MICROTENSILE BOND STRENGTH OF SELF-ADHESIVE RESIN COMPOSITE TO DENTIN

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Esthetic Restorative and Implant Dentistry
Faculty of Dentistry
Chulalongkorn University
Academic Year 2013

การศึกษากำลังแรงยึดแบบดึงระดับจุลภาคของเรซินคอมโพสิตชนิดยึดติดได้ด้วยตัวเองต่อเนื้อฟัน

นายศุภฤทธิ์ ฉันทะชัยมงคล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2556

Thesis Title	MICROTENSILE BOND STRENGTH OF SELF-ADHESIVE RESIN COMPOSITE TO DENTIN
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Field of Study	Esthetic Restorative and Implant Dentistry
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ศุภฤทธิ์ ฉันทะชัยมงคล : การศึกษากำลังแรงยึดแบบดึงระดับจุลภาคของเรซินคอมโพสิตชนิดยึดติดได้ด้วยตัวเองต่อเนื้อฟัน. (MICROTENSILE BOND STRENGTH OF SELF-ADHESIVE RESIN COMPOSITE TO DENTIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.ศิริวิมล ศรีสวัสดิ์, 88 หน้า.

วัตถุประสงค์: เพื่อประเมินผลของการปรับสภาพผิวเนื้อฟันและกระบวนการเสื่อมสลายด้วยวิธีการแช่น้ำต่อกำลังแรงยึดแบบดึงระดับจุลภาคของเรซินคอมโพสิตชนิดยึดติดได้ด้วยตัวเองต่อเนื้อฟัน วิธีการทดลอง: คัดเลือกฟันกรามแท้ที่ถูกถอนจำนวน 72 ซี่ เพื่อนำมาประเมินกำลังแรงยึดแบบดึงระดับจุลภาคของเนื้อฟันที่ระดับกึ่งกลางตัวฟัน โดยคำนึงถึงวิธีการปรับสภาพผิวเนื้อฟัน ชนิดของวัสดุ และระยะเวลาในการแช่น้ำ โดยฟันจะแบ่งเป็น 4 กลุ่ม ด้วยวิธีการสุ่ม ได้แก่ กลุ่มควบคุม ปรับสภาพเนื้อฟันด้วยซิงเกิล บอนด์ ยูนิเวอร์แซล ตามด้วยการบูรณะโดยใช้ฟิลเทค ซี 350 เอ็กซ์ที โฟลเอเบิล คอมโพสิต กลุ่มที่ไม่มีการปรับสภาพเนื้อฟัน ตามด้วยบูรณะโดยใช้เวอร์ทิส โฟล กลุ่มที่มีการปรับสภาพเนื้อฟันด้วยกรดฟอสฟอริกความเข้มข้น ร้อยละ 37.5 ตามด้วยการบูรณะโดยใช้เวอร์ทิส โฟล และกลุ่มที่มีปรับสภาพเนื้อฟันด้วยกรดฟอสฟอริกความเข้มข้น ร้อยละ 37.5 ร่วมกับออพติบอนด์ โซโล พลัส ตามด้วยการบูรณะโดย เวอร์ทิส โฟล หลังจากนั้นจะทำการแบ่งแต่ละกลุ่มเป็น 2 กลุ่มย่อยด้วยวิธีการสุ่ม ได้จำนวนทั้งสิ้น 8 กลุ่ม ตามระยะเวลาการแช่น้ำที่ 24 ชั่วโมงหรือ 3 เดือน ทำการทดสอบกำลังแรงยึดแบบดึงระดับจุลภาคของชิ้นงานตัวอย่าง จะทำหลังการแช่น้ำเสร็จสิ้น และจะมีการบันทึกชนิดของการล้มเหลว ข้อมูลที่ได้จะนำมาวิเคราะห์ทางสถิติด้วยสถิติความแปรปรวนแบบสองทางและเปรียบเทียบด้วยวิธีการของบอนเฟอโรนี ที่ระดับนัยสำคัญ 0.05 ผลการทดลอง: ค่ากำลังแรงยึดแบบดึงระดับจุลภาคสูงสุดพบในกลุ่มที่ปรับสภาพเนื้อฟันด้วยกรดฟอสฟอริกความเข้มข้น ร้อยละ 37.5 ร่วมกับออพติบอนด์ โซโล พลัส ตามด้วยการบูรณะโดย เวอร์ทิส โฟล ที่ 24 ชั่วโมง (42.63±4.57 เมกะปาสคาล) และ ค่ากำลังแรงยึดแบบดึงระดับจุลภาคต่ำสุดพบในกลุ่มที่ไม่มีการปรับสภาพเนื้อฟัน ตามด้วยบูรณะโดยใช้เวอร์ทิส โฟล ที่ 3 เดือน (23.39±3.88 เมกะปาสคาล) พิจารณาถึงผลของการปรับสภาพผิวเนื้อฟันพบว่า กลุ่มที่มีการปรับสภาพผิวเนื้อฟันจะมีค่ากำลังแรงยึดแบบดึงระดับจุลภาคสูงกว่ากลุ่มที่ไม่มีการปรับสภาพผิวเนื้อฟัน พิจารณาถึงผลของการแช่น้ำพบว่ากำลังแรงยึดแบบดึงระดับจุลภาคที่ 24 ชั่วโมง เปรียบเทียบกับ 3 เดือน จะมีค่าลดลงอย่างมีนัยสำคัญ สรุป: การปรับสภาพผิวเนื้อฟัน และการแช่น้ำมีผลต่อกำลังยึดแบบดึงระดับจุลภาค

สาขาวิชา ทันตกรรมบูรณะเพื่อความสวยงามและทันตกรรมรากเทียม ลายมือชื่อนิสิต .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2556

5376149132 : MAJOR ESTHETIC RESTORATIVE AND IMPLANT DENTISTRY
KEYWORDS: AGING / DENTIN ADHESION / FLOWABLE RESIN COMPOSITE / MICROTENSILE BOND STRENGTH / SELF- ADHESIVE RESIN COMPOSITE

SUPARIT CHANTCHAIMONGKOL: MICROTENSILE BOND STRENGTH OF SELF-ADHESIVE RESIN COMPOSITE TO DENTIN. ADVISOR: ASST. PROF. SIRIVIMOL SRISAWASDI, Ph.D., 88 pp.

Objective: To evaluate the effect of pretreatment and aging process, by means of water storage, on microtensile bond strength of a self-adhesive resin composite to dentin. Materials and Methods: 72 extracted human molars were selected. Microtensile bond strength was evaluated using mid-coronal dentin. According to pretreatment methods, type of materials and water storage time, teeth were randomly divided into 4 groups; pretreatment dentin with Single Bond Universal followed by Filtek Z350 XT Flowable (control group), group without pretreatment followed by Vertise Flow, group with pretreatment with 37.5% phosphoric acid followed by Vertise Flow, and group with pretreatment with 37.5% phosphoric acid and Optibond Solo Plus followed by Vertise Flow. Each group was further randomly divided into 2 subgroups, total of 8 groups to be tested for 24 hours or 3 months. The specimens were tested for microtensile bond strength after water storage, and failure modes were recorded. Data were analyzed using two-way ANOVA and Bonferroni post hoc test (p=0.05). Results: The microtensile bond strength revealed the highest in group of dentin pretreatment with 37.5% phosphoric acid and Optibond Solo Plus followed by Vertise Flow at 24 hours (42.63±4.57 MPa) and the lowest in group without pretreatment followed by Vertise Flow at 3 months (23.39±3.88 MPa). Considering the effect of pretreatment, groups with pretreatment showed significantly higher microtensile bond strength than groups without pretreatment. In terms of influence of aging process, microtensile bond strengths were significantly decreased by water storage. Conclusion: Dentin pretreatment and water storage had effect on microtensile bond strength.

Field of Study: Esthetic Restorative and Implant Dentistry
Academic Year: 2013

Student's Signature .....

Advisor's Signature .....

ACKNOWLEDGEMENTS

This research would not have been possible without the ongoing support and guidance of the following people, for which I would like to take this opportunity to thanks.

I would like to express my sincere appreciation to my adviser, Assist. Prof. Dr. Sirivimol Srisawasdi for sharing her expertise and knowledge, her love and passion for research which had greatly motivated me to focus on my work, and provided me with the experience of high quality research and its required attitudes.

I profoundly pay my sincere homage to my program director and mentor; Assoc. Prof. Chalermpol Leevailoj. His understanding, advice and support throughout my whole postgraduate course are greatly appreciated.

I also would like to thanks my members of the Thesis Supervisory Committee, Assist. Prof. Dr. Sirichan Chiaraputt who provide a recommendation that helped me not faltering in anyways.

My gratitude is extended to Dr. Tewarit Somkotra for statistic consulting.

I wish to thanks staffs of the Dental Material Research Center for their assistance. In addition, many thanks are extended to staffs of Esthetic Restorative and Implant Dentistry Clinic for providing their assistance and co-operation throughout my graduate training program.

Finally, all of this would have been unachievable without the endless support, moral, caring and understanding from family and friends. I am grateful to all of them. For those who have not been named, their encouragement and friendship; I will cherish forever.

CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Background and Rationale

The use of direct resin composite materials has become an integral part of contemporary operative dentistry. Esthetic appearance and constantly improved properties have made these materials the main choice for direct restorations (1). However, resin composites, in common with the majority of dental materials undergo deterioration and degradation in the oral environment. Moreover, being technique-sensitive materials, failure at the tooth-restoration interface may also occur (2). As a result, managing of failed restorations is a common problem encountered in daily practice. Presently, many of the researches and innovations in dental materials are focused on simplification of the bonding procedures to eliminate technique-sensitivity and time consumption (3).

At the moment, resin composite materials that are able to adhere to dentin and enamel without the application of a separate phosphoric-acid etching and adhesive agent, the so-called "self-adhesive resin composite", have been developed. Although, self-adhesive resin composite is claimed to bond to tooth structure, the bonding performance of self-adhesive resin composite revealed that dentin bond strength of this material was significantly less than that of conventional flowable resin composite used in combination with other self-etch adhesives (4-6). Self-etch adhesives have been associated with lower bonding effectiveness as compared to total-etch adhesives. These adhesives are usually used according to

manufacturers' recommendations, but some have been found to perform better using modification of application technique, such as surface pretreatment (7-9). For this reason, further work is required to evaluate the performance regarding adhesion to dentin. Its capacity to increase bond strength with etchant or adhesive agent is still open to question. It raises the subject as how to improve or at least to maintain acceptable bond strengths overtime. Moreover, 24-hour bond strength test is the most frequently used tests. They revealed excellent short-term bonding effectiveness of dental adhesives (10). In oral cavity, many factors affect the bond strength and longevity of resin composite restorations due to hydrolytic degradation process at the dentin-adhesive interface. The most commonly used method to facilitate fluid exchange along the dentin bonded interface is artificial aging by water storage (11). Several studies reported a significant decrease in bond strength, even after relatively short water storage period (12-14).

This study investigated the effects of pretreatments and artificial aging, by means of water storage, on microtensile bond strength (μTBS) of one self-adhesive resin composite to dentin. A conventional flowable resin composite in combination with 1-step self-etch adhesive was selected as control material because the first layer of self-adhesive resin composite was acting, in effect, as 1-step self-etch adhesive (15).

Research questions

1. Do the different dentin treatments have a significant influence on microtensile bond strength of self-adhesive resin composite to dentin?

2. Does the aging process by means of water storage have a significant influence on microtensile bond strength of self-adhesive resin composite to dentin?

Objectives of the study

1. To compare microtensile bond strength of self-adhesive resin composite to dentin when using different dentin treatments as followings:

   i. No surface treatment

   ii. 37.5% phosphoric acid

   iii. 37.5% phosphoric acid and 2-step total-etch adhesive

2. To compare microtensile bond strength of self-adhesive resin composite to dentin when using different water storage times as following:

   i. 24 hours

   ii. 3 months

Statement of hypotheses

Null hypotheses:

1. There was no significant difference in microtensile bond strength of self-adhesive resin composite to dentin when using different dentin treatments.

2. There was no significant difference in microtensile bond strength of self-adhesive resin composite to dentin when using different water storage times.

Alternative hypotheses:

1. There was a significant difference in microtensile bond strength of self-adhesive resin composite to dentin when using different dentin treatments.
2. There was a significant difference in microtensile bond strength of self-adhesive resin composite to dentin when using different water storage times.

Scope of the study

This research is an experimental research for evaluate the effect of pretreatment and water storage on microtensile bond strength (μTBS) of one self-adhesive resin composite to dentin. Therefore, the results cannot be generalized to other part of tooth in different areas. Moreover, the results of this study may not be able to be extrapolated to other self-adhesive resin composites because different brands have different properties.

Assumptions

1. Microtensile bond strength of self-adhesive resin composite to dentin was increased when using different dentin treatment.
2. Microtensile bond strength of self-adhesive resin composite to dentin was decreased when using water storage.

Study limitation

Due to a limited budget, all brands cannot be evaluated. Thus, one self-adhesive resin composite was chosen to be tested in this study.

Keywords

Aging/ Dentin adhesion/ Flowable resin composite/ Microtensile bond strength/ Self-adhesive resin composite

The expected benefits

1. The results of this study might draw a clinical suggestion and information for proper use of self-adhesive resin composite.
2. The results of this study will be a benefit for further study.

CHAPTER II

REVIEW OF LITERATURES

This research is related to the microtensile bond strength of self-adhesive resin composite to dentin with different methods of dentin surface treatment after artificial aging by means of water storage. This review of literatures is provided in order to present the existing background information of self-adhesive resin composite particularly associated with concept of minimal intervention, bonding to dentin, bond strength to dentin, dentin surface treatment, microtensile bond strength test and aging process.

1. Concept of minimal intervention

Nowadays, restoring the tooth by minimal sacrifice of sound tooth structure to a long-term condition of health, function, and esthetic appearance as well as preventing caries recurrence are the significant goals of restorative dentistry. The philosophy of minimal intervention dentistry is a result of increased understanding of the caries process and the development of adhesive restorative materials. The term “minimal intervention dentistry” has been coined to describe a new approach to the treatment of the carious disease. It is now recognized that demineralized but non-cavitated enamel and dentin can be “healed” and that the surgical approach to the treatment of a caries lesion along with “extension for prevention” as proposed by G.V. Black is gradually shifted in the operative philosophy to “prevention for extension.” As a result, the traditional surgical approach to caries lesions has been steadily superseded by a biological approach, focusing on the individual caries risk

assessment, the disease control, and the healing potential of early carious lesions (16).

According to the minimal intervention concept, conventional resin composites are still being widely used. Later, new materials and, consequently, new techniques have been developed for tooth restoration using a flowable resin composite. A new type of composite resin was developed; differing from conventional composites, flowable resin composites have low viscosity and high flowability. Their main advantage is high wettability of the tooth surface. This allows the material to adapt closely to the microstructural and macrostructural defects in the floor and walls of the cavity and ability to form layers of minimum thickness, therefore improving or eliminating air inclusion or entrapment (17). However, flowable resin composites do not have adhesive properties themselves. The combination use of an adhesive agent is necessary.

Among dental adhesive systems, 1-step self-etch adhesive systems are simplified handling. Their adhesion process is based on the self-etch approach, which combines etching, priming and bonding into single application step. The exclusion of rinsing and drying steps of the etching process is a clinical advantage of 1-step self-etch adhesive systems, since the contamination risk is reduced and the bonding procedure is less sensitive to possible over-drying or over-wetting mistakes (18, 19).

Recently, an innovative resin-based material, combining the properties of self-etch adhesive system and flowable resin composite was developed. This restorative

material has been defined as “self-adhesive resin composite.” It has adhesive properties within itself utilizing self-etch adhesive bonding mechanism and a high adaptability that is the properties of the flowable resin composite. Therefore, self-adhesive resin composite is a material that is suitable for using in accordance with minimal intervention concept (20).

2. Self-adhesive resin composite

One of the recent developments in material science is the development of self-adhesive flowable resin composite. It incorporated adhesive technology into the flowable resin composite, such as Vertise® Flow™ (Kerr, Orange, CA, USA) and Fusio™ Liquid Dentin (Pentron, Orange, CA, USA). They have different functional monomer. Vertise® Flow™ uses Glycerol Phosphate Dimethacrylate Adhesive Monomer (GPDM) while Fusio™ Liquid Dentin uses 4-methacryloxyethyl tremellitic acid (4-MET). The bonding mechanism of Vertise® Flow™ has been claimed to be two-fold. Firstly, chemical adhesion is speculated to occur by the phosphate functional group of the GPDM monomer united with the calcium ions within the tooth structure, however not proven. Secondly, through a micromechanical interlocking by etching process as a result of an interpenetrating network formed between the polymerized monomers and collagen fibers of dentin (1, 20). Fusio™ Liquid Dentin contains 4-MET. Yoshida et al. showed that 4 MET was able to establish chemical bond between ionic bond and calcium in hydroxyapatite (21), so Fusio™ Liquid Dentin has been speculated to attributed chemical bonding potential to hydroxyapatite and tooth tissue.

