ผลของสารคอนดิชันเนอร์ที่มีต่อความแข็งแรงพันธะเฉือนระยะแรกของ
เรซินมอดิฟายด์กลาสไอโอโนเมอร์ต่อเคลือบฟัน

นางสาวณัฐพร เลาห์ทวีรุ่งเรือง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาทันตกรรมจัดฟัน ภาควิชาทันตกรรมจัดฟัน
คณะทันตแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

EFFECT OF THE ENAMEL CONDITIONERS ON INITIAL SHEAR BOND STRENGTH OF RESIN-MODIFIED GLASS IONOMER ADHESIVE TO ENAMEL

Miss Nattaporn Laotaveerungrueng

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Orthodontics
Department of Orthodontics
Faculty of Dentistry
Chulalongkorn University
Academic Year 2014

Thesis Title	EFFECT OF THE ENAMEL CONDITIONERS ON INITIAL SHEAR BOND STRENGTH OF RESIN-MODIFIED GLASS IONOMER ADHESIVE TO ENAMEL
By	Miss Nattaporn Laotaveerungrueng
Field of Study	Orthodontics
Thesis Advisor	Assistant Professor Paiboon Techalertpaisarn, Ph.D.

Accepted by the Faculty of Dentistry, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

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

THESIS COMMITTEE

.....Chairman
(Associate Professor Porntip Chiewcharat)

.....Thesis Advisor
(Assistant Professor Paiboon Techalertpaisarn, Ph.D.)

.....External Examiner
(Clinical Professor Piyarat Apivatanagul)

ณัฐพร เลาห์ทวีรุ่งเรือง : ผลของสารคอนดิชันเนอร์ที่มีต่อความแข็งแรงพันธะเฉือนระยะแรกของเรซินมอดิฟายด์กลาสไอโอโนเมอร์ต่อเคลือบฟัน (EFFECT OF THE ENAMEL CONDITIONERS ON INITIAL SHEAR BOND STRENGTH OF RESIN-MODIFIED GLASS IONOMER ADHESIVE TO ENAMEL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ทพ. ดร. ไพบูลย์ เตชะเลิศไพศาล, หน้า.

วัตถุประสงค์ เพื่อศึกษาผลของการปรับสภาพผิวฟันด้วยสารคอนดิชันเนอร์ต่างๆต่อความแข็งแรงพันธะเฉือนระยะแรกของเรซินมอดิฟายด์กลาสไอโอโนเมอร์ต่อเคลือบฟัน

วัสดุและวิธีการ แบ่งฟันกรามน้อย 55 ซี่ ออกเป็น 5 กลุ่ม (กลุ่มละ 11 ซี่) กลุ่มที่ 1 เป็นกลุ่มควบคุมซึ่งไม่ใช้สารคอนดิชันเนอร์ กลุ่มที่ 2-4 เป็นกลุ่มทดลอง ซึ่งใช้สารคอนดิชันเนอร์ดังนี้ กรดพอลิอะคริลิกเข้มข้นร้อยละ 20 กรดฟอสฟอริกเข้มข้นร้อยละ 37 และสารเซลฟ์เอชชิงไพร์มเมอร์ ตามลำดับ กลุ่มที่ 1-4 ยึดติดแบร็กเกตด้วยสารยึดติดเรซินมอดิฟายด์กลาสไอโอโนเมอร์ (Fuji ortho LC) กลุ่มที่ 5 เป็นกลุ่มสำหรับเปรียบเทียบสมรรถนะ ซึ่งใช้สารคอนดิชันเนอร์กรดฟอสฟอริกและสารยึดติดเรซินคอมโพสิต (Transbonds XT) ภายหลังการฉายแสง 5 นาที นำตัวอย่างกลุ่มละ 10 ชิ้นไปวัดความแข็งแรงพันธะเฉือนโดยเครื่องทดสอบสากล บันทึกค่าความแข็งแรงพันธะเฉือนที่ทำให้แบร็กเกตหลุดจากผิวฟัน ทำการเปรียบเทียบค่าเฉลี่ยระหว่างกลุ่มด้วยค่าสถิติการวิเคราะห์ความแปรปรวนแบบทางเดียว วัดค่าการเหลืออยู่ของสารยึดติดบนตัวฟันด้วยค่าดัชนีการเหลืออยู่ของสารยึดติดและทดสอบด้วยค่าสถิติไคสแควร์ ที่ระดับความเชื่อมั่นร้อยละ 95 นำตัวอย่างกลุ่มละ 1 ชิ้นไปศึกษาการแทรกซึมของเรซินเข้าไปในเคลือบฟันด้วยกล้องจุลทรรศน์อิเล็คตรอนแบบส่องกราด

