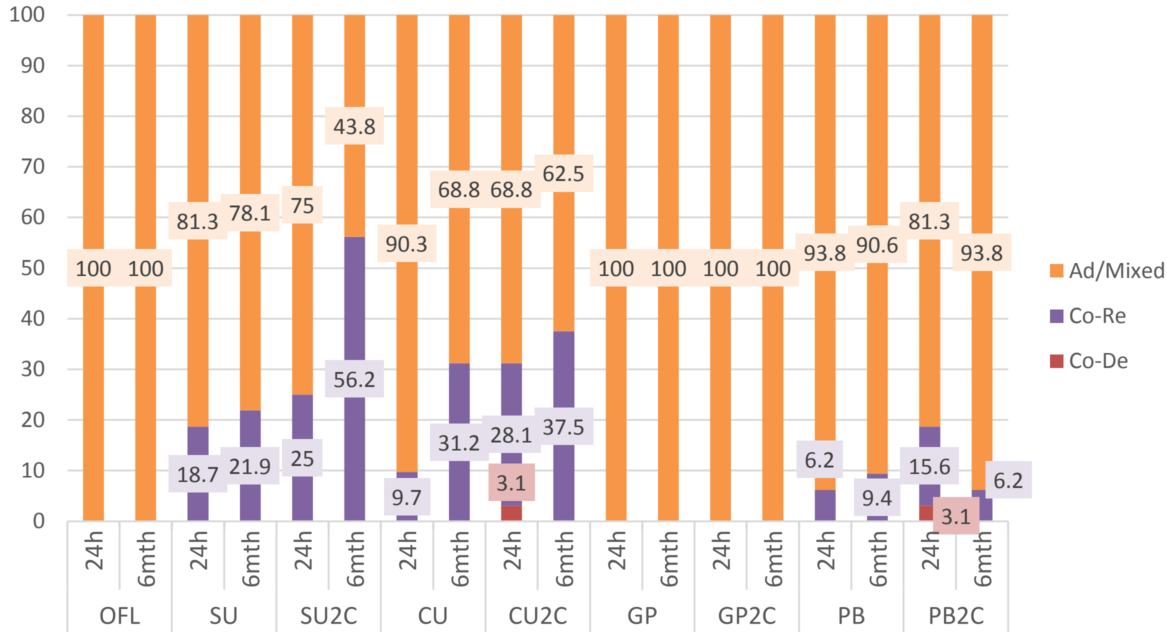


Figure 5 Failure modes of Optibond FL adhesive and the four universal adhesives bonded to dentine in the etch-and-rinse mode at 24 hours and after 6 months of water storage.

Failure type frequencies were given by group in Figure 5. Adhesive failure was noticed to be a major finding in all testing groups. Cohesive failure in resin composite was found in Single Bond Universal more than any other groups. No pre-test failure was recorded for any other adhesives tested.

Representative SEM observations of resin-dentin interface of all adhesives were presented uniformly hybrid layer and the penetration of adhesive in Figure 6 (a-r). All adhesives produced resin tags in dentinal tubules. The resin-dentin interface of G-Premio BOND (Figure 6 k,l,m,n) showed fracture in the adhesive layer (white arrow)

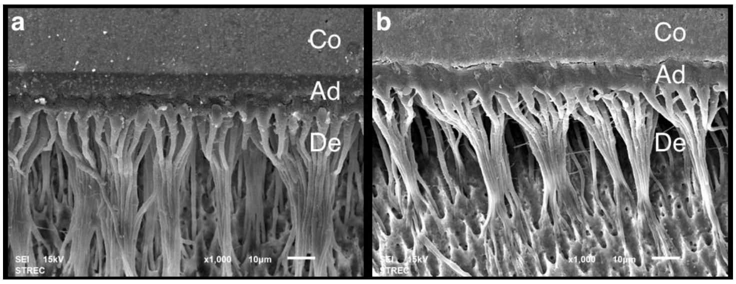


Figure 6: a, b Representative SEM micrograph of the resin-dentin interface formed by the Optibond FL (×1000 magnification).
a Samples were storage in water for 24 h. **b** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

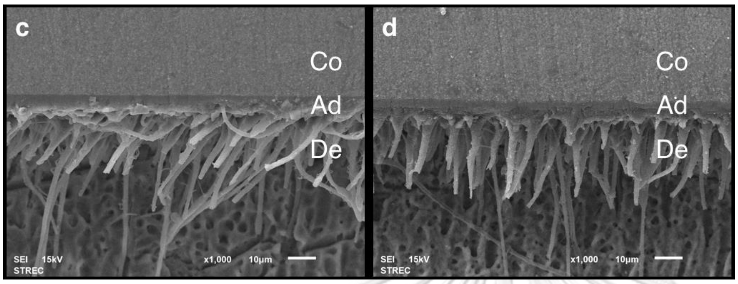


Figure 6: c, d Representative SEM micrograph of the resin-dentin interface formed by the Single Bond Universal adhesive using etch-and-rinse mode (×1000 magnification). **c** Samples were storage in water for 24 h. **d** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

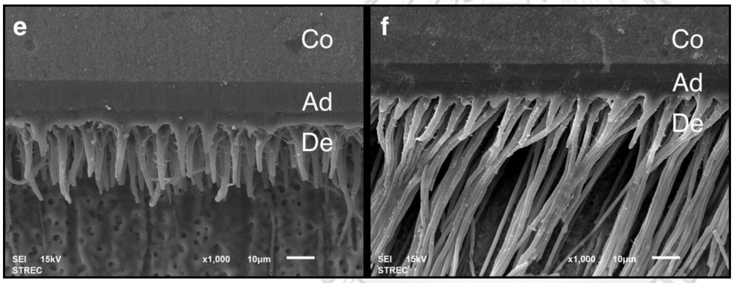


Figure 6: e, f Representative SEM micrograph of the resin-dentin interface formed by the Single Bond Universal adhesive using etch-and-rinse mode with double application (×1000 magnification).
e Samples were storage in water for 24 h. **f** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