The study by Tay et al. showed that 1-step self-etch adhesive systems permitted the passage of fluid and behaved as permeable membranes after polymerization. Water permeation through the polymerized adhesive layer occurs via osmotic pressure. The water molecules that migrate to the composite-adhesive interface mechanically disrupt coupling between the adhesive and resin composite; thus, the bond strength is decreased (22). This is especially important for self-adhesive resin composite, because the first layer was claimed to act as 1-step self-etch adhesive (15).

3. Bonding to dentin

Bonding to dentin is different from bonding to enamel due to the composition of the substrate between the enamel and dentin are different. Enamel contains about 86 vol % of an inorganic matrix, 2 vol% of an organic matrix, and 12 vol% of fluid but dentin contains about 45 vol% of an inorganic matrix, 33 vol% of an organic matrix and 22 vol % of fluid by volume. Dentin is more humid and more organic than enamel. The minerals are mostly in the form of apatite crystallites with dimensions that are smaller than those present in enamel. The organic matters consist mostly of type I collagen. Dentinal fluid has similar composition to plasma (23).

The structure and composition of dentin are highly variable from different areas even within the same tooth. Dentin is connected with the pulp via numerous fluid-filled channels or dentinal tubules. The orientation, size, and density of the dentinal tubules vary across the dentin due to the convergence of the dentinal

tubules towards the pulp. Dentinal tubule number is lowest at the dentino-enamel junction and highest at the predentin surface at the junction to the pulp chamber. As a result, the predentin surface at junction to the pulp contains moisture more than at the dentino-enamel junction (24).

The first documented use of dentin adhesive was published in 1952. Kramer and McLean reported their observation of an altered dentin surface after treatment with a self-polymerizing resin material (25). In 1955, Buonocore succeeded in using acid to increase the retention of enamel to adhesive resin (26). Next in 1956, Brudevold et al. observed adhesion of acrylic resin to dentin was to be improved by acid etching. Unfortunately, the bond was susceptible to hydrolysis and failed within a short period of time (27). Since then, a vast amount of effort has been invested in improving the bonding to dentin. Many systems of dentin bonding agents have evolved. Each system attempted to achieve an "optimal" bond through a different bonding mechanism. Currently, there have been two different strategies to achieve micromechanical retention between resin and dentin (3).

i. Complete removal of the smear layer

First approach, introduced by Fusayama et al. in 1979, aimed to remove the smear layers completely through acid etching and rinsing. Acid conditioner was simultaneously applied to enamel and dentin utilizing a total-etch technique (28). The surface smear layer and smear plugs were removed and the underlying dentin was partially demineralized. Infiltration of resin monomers enables micromechanical locking of the resin via the formation of hybrid layer and resin tags (29, 30). Three

separate steps, etching, priming, and application of adhesive, are required to enable coupling of resin composites to dentin. The use of 3-step adhesive systems, high initial bond strength could be achieved but the problems identified was the technique sensitive from dentin overwetting or overdrying (19) and the depth of demineralization may be greater than the depth of penetration by resin; consequently, this resulted in incomplete hybrid layer formation. Moreover, the presence of uninfiltrated demineralized dentin may affect the longevity of the resin-dentin bond (31).

Later, there was an innovation in adhesive technology by combining primer and adhesive into one component, resulting in 2-step total-etch systems. The problems that are derived from technique sensitivity and the wettability of primer/adhesive resins to properly penetrate into the demineralized dentin still remained (31, 32).

ii. Modification of smear layer

Second approach introduced to solve the problems of difficulty in determining the optimal level of dentin moisture and incomplete formation of hybrid layer, the smear layer was not completely removed but it was incorporated as part of the resin-dentin interface. This approach was based on the use of non-rinse acidic monomers that simultaneously condition and prime tooth structure, the so-called “2-step self-etch systems” (33). The combination of etching and priming not only reducing clinical application time but also avoiding the possibility of overdrying or overwetting that was seen when total-etch systems were applied due

to non-rinsing step to remove acid etching in this system. Moreover, self-etch adhesives eliminated the problem of incomplete resin infiltration within the hybrid layer, as etching and bonding were performed simultaneously (34). To further simplify bonding procedures, 1-step self-etch adhesive or all-in-one adhesive systems were subsequently introduced combining the etching, priming, and bonding procedures in a single step (35).

In addition, categorized according to acidity, there are basically three types of self-etch adhesive: mild, intermediate, or strong." “Strong” self-etch adhesives have a very low pH (<1) and exhibit a bonding mechanism and interfacial ultra-morphology in dentin resembling to that produced by total-etch adhesive systems. “Mild” self-etch adhesives (pH of around 2) dissolve the dentin surface only partially. Despite shallower resin tag formation, sufficient micromechanical interlocking and good bond strengths may be obtained. This may be the combined result of the simultaneous demineralization and resin monomer infiltration and a hydroxyapatite crystals remained within hybrid layer may serve as a receptor for additional chemical bonding (3).

Currently, a new category of resin composite, self-adhesive resin composite was introduced. This material did not require any pretreatment of the substrate. The study of Hanabusa et al. showed that self-adhesive resin composite was bonded to bur-cut dentin, and a resin infiltrated smear layer of maximum a few micrometers was formed but resin tags were not formed, since the smear plugs within the tubules were not dissolved. This kind of superficial interaction, along with the absence of

resin tag formation was typical of self-etch adhesives, especially in a rather thick and compact smear layer due to the low capacity of etching (15).

4. Bond strength to dentin

Many systems of dentin adhesive have evolved. Each system attempted to achieve optimal bond strength through a different bonding mechanism. Normally, self-etch adhesive systems produced hybrid layer with less thickness than that of total-etch adhesive systems. However, it has been recognized that the thickness of the hybrid layer was not related to the bonding ability of the adhesives (36). Studies comparing the performance of bonding to dentin between total-etch and self-etch adhesive systems have been performed. Vargas et al. showed that the shear bond strengths of some self-etch adhesive systems were similar to total-etch adhesive systems (37). Wilder et al. performed a study using bovine teeth, and found that although the mean shear bond strength of self-etch adhesive systems were lower than the total-etch adhesive system, the difference was not statistically significant (38).

The study of self-adhesive resin composite bond strength, Poitevin et al. revealed that Fusio™ Liquid dentin have a significantly higher dentin bond strength than Vertise® Flow™ (5) and the bonding performance of a self-adhesive resin composite, Fusio™ Liquid dentin (5) and Vertise® Flow™ (4-6), have significantly lower dentin bond strength than conventional flowable resin composite used in combination with other self-etch adhesives.

5. Dentin surface treatment

Adhesion to dentin is achieved by surface pretreatment with an acid, followed by application of an adhesive in total-etch adhesive systems and application of acidic primer to modify the smear layer in self-etch adhesive systems. The purpose of these steps is to remove or modify the smear layer and to demineralize the underlying dentin in order to expose the 3-dimensional collagen layer that can be infiltrated by adhesive resin monomers to form a hybrid layer between adhesive resin and dentin (34).

Calt et al. found that the more aggressive the conditioner, the more completely the smear layer was removed (39). Van Meerbeek et al. showed that phosphoric acid completely removed the smear layer and smear plugs while polyacrylic acid, lactic acid and citric acid exhibit milder actions on the smear layer (40). Meryon et al. stated that ethylene diamine tetra-acetic acid (EDTA) is a mild chelating agent that is also useful in removing the smear layer. Once the smear layer and smear plugs are dissolved, the permeability of dentin increases, and the etched dentin is covered with pulpal fluid that transudated from the dentinal tubules (41).

Although self-etch adhesive systems do not require pretreatment of the dental substrate, the use of adjunctive acid pretreatment with phosphoric acid has been suggested to improve bond strength of self-etching primers to dentin (7, 9). However, Perdigao et al. showed that the use of phosphoric acid in self-etch adhesive systems did not enhance bond strength because using phosphoric acid resulted in loss of the advantages of a self-etch adhesive systems and creating the

disadvantage as same as total-etch adhesive systems (42). The study of Gordon et al. showed that the use of phosphoric acid can improve bond strength because the compositions of self-etch primer have very low acidity that may not penetrate through the entire thickness of the smear layer (43).

A studies of pretreatment of self-adhesive resin composite, according to pretreatment enamel, Wajdowicz et al. found that shear bond strength of self-adhesive resin composite, Vertise® Flow™, was lower with uncut or aprismatic enamel, which is similar to using self-etch adhesive systems. For this reason, it is advisable to bevel, and/or etch aprismatic enamel beforehand. Furthermore, it was found that self-adhesive resin composite, Vertise® Flow™, had a significant higher in bond strength to enamel with the use of a phosphoric-acid etch (44). According to pretreatment dentin, Juloski et al. found that dentin bond strength of self-adhesive resin composite, Vertise® Flow™, was better but not significantly different when dentin was pretreated with phosphoric acid (45). Poitevin et al. showed dentin bond strength of self-adhesive resin composite, Vertise® Flow™, was significantly better when dentin was pretreated with phosphoric acid (5). The study of Bektas et al. found that dentin bond strength of self-adhesive resin composite, Vertise® Flow™, was significantly better when dentin was pretreated with adhesive agent (6).

6. Microtensile bond strength test

Tensile and shear bond strength tests have long been the most common laboratory tests to evaluate the adhesive strength of bonding systems to the tooth

substrate. Unfortunately, studies have shown that tensile and shear testing were significantly influenced by the variability in specimen geometry, loading conditions and material properties (46).

Tensile and shear bond strength tests were performed exclusively in specimens with relatively large bonded areas, usually 3–6 mm in diameter (approximately 7–12 mm^2). However, the validity of expressing bond strength in terms of nominal stress has been questioned due to the heterogeneity of the stress distribution at the bonded interface. Moreover, cohesive failure of both the composite and the dental substrate is a common occurrence, precluding an accurate assessment of the interfacial bond strength (47).

In 1994, Sano et al. developed the microtensile bond test (μTBS) to overcome tensile and shear bond tests limitations (48). Easier sample collection, ability to compare a variety of substrates and areas in the same tooth, more uniform loading stress distribution over a smaller bonded area are the advantages of the microtensile bond test (46, 47). However, the disadvantages of this technique are labor intensive, technically demanding; specimens easily dehydrate and damage (47).

In a typical microtensile test, bonded specimens were sectioned into rectangular or cylindrical sticks or bars, 0.5–1.5 mm thick, in the “non-trimming technique” or even further trimmed with burs at the adhesive interface to produce a dumbbell or hourglass appearance in case of the “trimming technique” (46, 48). The study of Betamar et al., a finite element analysis was used to study the stress

distribution of the test specimens in microtensile bond strength tests. The results indicated that stress distribution of a trimming specimen was better than that of a non-trimming specimen. However, improper preparation of trimming specimen result in geometries and more sensitive to flaws introduced during specimen preparation and pretest failures during trimming so the preparation of a specimen should be carefully performed (49).

A microshear bond strength test (μSBS) was introduced in 2002 (50). This test combines the ease of manipulation with the ability to test several specimens per tooth. The very fine composite build-up (cylinder) with a typical diameter of 0.7 mm, in combination with a relative thick adhesive layer may, however, result in considerable bending and variable and non-uniform loading conditions. Furthermore, it is impossible to confine the adhesive to the area tested. Basically due to these major shortcomings, this microshear bond strength test has not been adopted very well. In addition, Placido et al. summarized that microshear bond strength test results may worse represent shear bond strength than the traditional macroshear bond strength test (51).

At the present time, although lack of consensus of specimen design, a microtensile bond strength test appears to be able to discriminate adhesives better on their bonding performance than other bond strength tests. The microtensile bond strength approach with well controlled factor such as type of tooth, dentin depth, tooth storage and preparation of the specimen (52).

Research involving the bond strength test, both bovine and human teeth can be used as an in vitro specimen. Human teeth are more clinically relevant substrate tooth structure so the human teeth are the most common option chosen by researchers, especially human molar and premolar but using human molar gain more bonded area than premolar (53).

According to the ISO technical specification 11405:2003 (54), “ideally the bond strength test should be measured immediately post extraction, but this is not generally feasible. It appears that most changes occur in the initial days or weeks after extraction. For this reason, teeth 1 month, but not more than 6 months, after extraction should be used because teeth that has been extracted for longer than 6 months could undergo degenerative changes in dentinal protein. In general, teeth and teeth section can be storage successfully in a number of ways, the teeth that placed in alcohol or formalin solution can be affected to bond strength due to organic component change (55). The teeth when stored in distilled water or isotonic saline still have insufficient data to make a conclusion. There was a recommendation to store the teeth in 0.1% thymol solution because it inhibit the growth of bacteria and does not affect to bond strength (56, 57).

Another consideration is the methods to store specimens exposing to water, which two different strategies, direct and indirect water exposure have been used. In the indirect water exposure of the entire restored tooth, prior to sectioning would possibly simulate more closely to clinical situation. The water takes some time to diffuse from the external surface into the inner bonded interface (58). On the

contrary, direct water exposure of a small sample piece did not allow extrapolation of results to clinical practice (59).

Koibuchi et al. reported that bonding to a smooth (#600-grit) smear layer mediated by the self-etching adhesive system yielded excellent tensile bond strength. However, it was more clinically relevant by using “coarse” (#180-grit) smear layer because it not only was the tensile bond strength produced much lower than that found on “smooth” smear layers but also the deviation was large. Deviations were much narrower with the “smooth” smear layer specimens. In this way, #180-grit abrasive paper was recommended for the dentin preparation since these simulate smears formed clinically with dental burs (60).

7. Aging process

Even though the 24-hour bond strength test is the most frequently used tests, and they revealed excellent immediate and short-term bonding effectiveness of dental adhesives, the bond strength and longevity of resin bonded interfaces on dentin created by some bonding systems remain questionable because many intraoral factors such as thermal changes, acid and enzyme, and occlusal force affect the bond strength and longevity of resin composite restorations due to hydrolytic degradation process at the dentin-adhesive interface (61, 62). The bond strength tests that simulate intraoral condition have many methods, for instance, thermo-cycling, occlusal loading process, and storage media.

i Thermo-cycling

Thermo-cycling test is conventionally used to simulate thermal changes and water exposure that may occur in the oral cavity during eating, drinking, or even breathing. The ISO technical specification 11405:2003 (54) indicates that a thermo-cycling regimen comprised of 500 cycles in water between 5 and 55°C is an appropriate artificial aging method. A literature review of Gale and Darvell concluded that 10,000 cycles corresponded to approximately 1 year of in vivo function (63) due to the higher thermal contraction/expansion coefficient of the restorative material (as compared to that of tooth tissue), repetitive contraction/expansion stresses were generated at the tooth-biomaterial interface (64). These stresses may lead to cracks that propagated along bonded interfaces, and, once a gap was created, changing gap dimensions caused in- and outflow of oral fluids, a process known as “percolation” (63). A recent meta-analysis by Leloup et al., data published between 1992 and 1996 concluded that thermo-cycling had no significant effect on bond strength. Most studies included in the meta-analysis were carried out following the ISO standard of 500 cycles. This number of cycles was probably too low for an aging effect to be obtained. Also, specimen geometry has often not been taken into account. In most studies cited in that review, relatively large composite cylinders bonded to flat surfaces were thermo-cycled, prior to being pulled apart following a shear or tensile bond strength test protocol. As a result, the surrounding tooth and composite must have thermally protected a large part of the interface (65).

ii Mechanical loading

Mechanical loading may also affect resin adhesion to tooth structure. Again, to simulate this stress in vitro, it is important that one imposes stress similar to that occurring in vivo (66). One possibility is to “age” restored cavities in a chewing simulator and afterward measure the bonding effectiveness (67, 68). A better alternative is to study dynamic mechanical phenomena, such as crack initiation and propagation, in well controlled fracture toughness or fatigue test set-ups.

iii Water storage

The most commonly used artificial aging technique is long-term water storage. The bonded specimens are stored in fluid at 37°C for a specific period. This period may vary from a few months (12-14) up to 4-5 years or even longer (62, 69). Most studies report significant decreases in bond strengths, even after relatively short storage periods. In fact, the immediate dentin bond strength values do not always correlate with long-term bond stability since degradation throughout the dentin bonded interface occurs rapidly (13, 62, 69). De Munck et al. reported that within 3 months, all classes of adhesives exhibited mechanical and morphological evidence of degradation that resembles in vivo aging (18). Similarly, Toledano et al., found that bond strength of 2-step total-etch and 1-step self-etch adhesive systems decreased in 3-month water storage. (14). In the study of Breschi et al., most simplified 1-step self-etch adhesive systems were shown to be the least durable, while 3-step total-etch and 2-step self-etch adhesive systems showed better performances, as reported in the majority of studies (11).