ผลการศึกษา ค่าเฉลี่ยและค่าความแปรปรวนของความแข็งแรงพันธะเฉือนของกลุ่ม 1 2 3 4 และ 5 เท่ากับ 8.4 ± 2.0 10.8 ± 0.7 14.6 ± 3.0 13.7 ± 1.7 และ 21.5 ± 4.0 เมกะปาสคาล ตามลำดับ ไม่พบความแตกต่างอย่างมีนัยสำคัญของค่าเฉลี่ยความแข็งแรงพันธะเฉือนระหว่างกลุ่มที่ 3 และ 4 (p=0.328) ค่าดัชนีการเหลืออยู่ของสารยึดติดสัมพันธ์กับชนิดของสารคอนดิชันเนอร์ (p<0.05) พบว่ากลุ่ม 1 และ 2 เกิดการล้มเหลวของการยึดติดระหว่างผิวเคลือบฟันและสารยึดติด ส่วนกลุ่ม 3 4 และ 5 เกิดความล้มเหลวระหว่างฐานแบร็กเกตและสารยึดติด การศึกษาด้วยกล้องจุลทรรศน์อิเล็คตรอนแบบส่องกราดพบว่าลักษณะของแท่งเรซินในผิวฟันของกลุ่มที่ใช้สารเซลฟ์เอชชิงไพร์มเมอร์มีปริมาณมากและขนาดใหญ่กว่ากลุ่มทดลองอื่นๆ

สรุป การปรับสภาพผิวฟันด้วยกรดฟอสฟอริกหรือสารเซลฟ์เอชชิงไพร์มเมอร์มีผลเพิ่มความแข็งแรงพันธะเฉือนระยะแรกของเรซินมอดิฟายด์กลาสไอโอโนเมอร์

คำสำคัญ : *ความแข็งแรงพันธะเฉือนระยะแรก; เรซินมอดิฟายด์กลาสไอโอโนเมอร์; สารคอนดิชันเนอร์*

ภาควิชา ทันตกรรมจัดฟัน ลายมือชื่อนิสิต .....

สาขาวิชา ทันตกรรมจัดฟัน ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557

5675806932 : MAJOR ORTHODONTICS
KEYWORDS: ENAMEL CONDITIONER / INITIAL SHEAR BOND STRENGTH / RESIN-MODIFIED GLASS IONOMER

NATTAPORN LAOTAVEERUNGRUENG: EFFECT OF THE ENAMEL CONDITIONERS ON INITIAL SHEAR BOND STRENGTH OF RESIN-MODIFIED GLASS IONOMER ADHESIVE TO ENAMEL. ADVISOR: ASST. PROF. PAIBOON TECHALERTPAISARN, Ph.D., pp.

Objective: To study the effect of different enamel conditioners on initial shear bond strength of resin-modified glass ionomer adhesive to enamel.

Materials and methods: Fifty-five human premolars were divided into 5 groups (11 samples for each group). Group 1 was the controlled group without conditioner. Group 2-4 were experimental groups which were conditioned with 20% polyacrylic acid, 37% phosphoric acid and self-etching primer, respectively. For group 1-4, the brackets were bonded with resin-modified glass ionomer adhesive (Fuji ortho LC). Group 5 was the Benchmark group which was conditioned with 37% phosphoric acid and the brackets were bonded with composite resin adhesive (Transbond XT). After light activation for 5 minutes, the shear bond strengths of 10 specimens from each group were tested by universal testing machine. The forces that debonded the brackets from enamel surface were recorded. The mean of shear bond strength among groups were compared by one-way ANOVA. The adhesive remnant indices (ARI) in each group were measured and tested by the Chi-square at 95% confidence interval. One remaining specimen from each group was used to investigate of resin penetration pattern into enamel under scanning electron microscope.