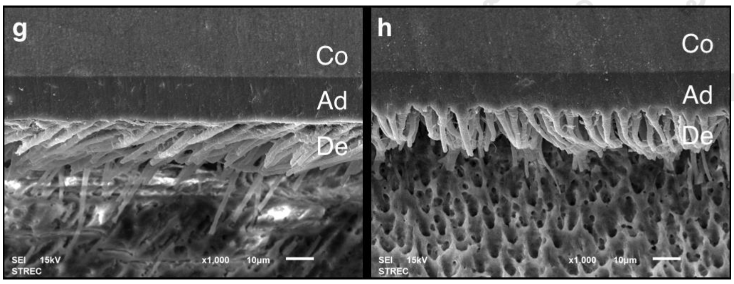


Figure 6: g, h Representative SEM micrograph of the resin-dentin interface formed by the Clearfil Universal Bond Quick adhesive using etch-and-rinse mode (×1000 magnification). **g** Samples were storage in water for 24 h. **h** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

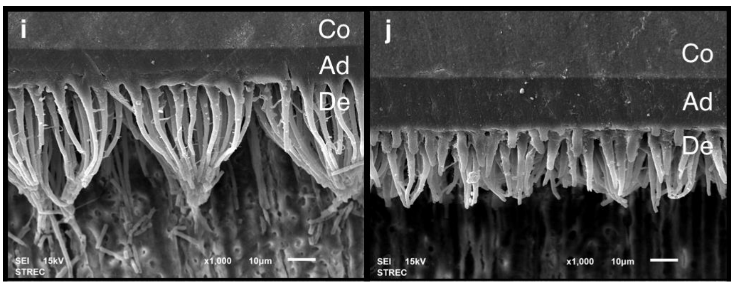


Figure 6: i, j Representative SEM micrograph of the resin-dentin interface formed by the Clearfil Universal Bond Quick adhesive using etch-and-rinse mode with double application (×1000 magnification). **i** Samples were storage in water for 24 h. **j** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

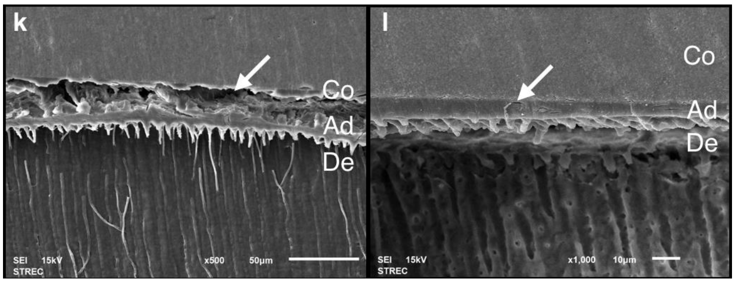


Figure 6: k, l Representative SEM micrograph of the resin-dentin interface formed by the G-Premio BOND using etch-and-rinse mode.
k Samples were storage in water for 24 h (×500 magnification). **l** Samples were storage in water for 6 months (×1000 magnification). (*Co* composite resin, *Ad* adhesive layer, *De* Dentin, *white arrow* fracture line)

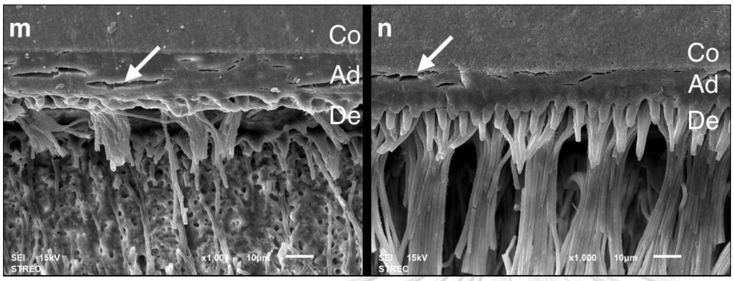


Figure 6: m, n Representative SEM micrograph of the resin-dentin interface formed by the G-Premio BOND using etch-and-rinse mode with double application (×1000 magnification).
m Samples were storage in water for 24 h. **n** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin, *white arrow* fracture line)

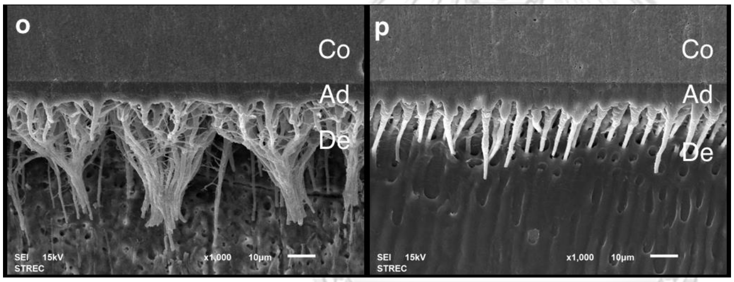


Figure 6: o, p Representative SEM micrograph of the resin-dentin interface formed by the Prime&Bond Universal using etch-and-rinse mode (×1000 magnification).
o Samples were storage in water for 24 h. **p** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