CHAPTER III

MATERIALS AND METHODS

Research design

Experimental research

Dental materials

1. Vertise® Flow™ (Kerr, Orange, CA, USA)
2. Filtek™ Z350 XT Flowable composite (3M ESPE, St. Paul, MN, USA)
3. OptiBond® Solo Plus™ (Kerr, Orange, CA, USA.)
4. Single Bond™ Universal (3M ESPE, St. Paul, MN, USA)
5. 37.5% Phosphoric acid – Kerr® Gel Etchant (Kerr, Orange, CA, USA)
6. Model Repair II Blue (Dentsply-Sankin, Ohtawara, Japan)
7. 0.1% Thymol solution (Merck KGaA, Darmstadt, Germany)
8. Dental wax, pink type (Pigeon, Shanghai,China)
9. A 180-grit silicon carbide paper (Weibao, Shanghai,China)
10. Standard grit, cylinder diamond bur 1.4 mm in diameter: B1, CrossTech Diamond bur #014 (CrossTech, Bangkok, Thailand)

Equipments

1. LED Light-Curing System: Demi™ Plus (Kerr, Orange, CA, USA)
2. Radiometer: Model 100 Optilux (Kerr, Orange, CA, USA)
3. Stereomicroscope: ML 9300 (MEIJI, Saitama, Japan)
4. Digital vernier caliper: Mitutoyo digital caliper (Mitutoyo Corp., Kanogawa, Japan)

5. Low-speed cutting machine: ISOMET® 1000 (Buehler, Lake Bluff, IL, USA)
6. Scanning electron microscope: JSM-5410LV (JEOL, Tokyo, Japan)
7. Universal testing machine: EZ-S Shimadzu (Shimadzu AG-IS, Kyoto, Japan)
8. Incubator: Contherm 160M (Contherm Scientific Ltd., Lower hut, New Zealand)

Methods

1. Sample description

The population of specimens was calculated from the result of the pilot study under a protocol approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (Pilot Study Code: P 2012-001). Seventy-two selected non-carious, and non-restored extracted human molars were collected, after informed consent was obtained under a protocol approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (Study Code: HREC-DCU 2012-049). All the selected teeth were debrided and stored in a 0.1% thymol solution at 4 °C for up to 1 month following extraction.

2. Preparation of samples

2.1 The roots of teeth were embedded into a dental wax, pink type (Pigeon, Shanghai, China) leaving the clinical crown exposed (Figure 1, 2).

2.2 The occlusal third of the embedded tooth was removed to expose mid-coronal dentin using a low-speed cutting machine (ISOMET® 1000; Buehler, IL, USA) (Figure 1, 3)

2.3 Teeth presented with enamel or pulp exposure were excluded when evaluated using a stereomicroscope (ML 9300; MEIJI, Saitama, Japan) at 40X magnification.

2.4 Smear layer on dentin was created by grinding the surface with a 180-grit silicon carbide paper in one direction under running water for 30 seconds.

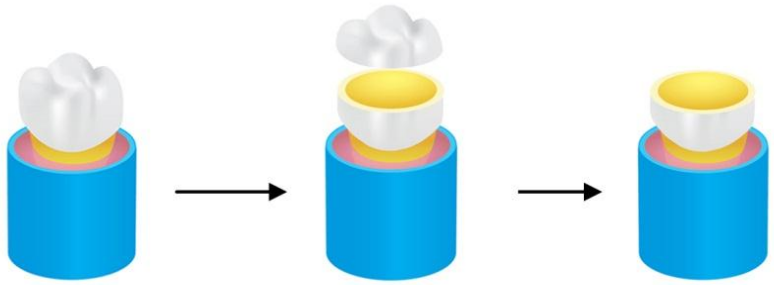


Figure 1 The preparation of specimens

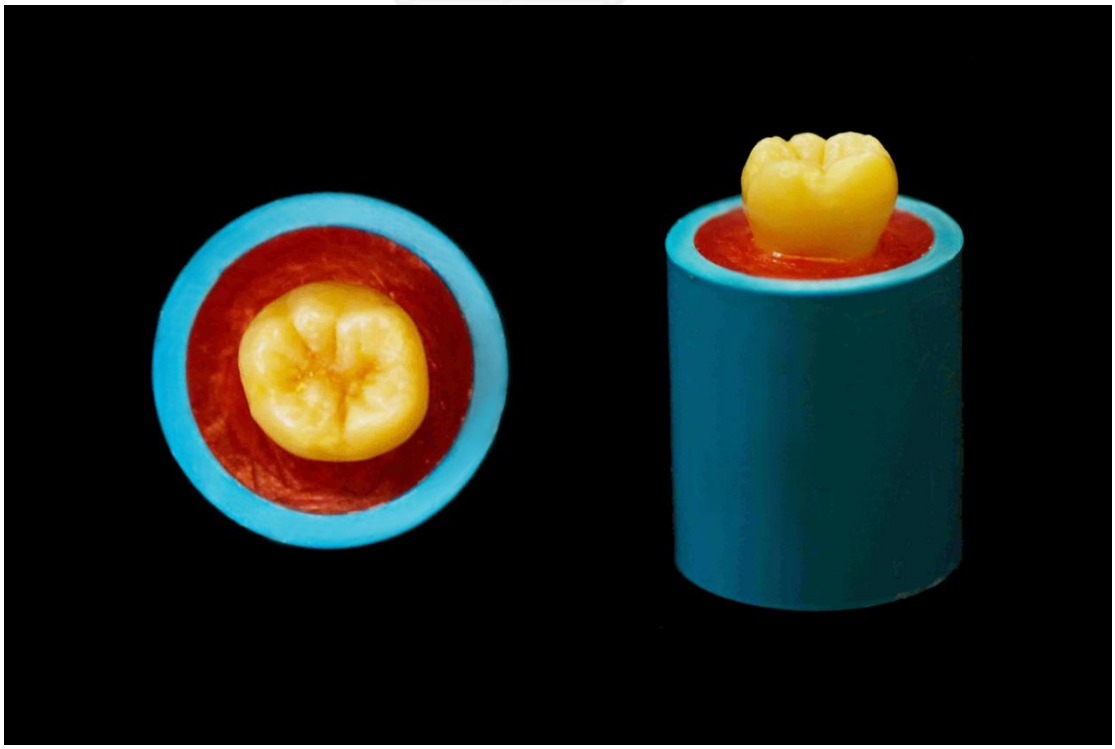


Figure 2 Embedded tooth was prepared

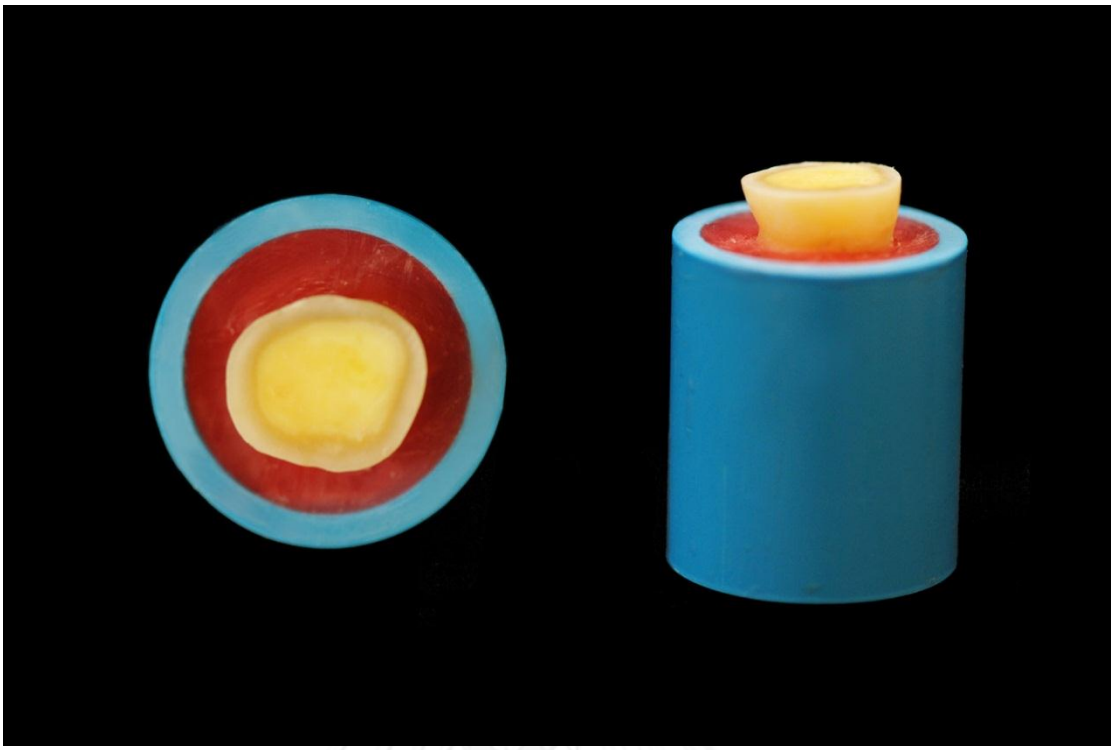


Figure 3 Crown was removed

3. Allocation technique

The compositions of resin composite and adhesive agents used in this study were summarized in Table 1. The teeth were randomly divided into four groups; each containing eighteen specimens as follows:

Group SF (control group): After application of 1-step self-etch adhesive (Single Bond™ Universal; 3M ESPE, St. Paul, MN, USA) following the manufacturer's instruction, flowable resin composite (Filtek™ Z350 XT Flowable; 3M ESPE, St. Paul, MN, USA) (shade A2) was injected into a silicone mold (6 x 6 x 4 mm^3) over the prepared dentin surface and light cured. Each 2 mm increments were polymerized for 20 seconds.

Group V: Self-adhesive flowable resin composite (Vertise® Flow™; Kerr, Orange, CA, USA) (shade A2) was agitated by brush for the first incremental for 20 seconds and light cured for 20 seconds. Next layer was injected into the silicone mold and light cured. Each 2 mm increment was polymerized for 20 seconds.

Group PV: After etching with 37.5% phosphoric acid (Kerr® Gel Etchant; Kerr, Orange, CA, USA) following the manufacturer's instruction, Vertise® Flow™ was agitated by brush for the first incremental for 20 seconds and light cured for 20 seconds. Next layer was injected into the silicone mold and light cured. Each 2 mm increment was polymerized for 20 seconds.

Group POV: After etching with 37.5% phosphoric acid (Kerr® gel etchant; Kerr, Orange, CA, USA) and application of 2-step total-etch adhesive (OptiBond® Solo Plus™; Kerr, Orange, CA, USA) following the manufacturer's instruction, Vertise® Flow™ was agitated for the first incremental by brush for 20 seconds and light cured for 20 seconds. Next layer was injected into the silicone mold and light cured. Each 2 mm increment was polymerized for 20 seconds.

Note: In this study, the conventional flowable resin composite in combination with 1-step self-etch adhesive was selected as control materials because the first layer of self-adhesive resin composite was acting, in effect, as 1-step self-etch adhesive (15).

Table 1 Materials used in this study

Materials	Composition	Application
Vertise® Flow™ (self-adhesive flowable resin composite) (Kerr, Orange, CA, USA)	**Resin:** GPDM and methacrylate co-monomers **Filler**: prepolymerized filler, 1 μm barium glass, nano-sized colloidal silica, nano-sized ytterbium fluoride	0.5 mm thin layer created by 20-second agitation by brush, light cure (20 seconds), build more restoration in increments of 2 mm or less, light cure (20seconds)
Filtek™ Z350 XT Flowable (flowable resin composite) (3M ESPE, St. Paul, MN, USA)	**Resin**: Bis-GMA, TEGDMA and Bis-EMA **Filler**: non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 75 nm silica filler, aggregated zirconia/silica cluster filler (consisted of 20 nm silica and 4-11 nm zirconia particles), 0.6-10 μm cluster particles, 0.1-0.5 μm ytterbium trifluoride particle	The thickness of the individual increments must not exceed 2.0 mm, light cure (20 seconds)
OptiBond® Solo Plus™ (2-step total-etch adhesive) (Kerr, Orange, CA, USA)	**Etchant**: 37.5% H_3PO_4 **Adhesive:** Bis-GMA, HEMA, GPDM, ethanol, barium,	Acid etch (15 seconds), rinse (15 seconds) and gentle air stream (do not

	aluminoborosilicate glass, fumed silica (silicon dioxide), sodium hexafluorosilicate, CQ	desiccate), apply adhesive (15 seconds with agitation), air dried thin (3 seconds), light cure (20 seconds)
Single Bond™ Universal (1-step self-etch adhesive) (3M ESPE, St. Paul, MN, USA)	Bis-GMA, HEMA, water, ethanol, silane-treated silica, 10-MDP, 2-propenoic acid, 2-methyl-, reaction products with 1,10-decanediol and P_2O_5, copolymer of acrylic and itaconic acid, dimethylaminobenzoat (-4), CQ, (dimethylamino) ethyl methacrylate, methyl ethyl ketone, silane	Gentle bond agitation (20 seconds), air stream (5 seconds) , light cure (10 seconds)

Abbreviation – 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA: bisphenol A glycol dimethacrylate; Bis-EMA: ethoxylated bisphenol A glycol dimethacrylate CQ: camphorquinone; GPDM: glycerol phosphate dimethacrylate; H_3PO_4: phosphoric acid; HEMA: 2-hydroxyethyl methacrylate; P_2O_5: phosphorous oxide; TEGDMA: triethyleneglycol-dimethacrylate

4. Restorative procedure

After pretreatment according to the experimental groups, by following the manufacturer's instruction, Vertise® Flow™ (Kerr, Orange, CA, USA) or Filtek™ Z350 XT Flowable (3M ESPE, St. Paul, MN, USA) in shade A2 was built up by following the

manufacturer's instruction to approximately 6 x 6 x 4 mm^3 using silicone mold onto the treated dentin surface (Figure 4-6). Each 2 mm increment was polymerized using a LED light-curing system (Demi™ Plus; Kerr, Orange, CA, USA) with 1,100 mW/cm^2 intensity. The light guide was held perpendicularly and within 1 mm superior to the silicone mold. Light output from the light-polymerizing unit was checked using a radiometer (Model 100 Optilux; Kerr, Orange, CA, USA) throughout the experiment.