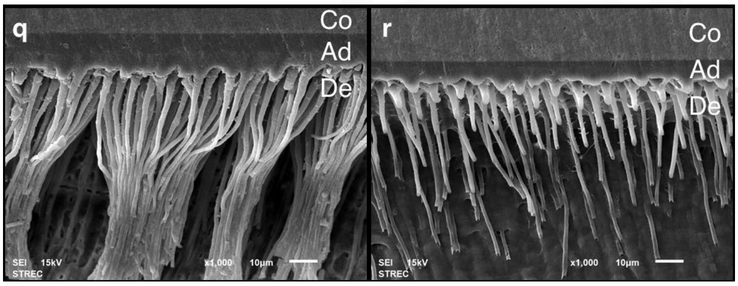


Figure 6: q, r Representative SEM micrograph of the resin-dentin interface formed by the Prime&Bond Universal using etch-and-rinse mode with double application (×1000 magnification).
q Samples were storage in water for 24 h. **r** Samples were storage in water for 6 months. (*Co* composite resin, *Ad* adhesive layer, *De* Dentin)

Figure 6 Representative SEM micrographs of the resin-dentin interface formed by the Optibond FL (a, b), Single Bond Universal adhesive (c, d, e, f), Clearfil Universal Bond Quick adhesive (g, h, I, j), G-Premio BOND (k, l, m, n), Prime&Bond Universal (o, p, q, r)

CHAPTER V DISCUSSION AND CONCLUSIONS

Discussion

This study was conducted to investigate μTBS of the universal adhesives utilizing etch and rinse mode in various application methods and the three-step etch and rinse adhesive at 24 hours and after 6 months water-storage. G-Premio Bond applied following manufacturer's instruction and modified double application method had statistically lower μTBS than other adhesive systems except Prime&bond universal applied following manufacturer's instruction at 24-hour water storage, Optibond FL and Clearfil universal bond quick applied following manufacturers' instructions after 6-month water storage. Therefore, the first null hypothesis was rejected.

The μTBS of universal adhesive systems with double application method was not significantly increased when compared to the manufacturer's instruction technique at 24 hr (p = 0.652) and after 6 month water storage (p = 0.173). Thus, the second null hypothesis was not rejected.

Moreover, there was statistically significant decrease in μTBS of the adhesive systems after 6 month water-storage comparing to those in 24 hours in several treatment groups, such as Optibond FL, Single Bond Universal Adhesive when applied in double application method, G-Premio BOND when followed the manufacturer's instruction and applied in double application method. Therefore, the other null hypothesis was rejected.

The universal adhesives are recommended by manufacturers to be used with or without acid pretreatment of enamel or dentin surfaces. Many studies have reported that the performance of bond strength and clinical appearance do not depend on the etching mode (80, 81). In 2016, Jang et al. presented that the dentin bond strengths of universal adhesive were comparable to contemporary multi-step adhesives (82).

While Optibond FL adhesive contains a mixture of cross-linking monomers including TEGDMA, UDMA, and GPDM, the universal adhesives contain multiple functional monomers, especially 10-MDP, 4-MET and PENTA. Solvents are another composition which can influence bond strength. Ethanol in Single Bond Universal and Clearfil Universal Bond Quick, is used as co-solvent combined with water, known as ‘azeotropic’(83), resulting in a better evaporation of solvent compared to pure water (84). Isopropanol in Prime&Bond Universal, offered the best balance between polarity and surface wetting properties under various amounts of residual moisture, revealing advantages in terms of storage stability of the formulation, economical dosing and on-demand removability of the water-alcohol vapor (85). Acetone in G-Premio BOND, has highly evaporation ability and high dipole moment, thus it can rapidly penetrate into etched dentin, so-called “water-chasing” effect (86, 87). However, adhesive may not sufficiently penetrate in some conditions (82) and evaporation of acetone caused water derived from the adhesive or the underlying dentin to be trapped as water bubbles (88). Moreover, G-Premio BOND, a HEMA-free adhesive, was prone to phase separation affecting lower bonding quality (89), however, this effect was not seen in the present study. The *in vitro* performance of universal adhesives has been reported as material-dependent, due to complexity of their chemical composition (13, 17).

Application method of universal adhesives was one of the factors which had an influence on dentin μTBS. It was speculated that higher solvent evaporation occurred under active application increasing penetration of monomers into tooth substrates. The active application also enhanced chemical interaction with dentin substrate and improved polymerization efficacy. These led to increase resin–dentin bond strength (90, 91). Universal adhesives were mostly recommended to apply with scrub or rub motion except G-Premio BOND. It was the only material that the manufacturer recommended to apply and to leave it undisturbed. This might be one of the reasons why G-Premio BOND had the lowest dentin μTBS at 24-hour and after 6-month water storage in this present study (13, 51).

In order to clarify the effect of double application method on bond strength, some studies recommended multiple-layer application to increase bond strength of adhesives (21, 92). Additional coats of adhesive in the etch and rinse approach may eliminate water from etched dentin, allowing resin infiltration into the collagen fibril network, as shown by Hashimoto and colleagues (23). Moreover, multiple coats may increase rate of solvent evaporation owing to active application and extended period of time that the adhesive remained on dentin surface before light-curing. Consequently, this could lead to an increase in the concentration of monomers inside the hybrid layer after each coat was applied, creating the bonding interface less permeable and also more tolerable to the effects of degradation over the time (22, 93-95). Clinically, double coats of adhesive could take more time when doing a restoration, which is opposite to what clinicians in general would desire, which is to reduce the application time. Moreover, the overall results of this study demonstrated that universal adhesives used in double application method presented no significant higher μTBS than single application.

After 6-month water storage, it was noticed that μTBS of adhesive-dentin bond of Optibond FL was significantly decreased about 49.5% more than the other adhesive systems. This observation confirmed that the bonding performance of the 3-step etch-and-rinse adhesive Optibond FL appeared not stable during the 6-month water exposure. In the previous study, it was hypothesized that the reduction in bond strength occurred from degradation of the hybrid layer components and not from the overlying adhesive resin or resin composite (96). However, μTBS of adhesive-dentin bond of Single Bond Universal Adhesive applied following manufacture's instruction, Clearfil Universal Bond Quick and Prime&Bond Universal adhesive when followed the manufacturer's instruction, and when applied in double application method showed no significant decrease in bond strengths after 6 months of water storage. In the previous study, Marchesi, G., et al. demonstrated that the bond strength to dentin after 6 months was material-dependent since aging in artificial saliva reduced the bond strength of Scotchbond Universal compared to immediate values (12). In contrast, Munoz and others study which indicated that the bond strength of Single Bond Universal when used in self-etch mode and etch and rinse mode was stable after six months of water storage (25). For Prime&Bond Universal, it contained a newly developed resin components and hydrolysis stable crosslinker to avoid undesired phase separation (97).

In the failure mode analysis, adhesive and mixed failures were predominant in all adhesive. The highest of cohesive failures in resin composite was found in Single Bond Universal Adhesive when applied in double application method after aging in water for 6 months. This indicated that the development of universal adhesives for the preservation of the bonding interface has been achieved (98). Therefore, cohesive failure in resin composite was higher after water storage.

Accordingly, SEM observations of resin–dentin interfaces showed excellent adaptation to the dentin surface regardless of the etching mode of all adhesives except G-Premio Bond, which had a fracture line at the interface (Fig 3 k, l, m and n). Optibond FL, Clearfil Universal Bond Quick, Prime&Bond Universal presented the complex of long resin tags (Fig 3 a, b, i, o and q), whereas the others presented the broken resin tags. Optibond FL formed an adhesive layer of approximately 10 μm thick (Fig 3a and b). For the adhesive layer of Single Bond Universal Adhesive, G-Premio BOND with single application were approximately 4-6 μm thick, and Prime&Bond Universal 7-9 μm. Moreover, the adhesive layer of Clearfil Universal Bond Quick using single application was about 13-15 μm thick. Single Bond Universal Adhesive, Clearfil Universal Bond Quick, G-Premio BOND and Prime&Bond Universal's layer using double application method were approximately 13-15 μm thick too. Mostly, the adhesive layer of universal adhesives when using double application method were thicker than those adhesives following the manufactures' instructions, except Clearfil Universal Bond Quick. These finding related to Takamizawa's study which found that Single Bond Universal Adhesive applied following the manufactures' instruction in etch and rinse mode formed an adhesive layer of approximately 6–8 μm thick (16), as comparable to seen in this study.

The application method of universal adhesives following manufacturer's instruction is recommended. Thus, further study should be done focusing on other mechanical properties of universal adhesives after 6 months of water storage, whether or not agitated application of G-Premio BOND would improve the bond strength, and whether or not the use of a hydrophobic resin coat would improve. In addition, μTBS of the universal adhesive systems used in the present study in self-etch mode after long-term water storage should be investigated.

Limitations

1) This study was an in vitro study, therefore, the results of this study could not be inferred totally to the clinical situation. However, the researcher tried to control confounding factors and closely simulate a clinical situation.

2) This study focused on only 5 systems of adhesives, which were Optibond FL, Single Bond Universal, Clearfil Universal Bond Quick, G-Premio BOND, Prime&Bond Universal. Thus, the results of this study could not be inferred to other adhesive systems.

Suggested further studies

Further study should be done focusing on other mechanical properties of universal adhesives after 6 months of water storage, whether or not agitated application of G-Premio BOND would improve the bond strength, and whether or not the use of a hydrophobic resin coat would improve. In addition, μTBS of the universal adhesive systems used in the present study in self-etch mode after long-term water storage should be investigated.

Conclusions

1. Modified double application of universal adhesives did not improve the bond strength of tested universal adhesives at 24 hours and after 6 months of water storage.

2. Utilizing etch and rinse mode of Single Bond Universal used following manufacturer's instruction, Clearfil Universal Bond Quick and Prime&Bond Universal used both following manufacturer's instruction and modified double application method, had stable dentin microtensile bond strength, while Optibond FL and G-Premio BOND used both techniques had deceased bond strength after 6 months of water storage.

Clinical implication

Universal adhesives can be used clinically as suggested by manufacturers. Modification of application method, such as double application is not needed.

Declaration of Conflicting Interest

The authors declare no conflicts of interest in this study.

REFERENCES

1. Pashley DH, Carvalho RM. Dentine permeability and dentine adhesion. J Dent. 1997;25(5):355-72.

2. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. Dent Mater. 2005;21(9):864-81.

3. Irmak O, Baltacioglu IH, Ulusoy N, Bagis YH. Solvent type influences bond strength to air or blot-dried dentin. BMC Oral Health. 2016;16(1):77.

4. Tay FR, Pashley DH. Dental adhesives of the future. J Adhes Dent. 2002;4(2):91-103.

5. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24(1):90-101.

6. Perdigao J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent Mater. 2010;26(2):e24-37.

7. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28(3):215-35.

8. Van Meerbeek B, Kanumilli PV, De Munck J, Van Landuyt K, Lambrechts P, Peumans M. A randomized, controlled trial evaluating the three-year clinical effectiveness of two etch & rinse adhesives in cervical lesions. Oper Dent. 2004;29(4):376-85.

9. Zhang Y, Wang Y. Effect of application mode on interfacial morphology and chemistry between dentine and self-etch adhesives. J Dent. 2013;41(3):231-40.

10. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005;84(2):118-32.

11. Suppa P, Breschi L, Ruggeri A, Mazzotti G, Prati C, Chersoni S, et al. Nanoleakage within the hybrid layer: A correlative FEISEM/TEM investigation. J Biomed Mater Res B. 2005;73b(1):7-14.