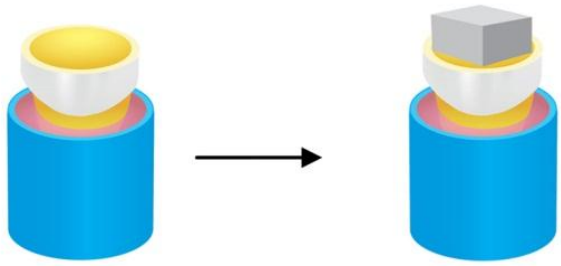


Figure 4 Restorative procedure

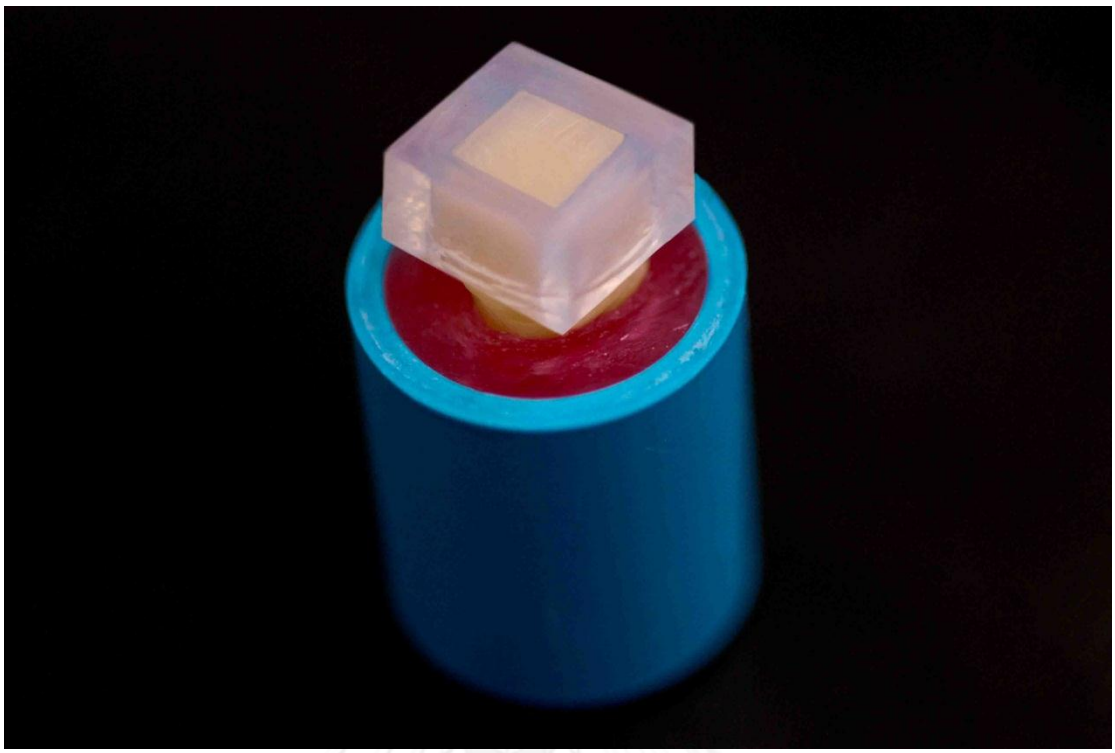


Figure 5 Silicone mold that was used in restorative procedure

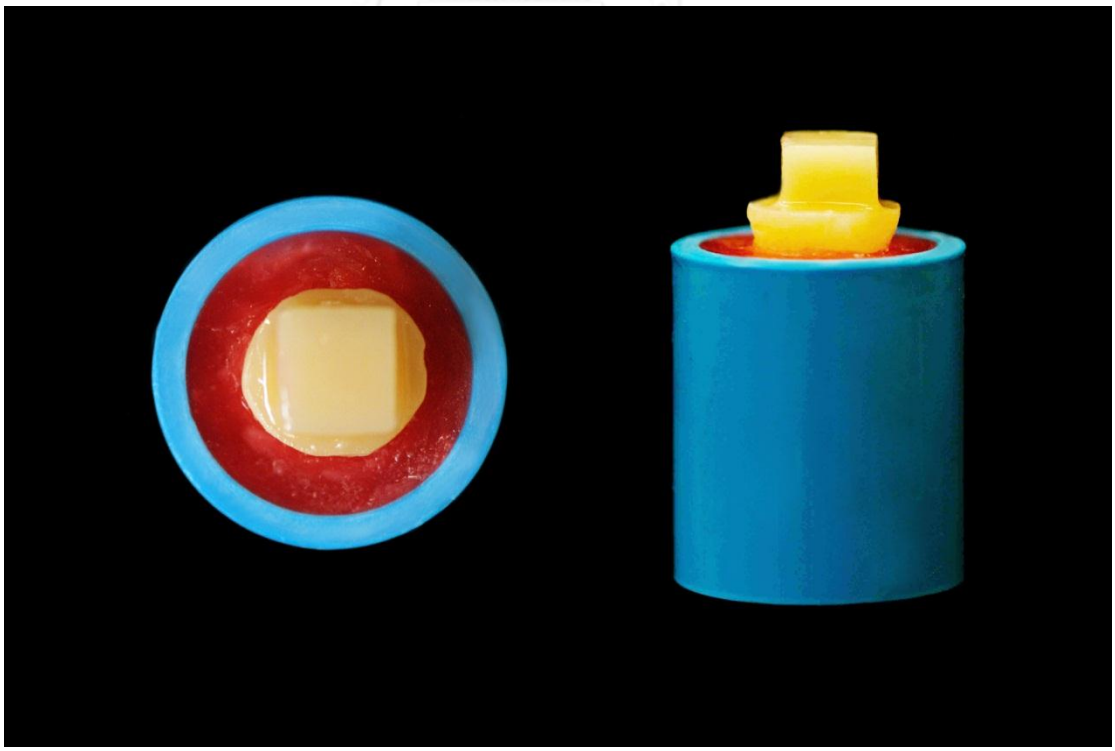


Figure 6 Composite was built up

5. Water storage

After the restorative procedure, each group was further randomly divided into two subgroups (n = 9 each) to be tested for 24 hours or 3 months after artificial aging by means of water storage in distilled water at 37 °C in an incubator (Contherm 160M; Contherm Scientific Ltd., Lower hut, New Zealand) as followed (Figure 7):

- Group SF24h, V24h, PV24h, POV24h, each group was storaged in distilled water for 24 hours.
- Group SF3m, V3m, PV3m, POV3m, each group was storaged in distilled water for 3 months.

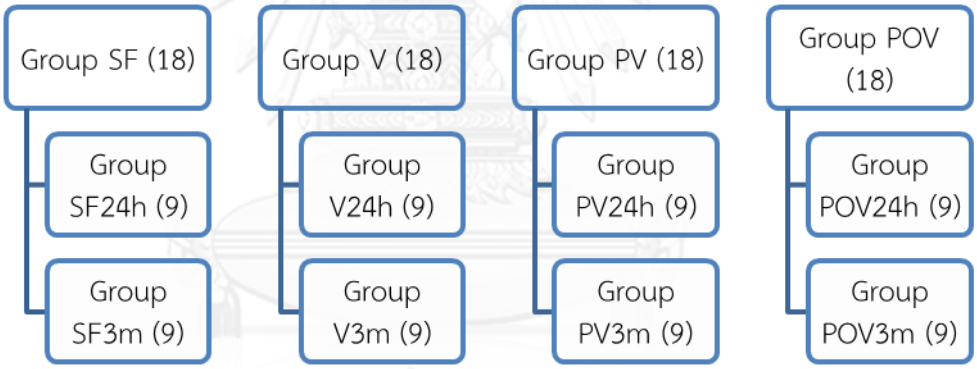


Figure 7 Diagram of experimental groups

6. Microtensile bond strength test preparation

After storage, teeth were sectioned perpendicular to the adhesive-tooth interface using low-speed cutting machine (ISOMET® 1000; Buehler Ltd., Lake Bluff, IL, USA) to obtain four rectangular samples of about 1 x 4 x 8 mm-slap from each tooth. Nine teeth from each group yielded thirty-six slabs for bond strength evaluation (n = 36 per group). Each slab was trimmed to an hourglass-shape with standard grit,

cylinder diamond bur of 1.4 mm in diameter (B1, CrossTech Diamond bur #014; CrossTech, Bangkok, Thailand) under air-water irrigation with a cross-sectional area of approximately 1 mm^2 (Figure 8-12). The exclusion criteria included presence of any obvious flaw or specimens debonding prior to testing.

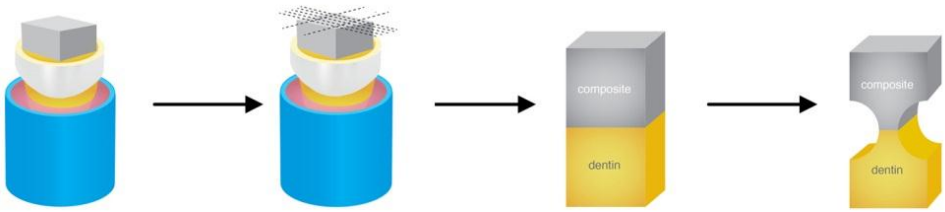


Figure 8 Microtensile bond strength test preparation

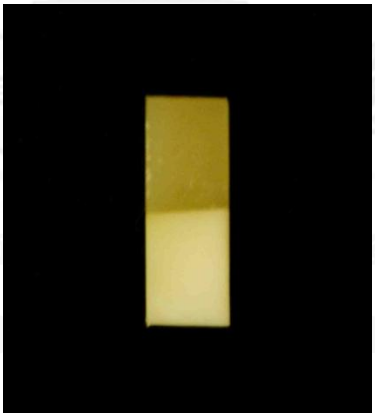


Figure 9 Rectangular slap was obtained for specimen restoration

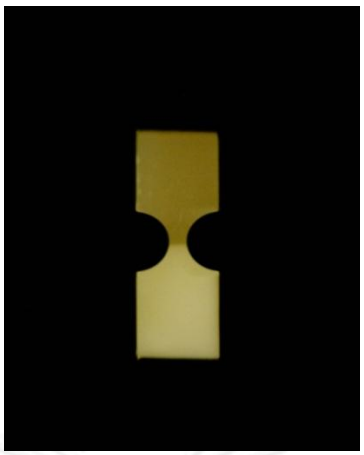


Figure 10 Each slap was prepared for hour-glass specimen

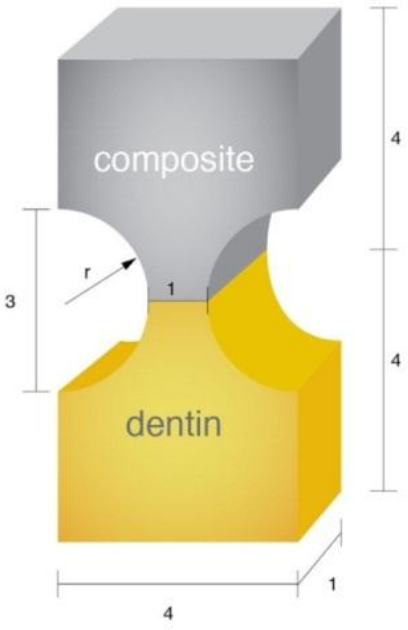


Figure 11 Geometric characteristics of hourglass specimen

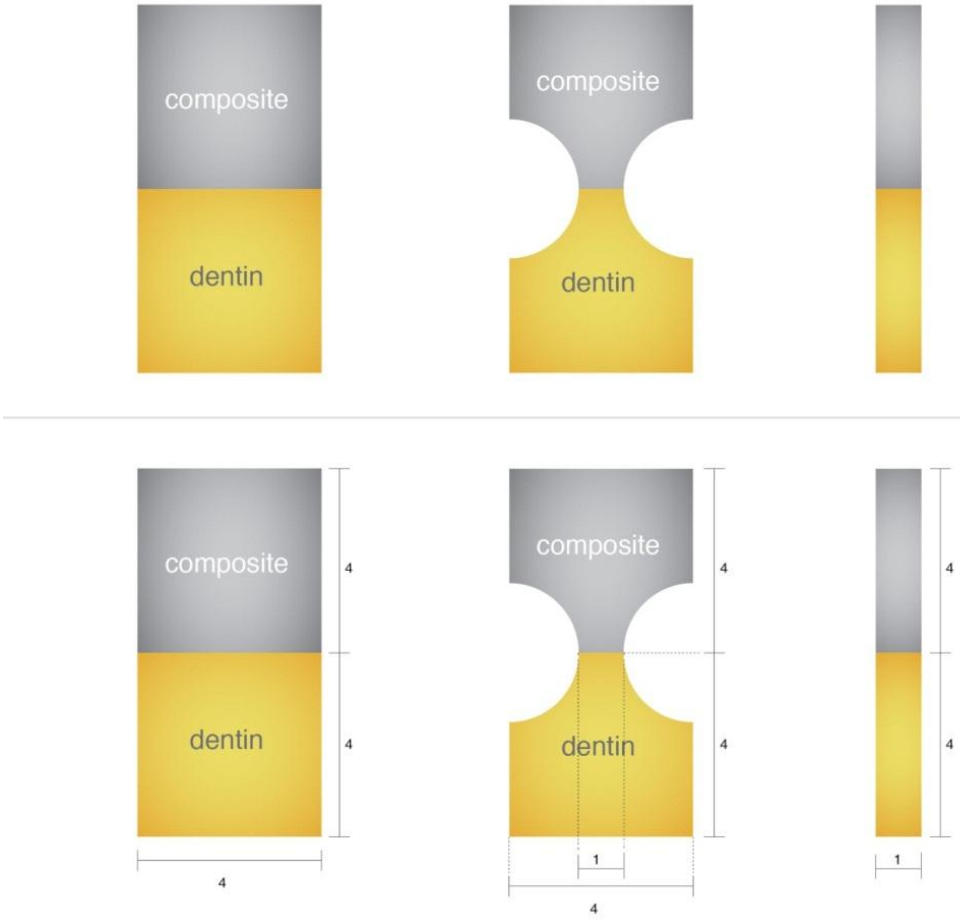


Figure 12 The dimension of hourglass specimen

7. Microtensile bond strength test

The dimension of each slap was measured using a digital vernier caliper (Mitutoyo digital caliper; Mitutoyo Corp., Kanogawa, Japan). All slabs were then attached to the testing apparatus using a cyanoacrylate adhesive (Model Repair II Blue; Dentsply-Sankin, Ohtawara, Japan), and stressed to failure in tension using a universal testing machine (EZ-S Shimadzu; Shimadzu AG-IS, Kyoto, Japan) at a cross-head speed of 1 mm/minute (Figure 13, 14).

Figure 13 The universal testing machine

Figure 14 The mouted specimen to the apparatus

8. Outcome measurement

8.1 This study measured the microtensile bond strength after 24-hour or 3-month storage in distilled water. The microtensile bond strength, given in MPa, was recorded when a fracture occurred.

8.2 Modes of failure of all specimens were recorded using a stereomicroscope (ML9300; MEIJI, Saitama JAPAN) at x40 magnification. Fracture mode was classified into one of four type categories as:

Type I: Adhesive failure at the resin/dentin interface (>75% of failure between resin/dentin interface)

Type II: Cohesive failure exclusively within resin composite (>75% of the failure is within the resin composite)

Type III: Cohesive failure exclusively within dentin (>75% of the failure is within the dentin)

Type IV: Mixed (failure at resin/dentin interface that included cohesive failure of the neighboring substrates).

In this study, slaps with pretest failures were not included in the compilation of mean microtensile bond strength as well as the failure mode assessment.

8.3 Some representative fracture surfaces were processed for scanning electron microscopy (JSM-5410LV; JEOL, Tokyo, Japan) in order to confirm the assigned mode of failure.

9. Data collection

9.1 Microtensile bond strength (μTBS)

The microtensile bond strength (MPa) at breaking point was recorded automatically by the testing machine.

9.2 Mode of failure

Data from this study was recorded in table in terms of the frequency of fracture modes.

10. Data analysis

All data were analyzed statistically using SPSS statistics for Windows, version 17.0 (Chicago, IL, USA). The microtensile bond strength data were analyzed with two-way ANOVA test and Bonferroni post hoc test ($p = 0.05$).

11. Sample size calculation

Sample description

1. According to pilot study, the population was sixteen selected non-carious and non-restored extracted human molars that collected after informed consent has

been obtained under a protocol approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University. (Pilot Study Code: P 2012-001)

2. Sample size calculation (n per group) was calculated from this formula;

$$n = \frac{2\sigma^2(Z_\alpha + Z_\beta)^2}{(\mu_1 - \mu_2)^2}$$

$$\sigma^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}$$

Where:

- 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)
- $Z\beta$ is the value of the standardized score cutting off the upper proportion
- σ^2 is the assumed common variance in the two groups (S)
- μ1-μ 2 is the difference in means of the two groups

In this study determines

$Z\alpha$ = 1.96 at 95 % confidence interval, $Z\beta$ = 0.84 at 80% power of test

The pilot study was randomly allocated four teeth per group. One tooth can prepare four hourglass slabs for the microtensile bond strength test (n = 16 per group). The test was processed after bond the resin composite to dentin and the restored teeth were storage in distilled water for 24 hours. In the Table 2, mean and standard deviation of microtensile bond strength test from the pilot study were shown.

Table 2 Data of microtensile bond strength test from pilot study

	Group SF	Group V	Group PV	Group POV
Mean	44.3814	31.7075	37.8217	41.1950
Standard deviation	5.1384	7.0797	6.0476	3.2680

According to the result of pilot study, mean and standard deviation were used to calculate the sample size by comparing each two group. In Table 3, the results from sample size calculation were shown.

Table 3 Sample size calculation

group comparison	μ_1	S_1	μ_2	S_2	σ_2	N
Group SF and V	44.3814	5.1384	31.7075	7.0797	38.2625	3.7398
Group SF and PV	44.3814	5.1384	37.8217	6.0476	31.4883	11.4891
Group SF and POV	44.3814	5.1384	41.1950	3.2680	18.5415	28.6711
Group V and PV	31.7075	7.0797	37.8217	6.0476	43.3478	18.2048
Group V and POV	31.7075	7.0797	41.1950	3.2680	30.4010	5.3025
Group PV and POV	31.7075	6.0476	41.1950	3.2680	23.6266	32.5979

Based on the sample size calculation, the maximum sample size per group was 32.5979 slaps. Four slabs were retrieved from 1 tooth. Therefore, this study used nine teeth per group.

CHAPTER IV

RESULTS

1. Results of microtensile bond strength test

The Shapiro-Wilk test showed a normal distribution of microtensile bond strength in all groups ($p > 0.05$). Two-way ANOVA revealed that the factor “pretreatment” and the factor “water storage time" influenced the microtensile bond strength ($p < 0.05$ for all factors). There was a significant two-factor interaction between “pretreatment” and the factor “water storage time" ($p < 0.05$) in all group.

Mean of microtensile bond strength, standard deviations, and numbers of pretest failures are shown in Table 4 and graph of mean microtensile bond strength of experimental groups are shown in Figure 15. The multiple comparison test revealed that microtensile bond strength was highest in Group POV24h (42.63±4.57 MPa) followed by Group SF24h (41.15±6.27 MPa), Group POV3m (37.86±6.47 MPa), Group PV24h (36.11±5.19 MPa), Group SF3m (34.89±6.36 MPa), Group V24h (32.30±5.62 MPa) and Group PV3m (28.89±5.13), respectively. The lowest microtensile bond strength was presented in Group V3m (23.39±3.88).

Considering the effect of pretreatment, groups with pretreatment gave significantly higher microtensile bond strength than groups without pretreatment at both 24 hours and 3 months, except when comparing between Group V24h and Group PV24h which showed no statistical difference ($p = 0.106$). Regarding influence

of water storage time, microtensile bond strength significantly was decreased by water storage ($p < 0.05$). The numbers of pretest failures during preparation were recorded in Table 4. The highest number of pretest failures were shown in Group V3m (n = 7) followed by Group PV3m (n = 5), Groups SF3m and POV3m (n = 1), respectively.