12. Marchesi G, Frassetto A, Mazzoni A, Apolonio F, Diolosa M, Cadenaro M, et al. Adhesive performance of a multi-mode adhesive system: 1-year in vitro study. J Dent. 2014;42(5):603-12.

13. Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Ende A, Van Meerbeek B, et al. Bonding effectiveness of a new 'multi-mode' adhesive to enamel and dentine. J Dent. 2012;40(6):475-84.

14. Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: A systematic review and meta-analysis. J Dent. 2015;43(7):765-76.

15. Wagner A, Wendler M, Petschelt A, Belli R, Lohbauer U. Bonding performance of universal adhesives in different etching modes. J Dent. 2014;42(7):800-7.

16. Takamizawa T, Barkmeier WW, Tsujimoto A, Berry TP, Watanabe H, Erickson RL, et al. Influence of different etching modes on bond strength and fatigue strength to dentin using universal adhesive systems. Dent Mater. 2016;32(2):E9-E21.

17. Munoz MA, Luque I, Hass V, Reis A, Loguercio AD, Bombarda NH. Immediate bonding properties of universal adhesives to dentine. J Dent. 2013;41(5):404-11.

18. Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives - Part II: etching effects on unground enamel. Dent Mater. 2001;17(5):430-44.

19. Erhardt MC, Osorio R, Pisani-Proenca J, Aguilera FS, Osorio E, Breschi L, et al. Effect of double layering and prolonged application time on MTBS of water/ethanol-based self-etch adhesives to dentin. Oper Dent. 2009;34(5):571-7.

20. Van Landuyt KL, Peumans M, De Munck J, Lambrechts P, Van Meerbeek B. Extension of a one-step self-etch adhesive into a multi-step adhesive. Dent Mater. 2006;22(6):533-44.

21. Ito S, Tay FR, Hashimoto M, Yoshiyama M, Saito T, Brackett WW, et al. Effects of multiple coatings of two all-in-one adhesives on dentin bonding. J Adhes Dent. 2005;7(2):133-41.

22. Pashley EL, Agee KA, Pashley DH, Tay FR. Effects of one versus two applications of an unfilled, all-in-one adhesive on dentine bonding. J Dent. 2002;30(2-3):83-90.

23. Hashimoto M, Sano H, Yoshida E, Hori M, Kaga M, Oguchi H, et al. Effects of multiple adhesive coatings on dentin bonding. Oper Dent. 2004;29(4):416-23.

24. Ferracane JL, Berge HX, Condon JR. In vitro aging of dental composites in water--effect of degree of conversion, filler volume, and filler/matrix coupling. J Biomed Mater Res. 1998;42(3):465-72.

25. Munoz MA, Luque-Martinez I, Malaquias P, Hass V, Reis A, Campanha NH, et al. In vitro longevity of bonding properties of universal adhesives to dentin. Oper Dent. 2015;40(3):282-92.

26. Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, et al. Bonding of universal adhesives to dentine--Old wine in new bottles? J Dent. 2015;43(5):525-36.

27. McLean DE, Meyers EJ, Guillory VL, Vandewalle KS. Enamel Bond Strength of New Universal Adhesive Bonding Agents. Oper Dent. 2015;40(4):410-7.

28. Loguercio AD, de Paula EA, Hass V, Luque-Martinez I, Reis A, Perdigao J. A new universal simplified adhesive: 36-Month randomized double-blind clinical trial. J Dent. 2015;43(9):1083-92.

29. Taschner M, Kummerling M, Lohbauer U, Breschi L, Petschelt A, Frankenberger R. Effect of double-layer application on dentin bond durability of one-step self-etch adhesives. Oper Dent. 2014;39(4):416-26.

30. Saikaew P, Chowdhury AF, Fukuyama M, Kakuda S, Carvalho RM, Sano H. The effect of dentine surface preparation and reduced application time of adhesive on bonding strength. J Dent. 2016;47:63-70.

31. Pashley DH, Ciucchi B, Sano H, Horner JA. Permeability of dentin to adhesive agents. Quintessence Int. 1993;24(9):618-31.

32. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27(1):1-16.

33. Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, et al. Microtensile bond strength of eleven contemporary adhesives to dentin. J Adhes Dent. 2001;3(3):237-45.

34. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res. 1992;71(8):1530-40.

35. Cardoso Pde C, Loguercio AD, Vieira LC, Baratieri LN, Reis A. Effect of prolonged application times on resin-dentin bond strengths. J Adhes Dent. 2005;7(2):143-9.

36. Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, et al. Microtensile bond strength of eleven contemporary adhesives to enamel. Am J Dent. 2003;16(5):329-34.

37. van Dijken JW. Clinical evaluation of three adhesive systems in class V non-carious lesions. Dent Mater. 2000;16(4):285-91.

38. Sarr M, Kane AW, Vreven J, Mine A, Van Landuyt KL, Peumans M, et al. Microtensile bond strength and interfacial characterization of 11 contemporary adhesives bonded to bur-cut dentin. Oper Dent. 2010;35(1):94-104.

39. Shirai K, De Munck J, Yoshida Y, Inoue S, Lambrechts P, Suzuki K, et al. Effect of cavity configuration and aging on the bonding effectiveness of six adhesives to dentin. Dent Mater. 2005;21(2):110-24.

40. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, et al. Four-year water degradation of total-etch adhesives bonded to dentin. J Dent Res. 2003;82(2):136-40.

41. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. Dent Mater J. 2005;24(1):1-13.

42. Frankenberger R, Kramer N, Petschelt A. Technique sensitivity of dentin bonding: effect of application mistakes on bond strength and marginal adaptation. Oper Dent. 2000;25(4):324-30.

43. Peschke A, Blunck U, Roulet JF. Influence of incorrect application of Optibond FL on the marginal adaptation. J Dent Res. 1998;77:680-.

44. Peutzfeldt A, Asmussen E. Adhesive systems: effect on bond strength of incorrect use. J Adhes Dent. 2002;4(3):233-42.

45. Cavalheiro A, Vargas MA, Armstrong SR, Dawson DV, Gratton DG. Effect of incorrect primer application on dentin permeability. J Adhes Dent. 2006;8(6):393-400.

46. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, Van Meerbeek B. A 13-year clinical evaluation of two three-step etch-and-rinse adhesives in non-carious class-V lesions. Clin Oral Investig. 2012;16(1):129-37.

47. Yoshihara K, Yoshida Y, Hayakawa S, Nagaoka N, Irie M, Ogawa T, et al. Nanolayering of phosphoric acid ester monomer on enamel and dentin. Acta Biomater. 2011;7(8):3187-95.

48. Sangwichit K, Kingkaew R, Pongprueksa P, Senawongse P. Effect of thermocycling on the durability of etch-and-rinse and self-etch adhesives on dentin. Dent Mater J. 2016;35(3):360-8.

49. Miyazaki M, Platt JA, Onose H, Moore BK. Influence of dentin primer application methods on dentin bond strength. Oper Dent. 1996;21(4):167-72.

50. do Amaral RC, Stanislawczuk R, Zander-Grande C, Gagler D, Reis A, Loguercio AD. Bond strength and quality of the hybrid layer of one-step self-etch adhesives applied with agitation on dentin. Oper Dent. 2010;35(2):211-9.

51. Munoz MA, Sezinando A, Luque-Martinez I, Szesz AL, Reis A, Loguercio AD, et al. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives. J Dent. 2014;42(5):595-602.

52. Sano H, Shono T, Takatsu T, Hosoda H. Microporous dentin zone beneath resin-impregnated layer. Oper Dent. 1994;19(2):59-64.

53. Perdigao J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, et al. A New Universal Simplified Adhesive: 18-Month Clinical Evaluation. Oper Dent. 2014;39(2):113-27.

54. Ortengren U, Andersson F, Elgh U, Terselius B, Karlsson S. Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials. J Dent. 2001;29(1):35-41.

55. Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Effects of storage media on mechanical properties of adhesive systems. Am J Dent. 2004;17(2):104-8.

56. Deng D, Yang H, Guo J, Chen X, Zhang W, Huang C. Effects of different artificial ageing methods on the degradation of adhesive-dentine interfaces. J Dent. 2014;42(12):1577-85.

57. Fukushima T, Inoue Y, Miyazaki K, Itoh T. Effect of primers containing N-methylolacrylamide or N-methylolmethacrylamide on dentin bond durability of a resin composite after 5 years. J Dent. 2001;29(3):227-34.

58. Shono Y, Terashita M, Shimada J, Kozono Y, Carvalho RM, Russell CM, et al. Durability of resin-dentin bonds. J Adhes Dent. 1999;1(3):211-8.

59. Burrow MF, Satoh M, Tagami J. Dentin bond durability after three years using a dentin bonding agent with and without priming. Dent Mater. 1996;12(5):302-7.

60. Armstrong SR, Keller JC, Boyer DB. Mode of failure in the dentin-adhesive resin-resin composite bonded joint as determined by strength-based (muTBS) and fracture-based (CNSB) mechanical testing. Dent Mater. 2001;17(3):201-10.

61. Pashley DH, Ciucchi B, Sano H, Carvalho RM, Russell CM. Bond strength versus dentine structure: a modelling approach. Arch Oral Biol. 1995;40(12):1109-18.

62. Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, et al. Relationship between surface area for adhesion and tensile bond strength--evaluation of a micro-tensile bond test. Dent Mater. 1994;10(4):236-40.

63. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, et al. The microtensile bond test: a review. J Adhes Dent. 1999;1(4):299-309.

64. Ferrari M, Goracci C, Sadek F, Eduardo P, Cardoso C. Microtensile bond strength tests: scanning electron microscopy evaluation of sample integrity before testing. Eur J Oral Sci. 2002;110(5):385-91.

65. Armstrong SR, Boyer DB, Keller JC. Microtensile bond strength testing and failure analysis of two dentin adhesives. Dent Mater. 1998;14(1):44-50.

66. Gomes AL, Castillo-Oyague R, Lynch CD, Montero J, Albaladejo A. Influence of sandblasting granulometry and resin cement composition on microtensile bond strength to zirconia ceramic for dental prosthetic frameworks. J Dent. 2013;41(1):31-41.

67. Scherrer SS, Cesar PF, Swain MV. Direct comparison of the bond strength results of the different test methods: a critical literature review. Dent Mater. 2010;26(2):e78-93.

68. Bouillaguet S, Gysi P, Wataha JC, Ciucchi B, Cattani M, Godin C, et al. Bond strength of composite to dentin using conventional, one-step, and self-etching adhesive systems. J Dent. 2001;29(1):55-61.

69. Poitevin A, De Munck J, Van Landuyt K, Coutinho E, Peumans M, Lambrechts P, et al. Critical analysis of the influence of different parameters on the microtensile bond strength of adhesives to dentin. J Adhes Dent. 2008;10(1):7-16.

70. Pashley DH, Tao L, Boyd L, King GE, Horner JA. Scanning electron microscopy of the substructure of smear layers in human dentine. Arch Oral Biol. 1988;33(4):265-70.

71. Finger WJ. Dentin bonding agents. Relevance of in vitro investigations. Am J Dent. 1988;1 Spec No:184-8.

72. Tao L, Pashely DH, Boyd L. Effect of different types of smear layers on dentin and enamel shear bond strengths. Dent Mater. 1988;4(4):208-16.