Table 4 Microtensile bond strength (μTBS) values, standard deviations and statistical significant after 24 hours and 3 months of water storage

Group	Mean ± SD (MPa)		PTF (n)	
	24 hours	3 months	24 hours	3 months
SF	41.15±6.27 (n=36) A,#	34.89±6.36 (n=35) X,*	0	1
V	32.30±5.62 (n=36) B,#	23.39±3.88 (n=29) Y,*	0	7
PV	36.11±5.19 (n=36) B,#	28.89±5.13 (n=31) Z,*	0	5
POV	42.63±4.57 (n=36) A,#	37.86±6.47 (n=35) X,*	0	1

Groups having similar letters or symbols (# or *) (letters: column; symbols: row) are not significantly different in their μTBS (two-way ANOVA and Bonferoni post hoc-tests; $p < 0.05$). Samples with pretest failure were excluded in the calculation of mean μTBS. (Groups: SF = Single Bond™ Universal/ Filtek™ Z350 XT Flowable, V = Vertise® Flow™; PV = 37.5% phosphoric acid/ Vertise® Flow™; POV = 37.5% phosphoric acid/ OptiBond® Solo Plus™/ Vertise® Flow™; SD = standard deviation; PTF = pretest failures; n = number of specimens)

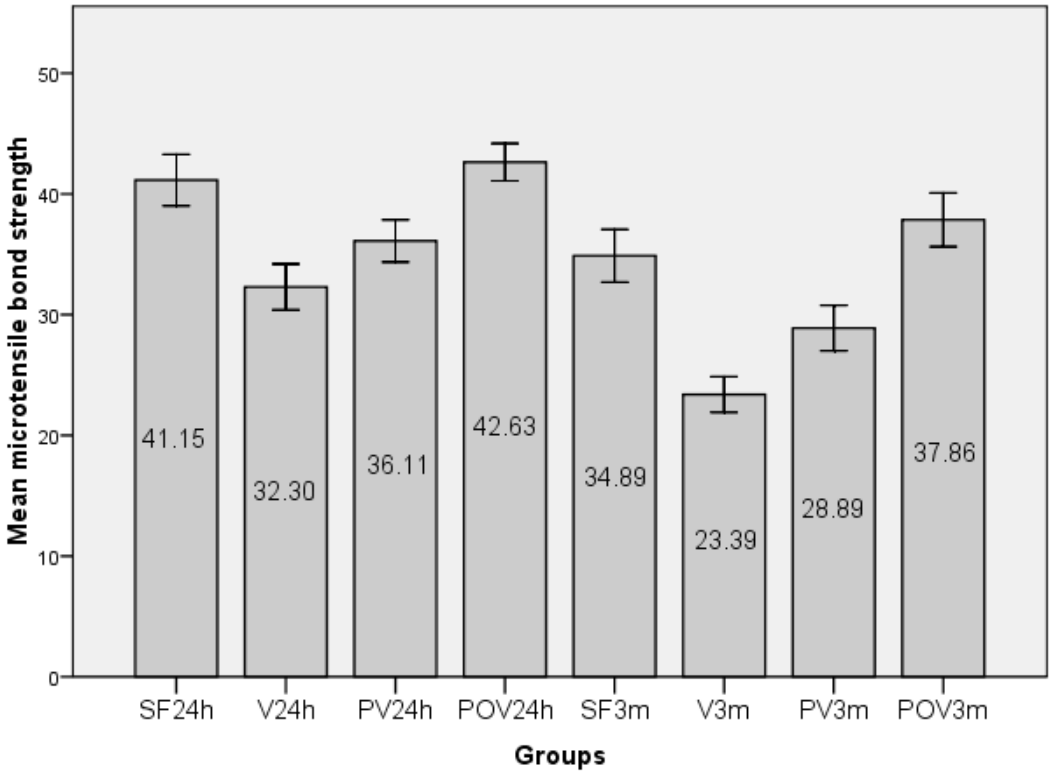


Figure 15 Graph of mean microtensile bond strength of experimental groups

2. Results of failure mode

The percentage distributions of failure modes were recorded using a stereomicroscope at x40 magnification. As shown in Table 5 and graph of percentage distribution of failure mode are shown in Figure 16, the majority of the failures were predominantly adhesive failure between resin composite and dentin. Group V3m showed the highest adhesive failures (100%), followed by Group PV3m (96.8%), Group SF3m (94.3%), Group V24h (91.7%), Group POV3m (91.4%), Group PV24h (88.9%), Groups SF24h and POV24h (86.1%), respectively. On the contrary, a few cohesive failure in composite were found in Group POV24h (11.1%), Group SF24h (8.3%), Group POV3m (5.7%), Groups V24h and PV24h (5.6%), and Group SF3m (2.9%), respectively. Mixed failures were also found in Groups SF24h and PV24h (5.6%),

Group PV3m (3.2%), Groups SF3m and POV3m (2.9%), and Groups V24h and POV24h (2.8%), respectively. In this study, cohesive failures within dentin were not found.

Table 5 Percentage distribution of failure mode

Group	Water storage time	Adhesive	Cohesive in resin	Cohesive in dentin	Mixed
SF	24 hours	31/36(86.1%)	3/36(8.3%)	-	2/36(5.6%)
V		33/36(91.7%)	2/36(5.6%)	-	1/36(2.8%)
PV		32/36(88.9%)	2/36(5.6%)	-	2/36(5.6%)
POV		31/36(86.1%)	4/36(11.1%)	-	1/36(2.8%)
SF	3 months	33/35(94.3%)	1/35(2.9%)	-	1/35(2.9%)
V		29/29(100%)	-	-	-
PV		30/31(96.8%)	-	-	1/31(3.2%)
POV		32/35(91.4)	2/35(5.7%)	-	1/35(2.9%)

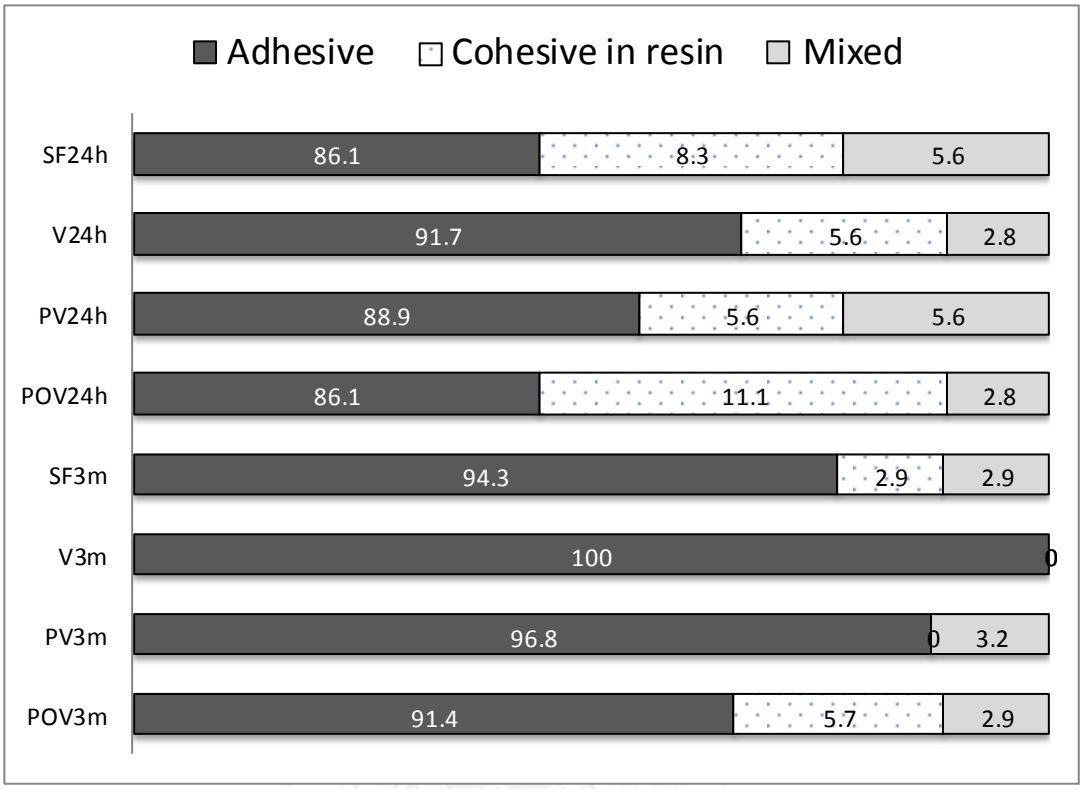
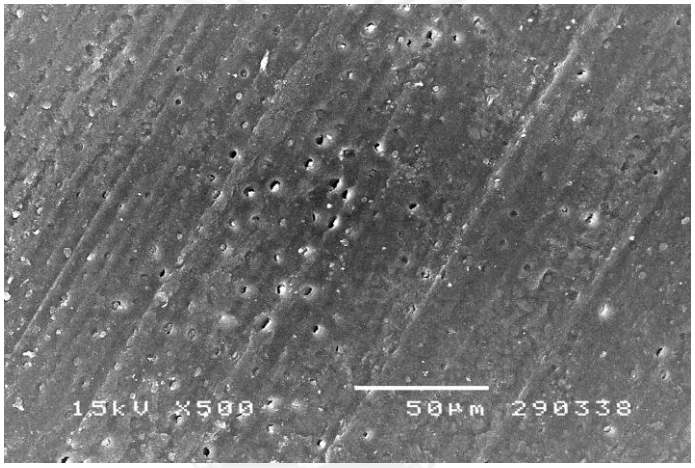


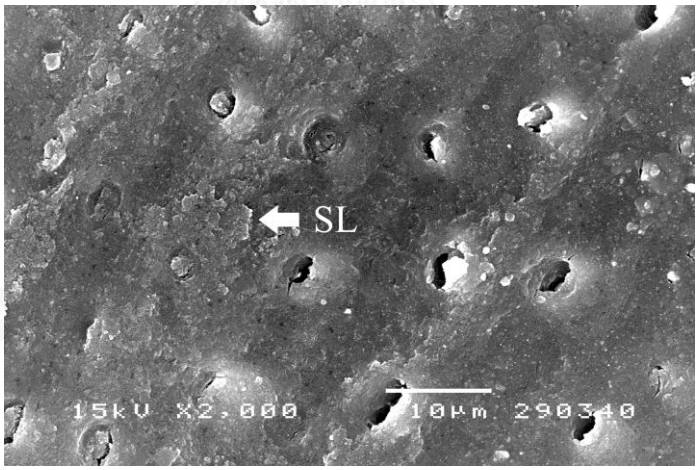
Figure 16 Graph of percentage distribution of failure mode

3. Results of SEM evaluation

SEM micrographs from representatives illustrating the fractured surfaces of the dentin side of the specimens are shown in Figure 17-20. Group SF24h (Figure 17); Figure 17A, at 500X, shows adhesive failure. Figure 17B, higher magnification at 2000X, shows dentin surface with smear layer remnants (SL). Dentinal tubules are covered by smear plugs.



A

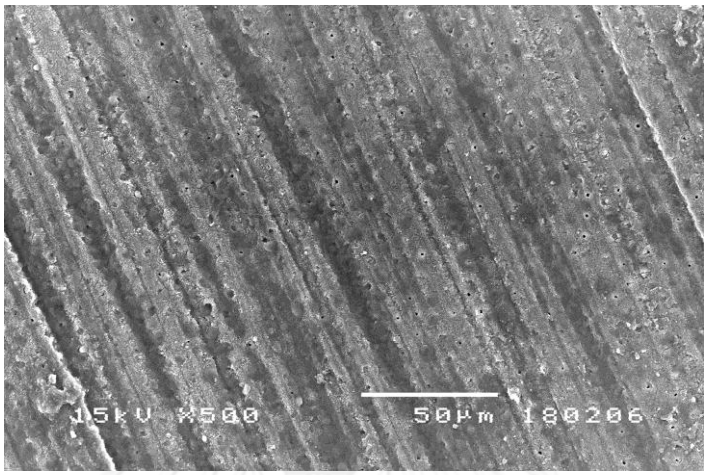


B

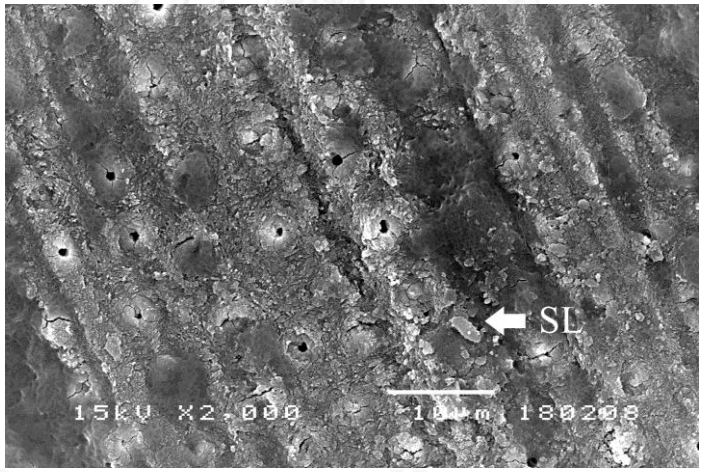
Figure 17 SEM micrographs from representatives illustrating the fractured surfaces of the dentin side of the specimens from Group SF24h. (A) at x500 magnification (B) at x2000 magnification.

(SL; smear layer)

In Group V24h (Figure 18); Figure 18A, at 500X, shows adhesive failure. Figure 18B, higher magnification at 2000X, shows dentin surface with smear layer remnants (SL). Dentinal tubules are covered by smear plugs.



A

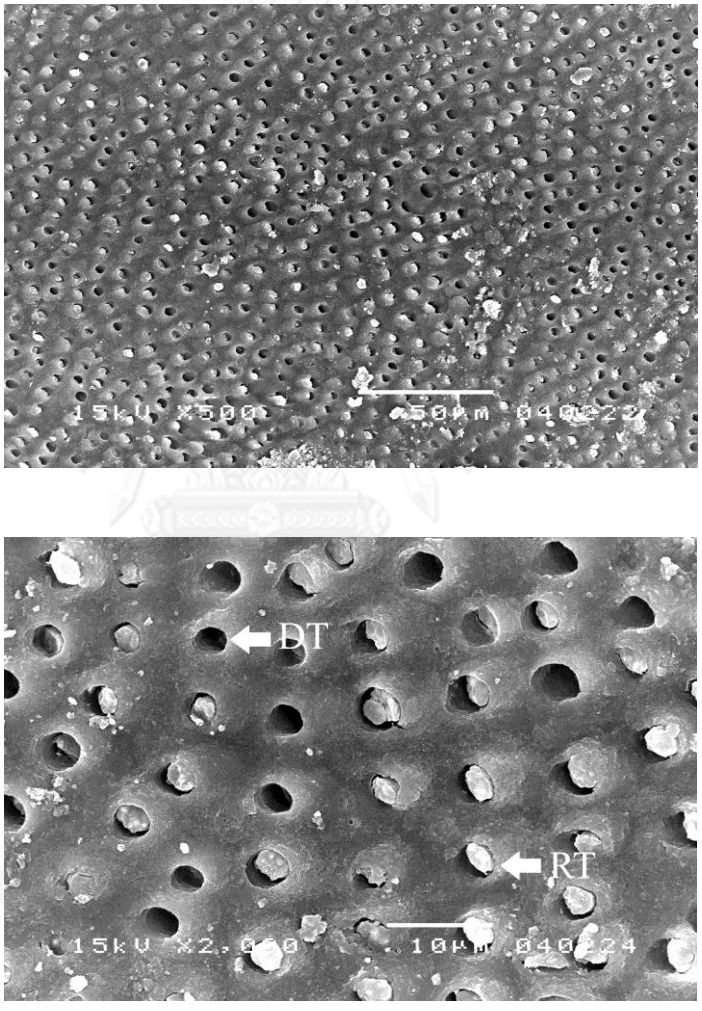


B

Figure 18 SEM micrographs from representatives illustrating the fractured surfaces of the dentin side of the specimens from Group V24h. (A) at x500 magnification (B) at x2000 magnification.

(SL; smear layer)

In Group PV24h (Figure 19); Figure 19A, at 500X, shows adhesive failure. Figure 19B, higher magnification at 2000X, smooth dentin surface is visible. Etching pattern can be observed, as smear layer have been completely dissolved. Opening dentinal tubules (DT) with or without resin tags (RT) can be observed.



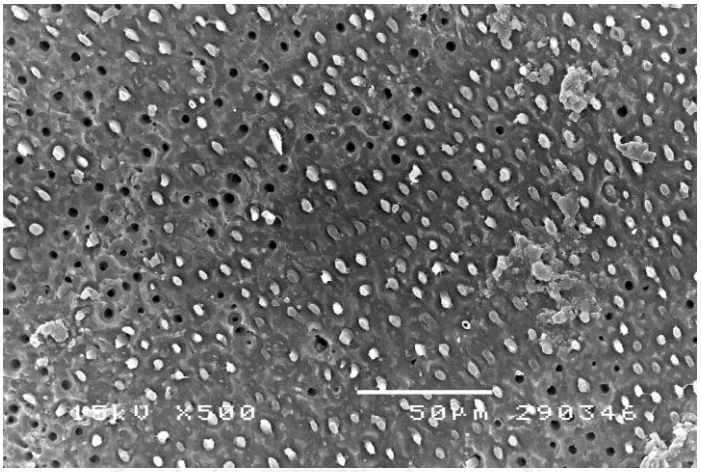
A

B

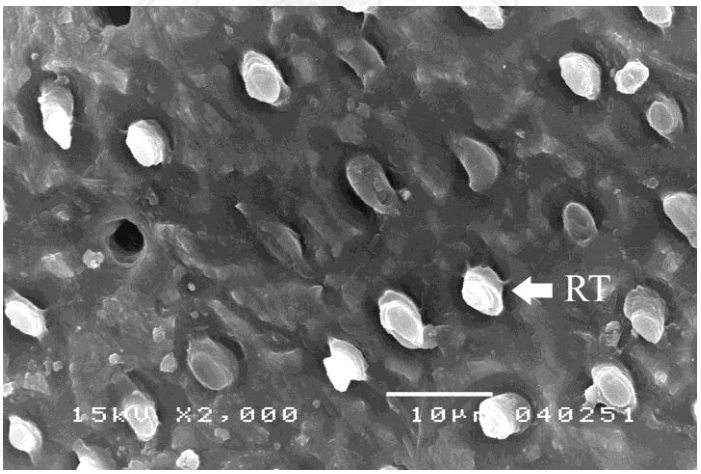
Figure 19 SEM micrographs from representatives illustrating the fractured surfaces of the dentin side of the specimens from Group PV24h. (A) at x500 magnification (B) at x2000 magnification.