73. Wei S, Shimada Y, Sadr A, Tagami J. Effect of double-application of three single-step self-etch adhesives on dentin bonding and mechanical properties of resin-dentin area. Oper Dent. 2009;34(6):716-24.

74. do Amaral RC, Stanislawczuk R, Zander-Grande C, Michel MD, Reis A, Loguercio AD. Active application improves the bonding performance of self-etch adhesives to dentin. J Dent. 2009;37(1):82-90.

75. Eckert GJ, Platt JA. A statistical evaluation of microtensile bond strength methodology for dental adhesives. Dent Mater. 2007;23(3):385-91.

76. De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, et al. Inhibition of enzymatic degradation of adhesive-dentin interfaces. J Dent Res. 2009;88(12):1101-6.

77. de la Macorra JC. Statistics: a nuisance, a tool, or a must? J Adhes Dent. 2007;9(5):424.

78. Armstrong S, Breschi L, Ozcan M, Pfefferkorn F, Ferrari M, Van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (muTBS) approach. Dent Mater. 2017;33(2):133-43.

79. Lafuente D. SEM analysis of hybrid layer and bonding interface after chlorhexidine use. Oper Dent. 2012;37(2):172-80.
80. Forgerini TV, Ribeiro JF, Rocha RO, Soares FZ, Lenzi TL. Role of Etching Mode on Bonding Longevity of a Universal Adhesive to Eroded Dentin. J Adhes Dent. 2017;19(1):69-75.
81. Burke FJT, Crisp RJ, Cowan AJ, Raybould L, Redfearn P, Sands P, et al. A Randomised Controlled Trial of a Universal Bonding Agent at Three Years: Self Etch vs Total Etch. Eur J Prosthodont Re. 2017;25(4):220-7.
82. Jang JH, Lee MG, Woo SU, Lee CO, Yi JK, Kim DS. Comparative study of the dentin bond strength of a new universal adhesive. Dent Mater J. 2016;35(4):606-12.
83. Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. Dent Mater. 2005;21(10):895-910.
84. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007;28(26):3757-85.
85. Gutierrez MF, Alegria-Acevedo LF, Mendez-Bauer L, Bermudez J, Davila-Sanchez A, Buvinic S, et al. Biological, mechanical and adhesive properties of universal adhesives containing zinc and copper nanoparticles. J Dent. 2019;82:45-55.
86. Jacobsen T, Soderholm KJ. Some Effects of Water on Dentin Bonding. Dent Mater. 1995;11(2):132-6.
87. Abate PF, Rodriguez VI, Macchi RL. Evaporation of solvent in one-bottle adhesives. J Dent. 2000;28(6):437-40.
88. Huang XQ, Pucci CR, Luo T, Breschi L, Pashley DH, Niu LN, et al. No-waiting dentine self-etch concept-Merit or hype. J Dent. 2017;62:54-63.
89. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. J Dent Res. 2005;84(2):183-8.
90. Zhang Y, Wang Y. Distinct photopolymerization efficacy on dentin of self-etch adhesives. J Dent Res. 2012;91(8):795-9.
91. Wang Y, Spencer P. Hybridization efficiency of the adhesive/dentin interface with wet bonding. J Dent Res. 2003;82(2):141-5.

92. Loguercio AD, Reis A. Application of. a dental adhesive using the self-etch and etch-and-rinse approaches - An 18-month clinical evaluation. J Am Dent Assoc. 2008;139(1):53-61.

93. Reis A, Grande RH, Oliveira GM, Lopes GC, Loguercio AD. A 2-year evaluation of moisture on microtensile bond strength and nanoleakage. Dent Mater. 2007;23(7):862-70.

94. Reis A, de Carvalho Cardoso P, Vieira LC, Baratieri LN, Grande RH, Loguercio AD. Effect of prolonged application times on the durability of resin-dentin bonds. Dent Mater. 2008;24(5):639-44.

95. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, et al. Degree of conversion and permeability of dental adhesives. Eur J Oral Sci. 2005;113(6):525-30.

96. Hashimoto M, Tay FR, Ohno H, Sano H, Kaga M, Yiu C, et al. SEM and TEM analysis of water degradation of human dentinal collagen. J Biomed Mater Res B. 2003;66(1):287-98.

97. Tsujimoto A, Shimatani Y, Nojiri K, Barkmeier WW, Markham MD, Takamizawa T, et al. Influence of surface wetness on bonding effectiveness of universal adhesives in etch-and-rinse mode. Eur J Oral Sci. 2019;127(2):162-9.

98. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjaderhane L, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater. 2018;34(1):78-96.

APPENDICES

Appendix A. Microtensile bond strength values of Optibond FL® after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
OFL-1	38.9527	21.6615
OFL-2	32.9122	23.3607
OFL-3	39.4824	21.9632
OFL-4	38.0726	20.0238
OFL-5	37.1287	12.8573
OFL-6	48.4060	26.7332
OFL-7	47.4756	19.0145
OFL-8	54.3029	24.3231
MEAN	42.0916	21.2421
SD	7.1682	4.1676

Appendix B. Microtensile bond strength values of Single Bond Universal® after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
SU-1	64.0448	55.2949
SU-2	59.0420	45.7378
SU-3	31.5573	36.1821
SU-4	66.7668	57.3618
SU-5	53.9610	45.5819
SU-6	55.5828	62.0367
SU-7	52.3268	49.6413
SU-8	37.1123	6.5733
MEAN	52.5492	44.8012
SD	12.3442	17.4254

Appendix C. Microtensile bond strength values of Single Bond Universal ® with double application methods after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
SU2C-1	54.2094	47.3173
SU2C-2	47.8734	47.4423
SU2C-3	58.6938	50.8202
SU2C-4	50.3263	45.0168
SU2C-5	45.7851	43.5609
SU2C-6	55.3301	53.7758
SU2C-7	56.2150	48.6959
SU2C-8	71.6094	49.1389
MEAN	55.0053	48.2210
SD	8.0114	3.2091