(DT: dentinal tubule; RT: resin tags)

In Group POV24h (Figure 20); Figure 20A, at 500X, shows adhesive failure. Figure 20B, higher magnification at 2000X, shows adhesive layer on dentin surface. Remnant of resin tags (RT) into the dentinal tubules can be observed. Some opening dentinal tubules can be observed (Fig. 2H).



A



B

Figure 20 SEM micrographs from representatives illustrating the fractured surfaces of the dentin side of the specimens from Group POV24h. (A) at x500 magnification (B) at x2000 magnification.

(RT: resin tags)

CHAPTER V

DISCUSSION

The objectives of this study were to determine the μTBS of a self-adhesive resin composite to dentin when using different pretreatment methods and water storage times, compared to a conventional flowable resin composite used in combination with an adhesive agent as a control. Single Bond^{TM} Universal was selected as an adhesive agent in control group because the first layer of self-adhesive resin composite was acting, in effect, as 1-step self-etch adhesive (15). In addition, it contains 10-MDP monomer that has been rated as the promising monomer for chemical bonding to hydroxyapatite (21). In this study, the first and second null hypotheses tested were rejected. Significant differences in μTBS among groups were shown when using different pretreatments and water storage times.

The μTBS test was chosen, because several specimens could be obtained from one tooth, and many researchers considered that μTBS was the most reliable technique to investigate "true" interfacial bond strength between an adhesive material and the substrate of interest (48, 70, 71). In addition, small bonded surface areas of approximately 1 mm^2 may provide higher bond strengths compared to conventional methods, which used a bonded area of 7–12 mm^2 (47, 70). In this study, μTBS specimens were trimmed with burs at the adhesive interface to produce an hourglass appearance called "trimming technique" (46, 48). Betamar et al. studied the stress distribution of the specimens in μTBS test using a finite-element analysis. The results indicated that stress distribution of trimmed specimen was better than

that of a non-trimmed specimen. However, improper preparation of trimmed specimen resulted in more sensitive to flaws introduced during specimen preparation and pretest failures during trimming, therefore the preparation of a specimen must be carefully performed (49). The smear layer, in this experiment, was produced by 180-grit abrasive paper. Koibuchi et al. recommended 180-grit abrasive paper for dentin preparation since it simulated smears formed clinically with dental burs (60).

The commonly used aging methods are water storage and thermocycling. Many studies have shown that using either or both methods as means of aging process have been well received (12, 13, 18, 64, 72). In this study, water storage was selected as aging method. The methods to store specimens exposing to water, which two different strategies, direct and indirect water exposure, have been used. In this study, the indirect water exposure of the entire restored tooth was selected because it possibly simulated more closely to clinical situation. Water takes some time to diffuse from the external surface into the inner bonded interface (58). On the contrary, direct water exposure of a small sample piece may not properly allow extrapolation of results to clinical practice (59).

Another consideration concerning μTBS test method is how to handle the specimens that fail before being tested. Some studies included the failures with a value of zero (73, 74). Others deleted the failures from further statistical analysis.(10, 12, 75-77) In this study, the pretest failure specimens (PTF in Table 2) were not taken into consideration during interpretation, because there was a certain amount of force generating failure during specimen preparation (78), not by the effect of aging process

by water storage. The pretest failures may have happened during trimming to produce hourglass specimens; hence, it was not suitable to attribute zero-value in the statistical analysis. However, when the calculation was based on the specimens surviving test, an overestimation of bonding potential must be taken into consideration (79).

Results of the present study revealed that the two-factor interactions (pretreatment and water storage time) were all significant to μTBS ($p < 0.05$). Therefore, one must take into account all involved factors including dentin pretreatment and aging process. In this study, at 24-hour water storage, μTBS of group PV24h, that 37.5% phosphoric acid was used as acid pretreatment, was increased when compared to group V24h, that Vertise® Flow™ was used alone. However this increase was not statistically significant. This result was in accordance with Juloski et al., 24-hour bonding performance of Vertise® Flow™ to dentin was better but not statistically significant after dentin pretreatment with acid etching. The increase in microporosities, produced by phosphoric acid etching, resulted in enhanced resin-interlocking and micromechanical retention (45). Poitevin et al. showed that dentin pretreatment with 37.5% phosphoric acid significantly improved bonding effectiveness of Vertise® Flow™ (5). In this study, at 3-month water storage, although group PV3m was significantly higher in μTBS when compared to group V3m, the further study should be perform using longer storage time, or using other means of aging such as thermocycling or cyclic loading, in order to determine bonding effectiveness of self-adhesive resin composite used in combination with phosphoric acid pretreatment.

In the present study, group POV, that 2-step self-etch adhesive was used as an adhesive agent, had significantly higher μTBS than group V, that Vertise® Flow™ was used alone, and group PV that pretreatment with 37.5% phosphoric acid. Moreover, even though the first layer of Vertise® Flow™ (0.5 mm thin layer created by 20-second agitation by brush) was acting, in effect, as 1-step self-etch adhesive (15). μTBS for the group V was significantly lower than group SF that used 1-step self-etch adhesive (Single Bond™ Universal) as an adhesive agent. This result is in accordance with Bektas et al., Vertise® Flow™ used with 1-step self-etch adhesive (OptiBond® All-In-One) provide significantly stronger bond strength than individual usage. It is possible that although Vertise® Flow™ incorporates adhesive technology similar to that found in OptiBond® products, adding other fillers may reduce the bond strength value (6). The previous studies showed that wetting of dentin surface was decreased due to higher viscosity of filled resin. This would decrease the penetration of monomers, thus reducing the μTBS (80, 81).

In this study, it is interesting to observe that μTBS of 1-step self-etch adhesive group (Group SF) compared to 2-step total-etch adhesive group (Group POV) at 24 hours or 3 months was not significantly different. The reason is possibly because Single Bond™ Universal contains 10-MDP monomer, structurally, the long carbonyl chain. Yoshida et al. described that 10-MDP was rated as the promising monomer for chemical bonding to hydroxyapatite. This monomer is capable of forming ionic bonds with calcium due to the low dissolution rate of the resulting Ca-salt in its own solution (21). In addition, Van landuyt et al. showed that the Ca-10-MDP was one of the most hydrolytically stable salts (82). However, according to the material's

technical profile, OptiBond® Solo Plus™ used different acidic monomer, namely GPDM. It possesses hydrophilic acidic phosphate group and the short spacer group in the adhesive monomer. To our knowledge, no data on chemical analysis on bonding mechanism of GPDM was available. Yoshida et al. showed that according to adhesion-decalcification concept, GPDM rather "etches" than "bonds" to hydroxyapatite compared to 10-MDP (21). Poitevin et al. suggested that to achieve self-adhesiveness, a relatively viscous self-adhesive flowable resin composite should contain a functional monomer that rather possesses an effective chemical bonding potential, as it cannot penetrate deeply (5).

The lowest μTBS was found in the group without pretreatment at 3 months water storage (Group V3m). Previous studies have shown that the aggressive version of simplified self-etch adhesives have been advocated to account for the suboptimal bonding performance, which may be due to 1) the stronger etching process may destabilize the collagen, leading to a decrease in bond strength (83), 2) weaker cohesive strength of the adhesive (10, 84), 3) the combination of acidic hydrophilic and hydrophobic monomers into a single step compromised the polymerization of the adhesive (62), and 4) a low degree of conversion of the resin monomer that was caused by the effect of oxygen inhibition (85).

A systematic review of Van Meerbeek et al. revealed that there were significant differences in the pooled mean bond strength, as the weighted bond strength means per adhesive class have the following ranges: 31 MPa for 3-step total etch adhesives, 29 MPa for 2-step total etch adhesives, 26 MPa for 2-step self-etch

adhesives, 20 MPa for 1-step self-etch adhesives (52), In present study, Vertise® Flow™ had a 23.39 MPa bond strength after 3-month water storage so it can be compared to other 1-step self-etch adhesives. In this study, all bond strengths obtained were more than 20 MPa. They were, supposedly, strong enough to resist contraction forces of resin composite because it has been postulated that minimum bond strength of 17 to 20 MPa to enamel and dentin was needed to resist contraction forces of resin composite materials (47). However, in clinical practice, the optimum bond strength of resin composite to dentin is not yet known. Bond strength was dependent not only on materials used, or storage time but also on other factors, such as, restorative technique, quality of substrate, isolation method, skills of operator and finishing technique, etc. Moreover, patient-related factors, such as oral hygiene, age, occlusal loading, and dentin quality, may be more influential than any material property (3, 86).

A factor known to promote bond degradation is long-term water exposure. The 3-month water storage applied in this experiment was regarded as a short period in comparison to life expectancy of restoration. Although, ISO technical specification 11405:2003 (54) recommendation for water storage is 6 months, several studies reported significant decreases in bond strengths in only a few months (12-14), since degradation throughout the dentin bonded interface occurred rapidly (58, 87). A recent study showed that μTBS significantly decreased after 3 months in water storage compared to 24 hours in all groups. Decrease in bonding effectiveness may be caused by degradation of interface components (88). Pashley et al. found that matrix metalloproteinases (MMPs) played an important role in degradation process.

These enzymes are hydrolases, which break peptide bonds by adding water. Therefore, in the presence of water, μTBS may decrease as a consequence of degradation of collagen (89). In addition, water infiltration decreased the mechanical properties of the polymer matrix by swelling and reducing the frictional forces between the polymer chains, a process known as "plasticization" (90, 91). Furthermore, some interface components, such as uncured monomers and break-down products of previous mechanisms, could elute and, therefore, weaken the bond overtime (92).

Regarding failure mode, in this study, the microscopic study showed the majority of failures were adhesive failure that occurred in adhesive interface. It can be assumed that bond strength value would be representing adhesive bond strength rather than cohesive bond strength. From the SEM evaluation, the failure mode of dentin side of fractured representatives was initially classified as adhesive failure. Group SF24h that used 1-step self-etch adhesive system as an adhesive agent (Figure 17), and group V24h that Vertise® Flow™ was applied alone (Figure18) showed similar pattern of smear layer remnant. These SEM evaluation results agreed with Hanabusa et al., which self-adhesive resin composite was bonded to bur-cut dentin, and resin infiltrated smear layer with a maximum of a few micrometers, but resin tags were not identified since the smear plugs within the tubules were not dissolved. This kind of superficial interaction, along with the absence of resin tag formation, was typical of self-etch adhesives (15). Group PV24h (Figure 19) showed etched pattern that was the result from phosphoric acid etching. Group POV24h (Figure 20) showed adhesive layer that was the result from application of 2-step total-etch adhesive.

In present study, using dentin pretreatment increased the bond strength of Vertise® Flow™. On the other hand, a limitation of this study is that only one self-adhesive resin composite was evaluated. Future investigation to improve bonding effectiveness may involve investigation of other self-adhesive resin composites and adhesive agents. The extended of water storage times may provide valuable information on longevity of material. In addition, clinical studies of self-adhesive resin composite are necessary to determine the bonding performance, and are useful for in vitro and in vivo comparison.

CHAPTER VI
CONCLUSIONS

This study was performed to determine the μTBS of a self-adhesive resin composite to dentin when using different pretreatment methods and water storage times. The results of the study indicate the following:

1. Vertise® Flow™, in combination with the use of 37.5% phosphoric acid etching and adhesive agent, OptiBond® Solo Plus™, gave significantly higher μTBS than using Vertise® Flow™ alone.
2. Pretreatment with 37.5% phosphoric acid and OptiBond® Solo Plus™ prior to the application of Vertise® Flow™ gave significantly higher μTBS than pretreatment with 37.5% phosphoric acid prior to the application of Vertise® Flow™.
3. μTBS of group that used phosphoric acid and OptiBond® Solo Plus™ prior to the application of self-adhesive resin composite, Vertise® Flow™, perform comparable to group that used Single Bond™ Universal in combination with conventional flowble resin composite, Filtek™ Z350 XT flowable.
4. The application of Vertise® Flow™ without pretreatment gave significantly lower μTBS than using Single Bond™ Universal in combination with conventional flowable resin composite, Filtek™ Z 350 XT Flowable.
5. Regarding influence of aging process, μTBS was significantly decreased by water storage time in all groups.

REFERENCES

1. Ferracane JL. Resin composite--state of the art. Dent Mater. 2011 Jan;27(1):29-38.
2. Soderholm KJ, Zigan M, Ragan M, Fischlschweiger W, Bergman M. Hydrolytic degradation of dental composites. J Dent Res. 1984 Oct;63(10):1248-54.
3. 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 May-Jun;28(3):215-35.
4. Fu J, Kakuda S, Pan F, Hoshika S, Ting S, Fukuoka A, et al. Bonding performance of a newly developed step-less all-in-one system on dentin. Dent Mater J. 2013;32(2):203-11.
5. Poitevin A, De Munck J, Van Ende A, Suyama Y, Mine A, Peumans M, et al. Bonding effectiveness of self-adhesive composites to dentin and enamel. Dent Mater. 2013;29(2):221-30.
6. Bektas OO, Eren D, Akin EG, Akin H. Evaluation of a self-adhering flowable composite in terms of micro-shear bond strength and microleakage. Acta Odontol Scand. 2013;71(3-4):541-6.
7. Frankenberger R, Perdigao J, Rosa BT, Lopes M. "No-bottle" vs "multi-bottle" dentin adhesives--a microtensile bond strength and morphological study. Dent Mater. 2001;17(5):373-80.
8. Ioannidis A, Stawarczyk B, Sener B, Attin T, Schmidlin PR. Influence of dentin and enamel pretreatment with acidic sulfur compounds on adhesive performance. Clin Oral Investig. 2013;17(8):1885-92.

9. Taschner M, Nato F, Mazzoni A, Frankenberger R, Kramer N, Di Lenarda R, et al. Role of preliminary etching for one-step self-etch adhesives. Eur J Oral Sci. 2010;118(5):517-24.

10. 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 Fall;3(3):237-45.

11. 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 Jan;24(1):90-101.

12. Armstrong SR, Vargas MA, Fang Q, Laffoon JE. Microtensile bond strength of a total-etch 3-step, total-etch 2-step, self-etch 2-step, and a self-etch 1-step dentin bonding system through 15-month water storage. J Adhes Dent. 2003 Spring;5(1):47-56.

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

14. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, Pashley DH, et al. Durability of resin-dentin bonds: effects of direct/indirect exposure and storage media. Dent Mater. 2007 Jul;23(7):885-92.

15. Hanabusa M, Mine A, Kuboki T, Momoi Y, Van Landuyt KL, Van Meerbeek B, et al. TEM interfacial characterization of an experimental self-adhesive filling material bonded to enamel/dentin. Dent Mater. 2011 Aug;27(8):818-24.

16. Tyas MJ, Anusavice KJ, Frencken JE, Mount GJ. Minimal intervention dentistry--a review. FDI Commission Project 1-97. Int Dent J. 2000 Feb;50(1):1-12.

17. Rada RE. The versatility of flowable composites. Dent Today. 1998 Apr;17(4):78-83.

18.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 Feb;84(2):118-32.

19.Van Meerbeek B, Perdigao J, Lambrechts P, Vanherle G. The clinical performance of adhesives. J Dent. 1998 Jan;26(1):1-20.

20.Salerno M, Derchi G, Thorat S, Ceseracciu L, Ruffilli R, Barone AC. Surface morphology and mechanical properties of new-generation flowable resin composites for dental restoration. Dent Mater. 2011 Dec;27(12):1221-8.

21.Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res. 2004 Jun;83(6):454-8.

22.Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent. 2002 Sep-Nov;30(7-8):371-82.

23.Marshall GW, Jr., Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent. 1997 Nov;25(6):441-58.

24.Pashley DH. Dynamics of the pulpo-dentin complex. Crit Rev Oral Biol Med. 1996;7(2):104-33.

25.Kramer IR, McLean JW. Alterations in the staining reactions of dentine resulting from a constituent of a new self-polymerizing resin. Br Dent J. 1952 Sep;93(6):150-53.

26.Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955 Dec;34(6):849-53.

27.Brudevold F, Buonocore M, Wileman W. A report on a resin composition capable of bonding to human dentin surfaces. J Dent Res. 1956 Dec;35(6):846-51.

28.Fusayama T, Nakamura M, Kurosaki N, Iwaku M. Non-pressure adhesion of a new adhesive restorative resin. J Dent Res. 1979 Apr;58(4):1364-70.