Appendix D. Microtensile bond strength values of Clearfil Universal Bond Quick® after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
CU-1	46.4422	21.7503
CU-2	53.0861	36.8681
CU-3	25.4829	43.0792
CU-4	41.6725	33.8287
CU-5	36.7778	15.3108
CU-6	30.7776	14.1687
CU-7	40.2549	53.5631
CU-8	59.9008	41.5267
MEAN	41.7993	32.5119
SD	11.2880	14.1672

Appendix E. Microtensile bond strength values of Clearfil Universal Bond Quick® with double application methods after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
SU-1	30.3502	43.6211
SU-2	49.3916	38.9659
SU-3	72.0031	45.1016
SU-4	26.7398	25.2444
SU-5	30.4530	41.5091
SU-6	47.5510	27.4290
SU-7	40.3713	44.9290
SU-8	48.3715	48.7469
MEAN	43.1540	39.4434
SD	14.7365	8.5904

Appendix F. Microtensile bond strength values of G-Premio BOND® after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
GP-1	25.7974	4.8502
GP-2	26.7866	24.0382
GP-3	31.3855	11.6368
GP-4	34.5404	25.1759
GP-5	19.4038	19.6417
GP-6	22.4774	20.0789
GP-7	20.1409	18.1255
GP-8	27.0504	21.0355
MEAN	25.1282	18.0728
SD	5.2635	6.7355

Appendix G. Microtensile bond strength values of G-Premio BOND® with double application methods after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
GP2C-1	31.9280	24.9054
GP2C-2	31.6778	28.8701
GP2C-3	20.6430	20.3504
GP2C-4	23.3023	20.4745
GP2C-5	21.1281	17.7388
GP2C-6	25.0663	16.1975
GP2C-7	21.7706	18.7693
GP2C-8	25.5100	16.9982
MEAN	25.1283	20.5380
SD	4.4723	4.3239

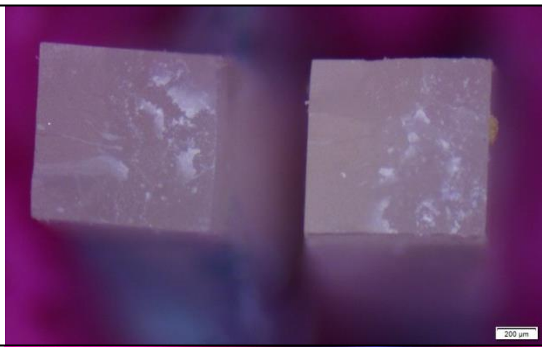
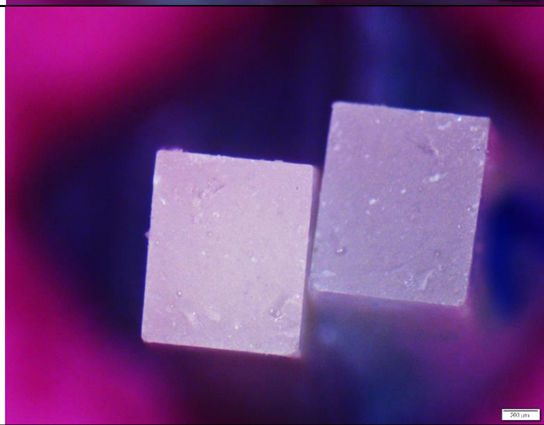
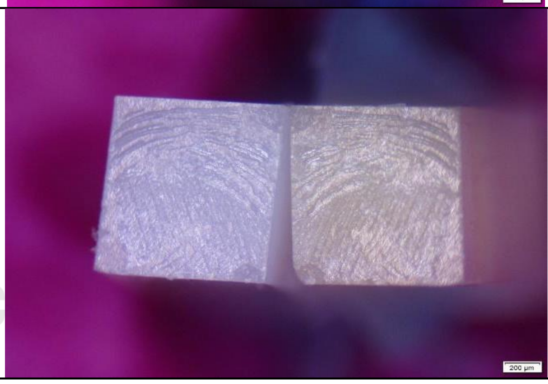
Appendix H. Microtensile bond strength values of Prime&Bond Universal® after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
PB-1	18.5741	17.8323
PB-2	28.4614	48.5922
PB-3	44.8281	46.5978
PB-4	51.5151	54.6121
PB-5	38.1947	41.2713
PB-6	37.9721	32.9825
PB-7	48.2165	39.9853
PB-8	60.5650	38.1380
MEAN	41.0409	40.0014
SD	13.2947	11.1906

Appendix I. Microtensile bond strength values of Single Bond Universal® after 24 hours vs 6 months water storage.

Units	24 Hours	6 months
SU-1	34.3926	47.6383
SU-2	30.2192	22.1020
SU-3	35.2554	43.4483
SU-4	65.5309	67.3609
SU-5	46.0337	43.1387
SU-6	44.3105	35.3625
SU-7	43.4113	37.5165
SU-8	43.7969	40.7311
MEAN	42.8688	42.1623
SD	10.8011	12.7607

Appendix J. Failure mode

Co-De	
Co-Re	
Ad/Mixed	

VITA

NAME	Suthatta Jeansuwannagorn
DATE OF BIRTH	10 September 1988
PLACE OF BIRTH	Bangkok, Thailand
INSTITUTIONS ATTENDED	2006-2012 Doctor of Dental Surgery, Faculty of Dentistry, Mahidol University, Bangkok, Thailand
HOME ADDRESS	35/30 Sivalee Village, Ratchpruk road, Bangkrui, Nonthaburi, Thailand