29.Gwinnett AJ. Quantitative contribution of resin infiltration/hybridization to dentin bonding. Am J Dent. 1993 Feb;6(1):7-9.

30.Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res. 1982 May;16(3):265-73.

31.Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. J Esthet Dent. 1991;3(4):133-8.

32.Van Meerbeek B, Lambrechts P, Inokoshi S, Braem M, Vanherle G. Factors affecting adhesion to mineralized tissues. Oper Dent. 1992;Suppl 5:111-24.

33.Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. Dent Mater. 2005;21(10):895-910.

34.Watanabe I, Nakabayashi N, Pashley DH. Bonding to ground dentin by a phenyl-P self-etching primer. J Dent Res. 1994 Jun;73(6):1212-20.

35.Hayakawa T, Kikutake-Sugiyama K, Fukushima T, Nemoto K. Development of self-etching primer adhesive in all-in-one bonding system. Dent Mater J. 2005;24(2):251-6.

36.Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength: demineralized dentin zone of the hybrid layer. Dent Mater. 2000 Nov;16(6):406-11.

37.Vargas MA, Cobb DS, Denehy GE. Interfacial micromorphology and shear bond strength of single-bottle primer/adhesives. Dent Mater. 1997 Sep;13(5):316-24.

38.Wilder AD, Jr., Swift EJ, Jr., May KN, Jr., Waddell SL. Bond strengths of conventional and simplified bonding systems. Am J Dent. 1998 Jun;11(3):114-7.

39.Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod. 2002;28(1):17-9.

40.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 Aug;71(8):1530-40.

41.Meryon SD, Tobias RS, Jakeman KJ. Smear removal agents: a quantitative study in vivo and in vitro. J Prosthet Dent. 1987;57(2):174-9.

42.Perdigao J, May KN, Jr., Wilder AD, Jr., Lopes M. The effect of depth of dentin demineralization on bond strengths and morphology of the hybrid layer. Oper Dent. 2000 May-Jun;25(3):186-94.

43.Gordan VV, Vargas MA, Denehy GE. Interfacial ultrastructure of the resin-enamel region of three adhesive systems. Am J Dent. 1998 Feb;11(1):13-6.

44.Wajdowicz MN, Vandewalle KS, Means MT. Shear bond strength of new self-adhesive flowable composite resins. Gen Dent. 2012 Mar-Apr;60(2):e104-8.

45.Juloski J, Goracci C, Rengo C, Giovannetti A, Vichi A, Vulicevic ZR, et al. Enamel and dentin bond strength of new simplified adhesive materials with and without preliminary phosphoric acid-etching. Am J Dent. 2012 Aug;25(4):239-43.

46.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 Oct;110(5):385-91.

47.Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion testing of dentin bonding agents: a review. Dent Mater. 1995 Mar;11(2):117-25.

48.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 Jul;10(4):236-40.

49.Betamar N, Cardew G, Van Noort R. Influence of specimen designs on the microtensile bond strength to dentin. J Adhes Dent. 2007 Apr;9(2):159-68.

50.McDonough WG, Antonucci JM, He J, Shimada Y, Chiang MY, Schumacher GE, et al. A microshear test to measure bond strengths of dentin-polymer interfaces. Biomaterials. 2002 Sep;23(17):3603-8.

51.Placido E, Meira JB, Lima RG, Muench A, de Souza RM, Ballester RY. Shear versus micro-shear bond strength test: a finite element stress analysis. Dent Mater. 2007;23(9):1086-92.

52.Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater. 2010;26(2):16.

53.Yassen GH, Platt JA, Hara AT. Bovine teeth as substitute for human teeth in dental research: a review of literature. J Oral Sci. 2011;53(3):273-82.

54.ISO. Dental-materials - Testing of adhesion to tooth structure. Tecnical specification-ISO/TS 11405. 2003:2-6.

55.Kimura S, Shimizu T, Fujii B. Influence of dentin on bonding of composite resin. Part 1. Effect of fresh dentin and storing conditions. Dent Mater J. 1985 Jun;4(1):68-80.

56.Rueggeberg FA. Substrate for adhesion testing to tooth structure - review of the literature. Dent Mater. 1991 Jan;7(1):2-10.

57.Santana FR, Pereira JC, Pereira CA, Fernandes Neto AJ, Soares CJ. Influence of method and period of storage on the microtensile bond strength of indirect composite resin restorations to dentine. Braz Oral Res. 2008;22(4):352-7.

58.Carrilho MR, Carvalho RM, Tay FR, Yiu C, Pashley DH. Durability of resin-dentin bonds related to water and oil storage. Am J Dent. 2005 Dec;18(6):315-9.

59.Loguercio AD, Uceda-Gomez N, Carrilho MR, Reis A. Influence of specimen size and regional variation on long-term resin-dentin bond strength. Dent Mater. 2005 Mar;21(3):224-31.

60.Koibuchi H, Yasuda N, Nakabayashi N. Bonding to dentin with a self-etching primer: the effect of smear layers. Dent Mater. 2001 Mar;17(2):122-6.

61.Sadr A, Ghasemi A, Shimada Y, Tagami J. Effects of storage time and temperature on the properties of two self-etching systems. J Dent. 2007 Mar;35(3):218-25.

62.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 Feb;82(2):136-40.

63.Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. J Dent. 1999 Feb;27(2):89-99.

64.Miyazaki M, Sato M, Onose H, Moore BK. Influence of thermal cycling on dentin bond strength of two-step bonding systems. Am J Dent. 1998 Jun;11(3):118-22.

65.Leloup G, D'Hoore W, Bouter D, Degrange M, Vreven J. Meta-analytical review of factors involved in dentin adherence. J Dent Res. 2001 Jul;80(7):1605-14.

66.Krejci I, Hausler T, Sagesser, Lutz F. New adhesives in Class V restorations under combined load and simulated dentinal fluid. Dent Mater. 1994 Sep;10(5):331-5.

67.Nikaido T, Kunzelmann KH, Chen H, Ogata M, Harada N, Yamaguchi S, et al. Evaluation of thermal cycling and mechanical loading on bond strength of a self-etching primer system to dentin. Dent Mater. 2002 May;18(3):269-75.

68.Frankenberger R, Strobel WO, Kramer N, Lohbauer U, Winterscheidt J, Winterscheidt B, et al. Evaluation of the fatigue behavior of the resin-dentin bond with the use of different methods. J Biomed Mater Res B Appl Biomater. 2003 Nov 15;67(2):712-21.

69.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 Mar;29(3):227-34.

70.Goracci C, Sadek FT, Monticelli F, Cardoso PE, Ferrari M. Influence of substrate, shape, and thickness on microtensile specimens' structural integrity and their measured bond strengths. Dent Mater. 2004 Sep;20(7):643-54.

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

72.Blunck U, Zaslansky P. Effectiveness of all-in-one adhesive systems tested by thermocycling following short and long-term water storage. J Adhes Dent. 2007;2:231-40.

73.Tay FR, Pashley DH, Yiu CK, Sanares AM, Wei SH. Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part I. Single-step self-etching adhesive. J Adhes Dent. 2003 Spring;5(1):27-40.

74.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 Feb-Mar;30(2-3):83-90.

75.Guzman-Armstrong S, Armstrong SR, Qian F. Relationship between nanoleakage and microtensile bond strength at the resin-dentin interface. Oper Dent. 2003 Jan-Feb;28(1):60-6.

76.Uno S, Inoue H, Finger WJ, Inoue S, Sano H. Microtensile bond strength evaluation of three adhesive systems in cervical dentin cavities. J Adhes Dent. 2001 Winter;3(4):333-41.

77.Xie B, Dickens SH, Giuseppetti AA. Microtensile bond strength of thermally stressed composite-dentin bonds mediated by one-bottle adhesives. Am J Dent. 2002 Jun;15(3):177-84.

78.Sadek FT, Monticelli F, Muench A, Ferrari M, Cardoso PE. A novel method to obtain microtensile specimens minimizing cut flaws. J Biomed Mater Res B Appl Biomater. 2006 Jul;78(1):7-14.

79.Van Noort R, Noroozi S, Howard IC, Cardew G. A critique of bond strength measurements. J Dent. 1989 Apr;17(2):61-7.

80.Miyazaki M, Ando S, Hinoura K, Onose H, Moore BK. Influence of filler addition to bonding agents on shear bond strength to bovine dentin. Dent Mater. 1995 Jul;11(4):234-8.

81.Nunes MF, Swift EJ, Perdigao J. Effects of adhesive composition on microtensile bond strength to human dentin. Am J Dent. 2001;14(6):340-3.

82.Van Landuyt KL, Yoshida Y, Hirata I, Snauwaert J, De Munck J, Okazaki M, et al. Influence of the chemical structure of functional monomers on their adhesive performance. J Dent Res. 2008 Aug;87(8):757-61.

83.Yoshiyama M, Carvalho R, Sano H, Horner J, Brewer PD, Pashley DH. Interfacial morphology and strength of bonds made to superficial versus deep dentin. Am J Dent. 1995 Dec;8(6):297-302.

84.Santini A, Plasschaert AJ, Mitchell S. Effect of composite resin placement techniques on the microleakage of two self-etching dentin-bonding agents. Am J Dent. 2001 Jun;14(3):132-6.

85.Nunes TG, Ceballos L, Osorio R, Toledano M. Spatially resolved photopolymerization kinetics and oxygen inhibition in dental adhesives. Biomaterials. 2005 May;26(14):1809-17.

86.Ermis RB, De Munck J, Cardoso MV, Coutinho E, Van Landuyt KL, Poitevin A, et al. Bond strength of self-etch adhesives to dentin prepared with three different diamond burs. Dent Mater. 2008;24(7):978-85.

87.Tay FR, Pashley DH, Suh BI, Hiraishi N, Yiu CK. Water treeing in simplified dentin adhesives--deja vu? Oper Dent. 2005 Sep-Oct;30(5):561-79.

88.De Munck J, Shirai K, Yoshida Y, Inoue S, Van Landuyt K, Lambrechts P, et al. Effect of water storage on the bonding effectiveness of 6 adhesives to Class I cavity dentin. Oper Dent. 2006 Jul-Aug;31(4):456-65.

89.Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res. 2004 Mar;83(3):216-21.

90.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 Dec 5;42(3):465-72.

91.Santerre JP, Shajii L, Leung BW. Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products. Crit Rev Oral Biol Med. 2001;12(2):136-51.

92.Hashimoto M, Ohno H, Sano H, Tay FR, Kaga M, Kudou Y, et al. Micromorphological changes in resin-dentin bonds after 1 year of water storage. J Biomed Mater Res. 2002;63(3):306-11.

APPENDIX

Appendix A Data of microtensile bond strength (μTBS)

Table 6 Data of microtensile bond strength (μTBS) test from Group SF24h

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.06	1.00	1.0600	57.7064	54.4400	Adhesive
2	1.04	1.03	1.1200	48.8208	43.5900	Adhesive
3	1.06	1.02	1.1200	55.1656	49.2550	Cohesive
4	1.05	1.02	1.0710	56.5890	52.8375	Cohesive
5	1.07	1.02	1.0914	49.4268	45.2875	Mixed
6	1.06	1.03	1.0918	45.7327	41.8875	Adhesive
7	1.05	1.03	1.0815	42.2677	39.0825	Adhesive
8	1.04	1.01	1.0504	44.8731	42.7200	Adhesive
9	1.05	1.02	1.0710	42.0448	39.2575	Mixed
10	1.06	1.01	1.0706	47.7567	44.6075	Cohesive
11	1.03	1.04	1.0712	42.0044	39.2125	Adhesive
12	1.03	1.03	1.0609	46.2393	43.5850	Adhesive
13	1.07	1.00	1.0700	54.3774	50.8200	Adhesive
14	1.08	1.01	1.0908	38.8816	35.6450	Adhesive
15	1.06	1.02	1.0812	44.4779	41.1375	Adhesive
16	1.07	1.03	1.1021	51.5094	46.7375	Adhesive
17	1.04	1.02	1.0608	48.2240	45.4600	Adhesive
18	1.03	1.02	1.0506	37.1570	35.3675	Adhesive
19	1.05	1.04	1.0902	40.6045	37.2450	Adhesive
20	1.04	1.03	1.0712	36.8225	34.3750	Adhesive
21	1.04	1.04	1.0816	43.0504	39.8025	Adhesive
22	1.03	1.04	1.0712	49.9875	46.6650	Adhesive
23	1.05	1.03	1.0815	47.4048	43.8325	Adhesive
24	1.03	1.02	1.0506	44.4509	42.3100	Adhesive
25	1.03	1.04	1.0712	44.4628	41.5075	Adhesive
26	1.05	1.02	1.0710	39.3673	36.7575	Adhesive
27	1.03	1.04	1.0712	49.6153	46.3175	Adhesive
28	1.03	1.04	1.0712	32.5751	30.4100	Adhesive
29	1.02	1.03	1.0506	31.0479	29.5525	Adhesive
30	1.03	1.03	1.0609	47.0721	44.3700	Adhesive

31	1.04	1.03	1.0712	36.2467	33.8375	Adhesive
32	1.03	1.04	1.0712	26.8014	25.0200	Adhesive
33	1.05	1.04	1.0920	41.6707	38.1600	Adhesive
34	1.04	1.03	1.0712	42.4972	39.6725	Adhesive
35	1.00	1.02	1.0200	41.4069	40.5950	Adhesive
36	1.03	1.05	1.0815	43.2708	40.0100	Adhesive

Table 7 Data of microtensile bond strength (μTBS) test from Group V24h

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.05	1.01	1.0605	20.8600	19.6700	Adhesive
2	1.04	1.00	1.0400	32.5805	31.3275	Cohesive
3	1.05	1.06	1.1130	29.9119	26.8750	Adhesive
4	1.06	1.04	1.1024	32.2452	29.2500	Adhesive
5	1.04	1.03	1.0712	29.0850	29.0850	Adhesive
6	1.03	1.03	1.0609	33.7605	31.8225	Adhesive
7	1.03	1.06	1.1124	28.2772	25.4200	Adhesive
8	1.05	1.04	1.0920	44.1223	40.4050	Adhesive
9	1.06	1.02	1.0812	37.8825	35.0375	Adhesive
10	1.04	1.01	1.0504	24.9207	23.7250	Adhesive
11	1.05	1.03	1.0815	42.9761	39.7375	Adhesive
12	1.05	1.05	1.1025	21.5649	19.5600	Adhesive
13	1.07	1.03	1.1021	43.9573	39.8850	Cohesive
14	1.06	1.02	1.0812	39.5882	36.6150	Mixed
15	1.05	1.04	1.0920	40.9418	37.4925	Adhesive
16	1.04	1.04	1.0816	44.7918	41.4125	Adhesive
17	1.04	1.05	1.0920	32.9921	30.2125	Adhesive
18	1.07	1.06	1.1342	42.1326	37.1475	Adhesive
19	1.03	1.02	1.0506	29.7950	28.3600	Adhesive
20	1.01	1.07	1.0807	33.0289	30.5625	Adhesive
21	1.03	1.04	1.0712	32.0476	29.9175	Adhesive
22	1.04	1.03	1.0712	31.3406	29.2575	Adhesive
23	1.02	1.01	1.0302	36.2038	35.1425	Adhesive
24	1.02	1.03	1.0506	34.4124	32.7550	Adhesive
25	1.04	1.04	1.0816	46.0167	42.5450	Adhesive
26	1.02	1.03	1.0506	32.8785	31.2950	Adhesive
27	1.04	1.05	1.0920	32.5007	29.7625	Adhesive
28	1.03	1.02	1.0506	33.1359	31.5400	Adhesive
29	1.02	1.01	1.0302	40.8371	39.6400	Adhesive
30	1.04	1.02	1.0608	36.8893	34.7750	Adhesive
31	1.05	1.03	1.0815	33.5860	31.0550	Adhesive
32	1.03	1.03	1.0609	31.7421	29.9200	Adhesive

33	1.04	1.06	1.1024	41.1526	37.3300	Adhesive
34	1.05	1.03	1.0815	30.4118	28.1200	Adhesive
35	1.03	1.04	1.0712	33.7562	31.5125	Adhesive
36	1.04	1.05	1.0920	37.6494	34.4775	Adhesive

Table 8 Data of microtensile bond strength (μTBS) test from Group PV24h

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.05	1.00	1.1025	38.0170	34.4825	Adhesive
2	1.06	1.01	1.0706	29.8242	27.8575	Adhesive
3	1.08	1.02	1.1016	40.6132	36.8675	Adhesive
4	1.08	1.01	1.0908	38.5080	35.3025	Adhesive
5	1.09	1.03	1.1227	34.1301	30.4000	Adhesive
6	1.08	1.03	1.1124	50.3027	45.2200	Adhesive
7	1.07	1.04	1.1128	45.2242	40.6400	Adhesive
8	1.09	1.00	1.0900	53.0394	48.6600	Adhesive
9	1.09	1.04	1.1336	44.6525	39.3900	Adhesive
10	1.05	1.00	1.0500	48.7095	46.3900	Adhesive
11	1.09	1.01	1.1009	44.1378	40.0925	Adhesive
12	1.07	1.03	1.1021	37.6147	34.1300	Cohesive
13	1.08	1.00	1.0800	31.5441	29.2075	Adhesive
14	1.04	1.01	1.0504	34.2588	32.6150	Adhesive
15	1.09	1.02	1.1118	45.4559	40.8850	Cohesive
16	1.08	1.08	1.1664	50.1639	43.0075	Adhesive
17	1.04	1.05	1.0920	31.2230	28.5925	Adhesive
18	1.05	1.05	1.1025	42.4131	38.4700	Adhesive
19	1.04	1.03	1.0712	32.2967	30.1500	Adhesive
20	1.06	1.02	1.0812	39.6314	36.6550	Adhesive
21	1.03	1.04	1.0712	37.7384	35.2300	Adhesive
22	1.05	1.04	1.0920	32.1731	29.4625	Adhesive
23	1.05	1.03	1.0815	38.1066	35.2350	Adhesive
24	1.02	1.03	1.0506	45.0418	42.8725	Adhesive
25	1.03	1.03	1.0609	35.0951	33.0850	Adhesive
26	1.03	1.01	1.0304	34.1887	33.1800	Adhesive
27	1.05	1.06	1.1130	35.9082	32.2625	Adhesive
28	1.06	1.03	1.0918	44.3189	40.5925	Adhesive
29	1.04	1.03	1.0712	33.4589	31.2350	Adhesive
30	1.04	1.04	1.0816	42.8422	39.6100	Mixed
31	1.03	1.05	1.0815	38.6231	35.7125	Mixed
32	1.06	1.04	1.1024	42.2605	38.3350	Adhesive

33	1.03	1.00	1.0300	35.4912	34.4575	Adhesive
34	1.02	1.02	1.0404	35.0927	33.7300	Adhesive
35	1.04	1.05	1.0920	35.3863	32.4050	Adhesive
36	1.03	1.03	1.0609	35.6356	33.5900	Adhesive

Table 9 Data of microtensile bond strength (μTBS) test from Group POV24h

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.07	1.06	1.1342	44.6563	39.3725	Adhesive
2	1.05	1.02	1.0710	46.2726	43.2050	Adhesive
3	1.08	1.00	1.0800	38.7180	35.8500	Adhesive
4	1.08	1.03	1.1124	45.7753	41.1500	Adhesive
5	1.00	1.03	1.0300	39.2095	38.0675	Adhesive
6	1.02	1.03	1.0506	44.3668	42.2300	Adhesive
7	1.09	1.04	1.1336	49.2748	43.4675	Adhesive
8	1.08	1.06	1.1448	45.5602	39.7975	Cohesive
9	1.09	1.02	1.1118	56.9297	51.2050	Cohesive
10	1.09	1.05	1.1445	46.9016	40.9800	Adhesive
11	1.08	1.05	1.1340	46.5110	41.0150	Adhesive
12	1.07	1.03	1.1021	47.0763	42.7150	Mixed
13	1.05	1.06	1.0815	42.5381	39.3325	Adhesive
14	1.09	1.05	1.1445	48.3408	42.2375	Adhesive
15	1.06	1.06	1.1236	42.9075	38.1875	Adhesive
16	1.07	1.08	1.1556	46.5793	40.3075	Adhesive
17	1.05	1.05	1.1025	40.4066	36.6500	Adhesive
18	1.03	1.06	1.0918	44.3708	40.6400	Adhesive
19	1.04	1.05	1.0920	41.3813	37.8950	Adhesive
20	1.07	1.04	1.1128	36.6668	32.9500	Adhesive
21	1.04	1.06	1.1024	46.8823	42.5275	Adhesive
22	1.03	1.04	1.0712	50.2071	46.8700	Adhesive
23	1.05	1.06	1.1130	52.8758	47.5075	Adhesive
24	1.06	1.04	1.1024	48.5028	43.9975	Adhesive
25	1.03	1.05	1.0815	49.0677	45.3700	Adhesive
26	1.06	1.03	1.0918	43.5000	39.8425	Adhesive
27	1.05	1.05	1.1025	58.8680	53.3950	Adhesive
28	1.04	1.06	1.1024	46.2843	41.9850	Adhesive
29	1.05	1.04	1.0920	48.0835	44.0325	Adhesive
30	1.04	1.04	1.0816	53.7880	49.7300	Adhesive
31	1.06	1.05	1.1113	53.2424	47.9100	Cohesive
32	1.07	1.03	1.1021	44.6957	40.5550	Adhesive

33	1.03	1.06	1.0918	43.0633	39.4425	Adhesive
34	1.05	1.05	1.1025	55.2959	50.1550	Adhesive
35	1.02	1.04	1.0508	47.9244	45.6075	Adhesive
36	1.06	1.04	1.1024	53.5325	48.5600	Cohesive

Table 10 Data of microtensile bond strength (μTBS) test from Group SF3m

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.04	1.05	1.1342	40.6752	35.8625	Adhesive
2	1.06	1.03	1.0710	30.7752	28.7350	Adhesive
3	1.02	1.05	1.0800	35.9235	33.2625	Adhesive
4	1.05	1.07	1.1124	43.4837	39.0900	Adhesive
5	1.06	1.07	1.0300	41.4704	40.2625	Adhesive
6	1.05	1.05	1.0506	27.8908	26.5475	Adhesive
7	1.04	1.05	1.1336	33.8408	29.8525	Adhesive
8	1.05	1.06	1.1448	54.6470	47.7350	Adhesive
9	1.06	1.07	1.1118	41.5480	37.3700	Adhesive
10	1.08	1.07	1.1445	45.0275	39.3425	Adhesive
11	1.04	1.05	1.1340	43.8546	38.6725	Adhesive
12	1.05	1.05	1.1021	48.4676	43.9775	Adhesive
13	1.03	1.02	1.0815	34.9027	32.2725	Adhesive
14	1.06	1.04	1.1445	30.2549	26.4350	Adhesive
15	1.03	1.04	1.1236	43.2502	38.4925	Adhesive
16	1.06	1.05	1.1556	47.4230	41.0375	Adhesive
17	1.02	1.03	1.0506	40.7081	38.7475	Adhesive
18	1.04	1.06	1.1024	27.6317	25.0650	Adhesive
19	1.05	1.03	1.0815	30.9309	28.6000	Adhesive
20	1.06	1.07	1.1342	37.3691	32.9475	Adhesive
21	1.06	1.05	1.1130	42.7086	38.3725	Adhesive
22	1.03	1.04	1.0712	40.8609	38.1450	Adhesive
23	1.05	1.03	1.0815	25.3260	23.4175	Adhesive
24	1.03	1.02	1.0506	39.8965	37.9750	Mixed
25	1.05	1.04	1.0920	41.8564	38.3300	Adhesive
26	1.05	1.03	1.0815	45.9854	42.5200	Adhesive
27	1.03	1.04	1.0712	44.0344	41.1075	Adhesive
28	1.04	1.05	1.0920	41.0619	37.6025	Adhesive
29	1.05	1.06	1.1130	35.3294	31.7425	Adhesive
30	1.07	1.07	1.1449	24.7098	21.5825	Cohesive
31	1.07	1.06	1.1342	28.7747	25.3700	Adhesive
32	1.05	1.04	1.0920	40.9254	37.4775	Adhesive

33	1.05	1.05	1.1025	43.6232	39.5675	Adhesive
34	1.05	1.06	1.1130	32.2742	28.9975	Adhesive
35	1.04	1.04	1.0816	37.3044	34.4900	Adhesive
36	-	-	-	-	-	-

Table 11 Data of microtensile bond strength (μTBS) test from Group V3m

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.03	1.05	1.1342	26.3787	23.2575	Adhesive
2	1.07	1.04	1.0710	32.3656	30.2200	Adhesive
3	1.03	1.02	1.0800	30.6099	28.3425	Adhesive
4	1.00	1.08	1.1124	27.9518	25.1275	Adhesive
5	1.07	1.06	1.0300	23.5381	22.8525	Adhesive
6	1.04	1.02	1.0506	23.0370	21.9275	Adhesive
7	1.03	1.03	1.1336	21.7339	19.1725	Adhesive
8	1.05	1.02	1.1448	26.0785	22.7800	Adhesive
9	1.03	1.04	1.1118	31.3305	28.1800	Adhesive
10	1.04	1.01	1.1445	25.6540	22.4150	Adhesive
11	1.04	1.07	1.1340	27.2925	24.0675	Adhesive
12	1.03	1.02	1.1021	30.8092	27.9550	Adhesive
13	1.05	1.02	1.0815	29.5304	27.3050	Adhesive
14	1.04	1.03	1.1445	27.8485	24.3325	Adhesive
15	1.03	1.02	1.1236	29.6406	26.3800	Adhesive
16	1.03	1.02	1.1556	16.4760	14.2575	Adhesive
17	1.04	1.05	1.0920	20.9746	19.2075	Adhesive
18	1.06	1.05	1.1130	16.3667	14.7050	Adhesive
19	1.05	1.09	1.1445	27.3335	23.8825	Adhesive
20	-	-	-	-	-	-
21	1.05	1.04	1.0920	23.4480	21.4725	Adhesive
22	1.03	1.02	1.0506	21.7737	20.7250	Adhesive
23	1.01	1.07	1.0807	20.0956	18.5950	Adhesive
24	-	-	-	-	-	-
25	1.06	1.05	1.1130	32.5191	29.2175	Adhesive
26	1.04	1.02	1.0608	25.0667	23.6300	Adhesive
27	1.03	1.07	1.1021	27.7619	25.1900	Adhesive
28	-	-	-	-	-	-
29	1.07	1.08	1.1556	24.5305	21.2275	Adhesive
30	1.08	1.02	1.1016	24.9457	22.6450	Adhesive
31	-	-	-	-	-	-
32	-	-	-	-	-	-

33	1.04	1.05	1.0920	27.3027	25.0025	Adhesive
34	1.02	1.03	1.0506	25.5664	24.3350	Adhesive
35	-	-	-	-	-	-
36	-	-	-	-	-	-

Table 12 Data of microtensile bond strength (μTBS) test from Group PV3m

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.04	1.03	1.1342	26.6991	23.5400	Adhesive
2	1.05	1.04	1.0710	31.6909	29.5900	Adhesive
3	1.04	1.06	1.0800	34.9758	32.3850	Adhesive
4	1.06	1.05	1.1124	28.8361	25.9225	Adhesive
5	1.05	1.05	1.0300	20.2318	19.6425	Adhesive
6	1.04	1.03	1.0506	29.8397	28.4025	Adhesive
7	1.05	1.07	1.1336	39.8715	35.1725	Adhesive
8	1.06	1.04	1.1448	40.0508	34.9850	Adhesive
9	1.05	1.05	1.1118	35.1329	31.6000	Adhesive
10	1.06	1.07	1.1445	34.9702	30.5550	Adhesive
11	1.07	1.06	1.1340	38.1024	33.6000	Adhesive
12	1.06	1.05	1.1021	39.2651	35.6275	Adhesive
13	1.04	1.03	1.0815	24.7636	22.8975	Adhesive
14	1.02	1.05	1.1445	23.8714	20.8575	Adhesive
15	1.05	1.05	1.1236	31.2052	27.7725	Adhesive
16	1.04	1.03	1.1556	38.2272	33.0800	Adhesive
17	1.07	1.06	1.1342	32.6508	28.7875	Mixed
18	1.05	1.06	1.1130	40.5215	36.4075	Adhesive
19	1.06	1.04	1.1024	32.3858	29.3775	Adhesive
20	1.06	1.05	1.1130	36.3561	32.6650	Adhesive
21	1.06	1.04	1.1024	33.3145	30.2200	Adhesive
22	1.07	1.06	1.1342	20.9288	18.4525	Adhesive
23	1.05	1.06	1.1130	33.7267	30.3025	Adhesive
24	1.06	1.05	1.1130	24.7086	22.2000	Adhesive
25	1.04	1.03	1.0712	33.2447	31.0350	Adhesive
26	1.02	1.01	1.0302	33.6180	32.6325	Adhesive
27	1.02	1.02	1.0404	23.8694	22.9425	Adhesive
28	-	-	-	-	-	-
29	1.06	1.07	1.1342	23.0243	20.3000	Adhesive
30	1.07	1.07	1.1449	35.5463	31.0475	Adhesive
31	-	-	-	-	-	-
32	-	-	-	-	-	-

33	1.04	1.03	1.0712	34.6265	32.3250	Adhesive
34	1.02	1.01	1.0302	32.2890	31.3425	Adhesive
35	-	-	-	-	-	-
36	-	-	-	-	-	-

Table 13 Data of microtensile bond strength (μTBS) test from Group POV3m

	(mm)	(mm)	Area (mm^2)	Force (N)	μTBS (MPa)	Failure
1	1.05	1.04	1.1342	51.1127	45.0650	Adhesive
2	1.04	1.03	1.0710	25.8620	24.1475	Adhesive
3	1.00	1.02	1.0800	43.4700	40.2500	Adhesive
4	1.04	1.03	1.1124	48.5924	43.6825	Adhesive
5	1.01	1.02	1.0300	39.6061	38.4525	Adhesive
6	1.03	1.02	1.0506	47.3505	45.0700	Mixed
7	1.02	1.03	1.1336	43.1193	38.0375	Adhesive
8	1.04	1.03	1.1448	39.8076	34.7725	Adhesive
9	1.04	1.05	1.1118	45.5699	40.9875	Adhesive
10	1.05	1.06	1.1445	40.9588	35.7875	Adhesive
11	1.06	1.06	1.1340	41.7681	36.8325	Adhesive
12	1.06	1.07	1.1021	43.1941	39.1925	Adhesive
13	1.05	1.06	1.0815	48.5756	44.9150	Adhesive
14	1.04	1.03	1.1445	45.2192	39.5100	Adhesive
15	1.05	1.05	1.1236	38.5058	34.2700	Adhesive
16	1.04	1.04	1.1556	38.1637	33.0250	Adhesive
17	1.03	1.02	1.0506	43.7995	41.6900	Adhesive
18	1.01	1.02	1.0302	42.7636	41.5100	Adhesive
19	1.02	1.03	1.0506	27.5204	26.1950	Adhesive
20	1.02	1.04	1.0608	23.5710	22.2200	Adhesive
21	1.05	1.06	1.1130	45.0821	40.5050	Adhesive
22	1.04	1.03	1.0712	41.8812	39.0975	Cohesive
23	1.04	1.02	1.0608	52.3213	49.3225	Cohesive
24	1.03	1.05	1.0815	35.7922	33.0950	Adhesive
25	1.05	1.04	1.0920	38.1627	34.9475	Adhesive
26	1.04	1.03	1.0712	45.7697	42.7275	Adhesive
27	1.02	1.04	1.0608	28.1404	26.5275	Adhesive
28	1.05	1.06	1.1130	42.3107	38.0150	Adhesive
29	1.03	1.03	1.0609	39.6299	37.3550	Adhesive
30	1.03	1.04	1.0712	48.8092	45.5650	Adhesive
31	1.04	1.02	1.0608	40.4218	38.1050	Adhesive
32	1.02	1.01	1.0302	43.3379	42.0675	Adhesive

33	1.02	1.03	1.0506	28.1823	26.8250	Adhesive
34	1.05	1.03	1.0815	50.1492	43.3700	Adhesive
35	1.03	1.05	1.0815	45.4203	41.9975	Adhesive
36	-	-	-	-	-	-

Appendix B Descriptive statistics of experimental groups

Table 14 Descriptive statistics of experimental groups

Group	Microtensile bond strength (MPa)				
	n	Min	Max	Mean	SD
SF24h	36	25.0200	54.4400	41.15	6.27
V24h	36	19.5600	42.5450	32.30	5.62
PV24h	36	27.8575	48.6600	36.11	5.19
POV24h	36	32.9500	53.3950	42.63	4.57
SF3m	35	22.2200	49.3225	34.89	6.36
V3m	29	14.2575	30.2200	23.39	3.88
PV3m	31	18.4525	36.4075	28.89	5.13
POV3m	35	22.2200	49.3225	37.86	6.47

Appendix C Statistical comparison of microtensile bond strength

Table 15 Statistical comparison of microtensile bond strength

Comparison	p-value
SF24h & V24h	0.000
SF24h & PV24h	0.004
SF24h & POV24h	1.000*
SF24h & SF3m	0.000
SF24h & V3m	0.000
SF24h & PV3m	0.000
SF24h & POV3m	0.365*
V24h & PV24h	0.106*
V24h & POV24h	0.000
V24h & SF3m	1.000*
V24h & V3m	0.000
V24h & PV3m	0.358*
V24h & POV3m	0.001
PV24h & POV24h	0.000
PV24h & SF3m	1.000*
PV24h & V3m	0.000
PV24h & PV3m	0.000
PV24h & POV3m	1.000*
POV24h & SF3m	0.000
POV24h & V3m	0.000
POV24h & PV3m	0.000
POV24h & POV3m	0.010
SF3m & V3m	0.000
SF3m & PV3m	0.000
SF3m & POV3m	0.714*
V3m & PV3m	0.004
V3m & POV3m	0.000
PV3m & POV3m	0.000

* Statistically significant difference at the 0.05 level

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RESEARCH GRANT: Supported by Chulalongkorn University Graduate School Thesis Grant