Results: Mean and standard deviations of shear bond strengths of group 1, 2, 3, 4 and 5 were 8.4 ± 2.0, 10.8 ± 0.7, 14.6 ± 3.0, 13.7 ± 1.7 and 21.5 ± 4.0 megapascal, respectively. There is no significant difference between group 3 and 4 (p=0.328). Types of enamel conditioners were statistically significant to ARI scores (p<0.05). SEM image revealed that the size and number of resin tags in self-etching primer group are greater than the other experimental groups.

Conclusion: The use of phosphoric acid or self-etching primer as a conditioner can increase initial shear bond strength of resin-modified glass ionomer adhesive

Department: Orthodontics
Field of Study: Orthodontics
Academic Year: 2014

Student's Signature .....
Advisor's Signature .....

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my research adviser, Assistant Professor Paiboon Techalertpaisarn, for his guidance, inspiration, mentorship, and full support throughout my master research years. His expertise and innovative advice have substantially broadened my knowledge and greatly benefited my career. I have been very fortunate to have this opportunity to work under his supervision. Moreover, I would like to acknowledge Faculty Research Grant (DRF 57018) Faculty of Dentistry, Chulalongkorn University for providing funding for this research.

I deeply appreciate Associate Professor Chaiwat Maneenut for his guidance, patience, and help with microhardness test. His advice enabled me to complete this research work. I would also like to thank the rest of my committee members, Associate Professor Porntip Chiewcharat and Clinical Professor Piyarat Apivatanagul for their interest and for providing invaluable advice in all aspects of my research. In addition, I would like to express my sincere gratitude to Associate Professor Vachara Phetcharakupt for his guidance on my thesis proposal. Although he could not join my thesis examination, I wish he will have a fast recovery and get better soon.

Also, I would like to thank all friends for their help, discussion, collaboration, and friendship. Many thanks to all scientists and technicians of the Dental Material Science Research Center for constant help with my research.

Lastly, but most importantly, I would like to thank my parents, my brothers and my aunt for their continuous love, encouragement, and support throughout my time. Nothing could have been achieved without their patience and kind support.

CONTENTS

Page

Page

Page

List of figures

List of tables

Chapter I
INTRODUCTION

Background and Rationale

Patients with fixed orthodontic appliances have elevated risk of caries and white spot lesion. The incidence of enamel demineralization after the use of fixed orthodontic appliance can occur in up to 50%.[1] Although the combination of fluoride methods which are dentifrice and mouth rinse, is efficient for reducing caries progression, the patient's cooperation is essential.[2] Excellent compliance with using the mouth rinse can be expected in less than 12% of patients. Therefore, the preventive measure that does not depend on patient compliance, such as the use of dental materials with fluoride-releasing properties, has been assessed. Glass ionomer cement (GIC) has been shown to release fluoride over the long term and generally at much higher levels than fluoride-releasing composites. GIC bond chemically to enamel, cementum and dentin. Moreover, the potential advantages of GIC are adhesion in a wet field and a non-etching technique. On the other hand, the disadvantage of GIC is their weak bond strength, as shown in several studies with either phosphoric acid or polyacrylic acid conditioning.[3]

Resin-modified glass ionomer adhesive (RMGI) was chosen for bonding the bracket due to its property to release fluoride of conventional glass ionomer and the composite resin's good adhesion. Due to previous study, RMGI showed the reduction of enamel demineralization around orthodontic brackets.[4] Although RMGI exhibit cariostatic activity, the bond strength of RMGI was lower than composite adhesive.[5] Therefore, many studies have been done in order to search for the better enamel conditioner which could improve shear bond strength of RMGI.

Nowadays, there is no further study the shear bond strength of RMGI within five minutes. The purpose of this study is to determine the effects of the type of enamel conditioner on the initial shear bond strength of RMGI. In practical, the

orthodontists will ligate the arch wire after all brackets attachment immediately, therefore the experiment will be done within five minutes.

Furthermore, the pilot study is to determine the effects of the type of enamel conditioner on microhardness of enamel. In appendix section, the microhardness is tested for selecting the type of conditioner which can provide high shear bond strength and less enamel loss.

Research questions

1. Does the type of enamel conditioners of resin-modified glass ionomer adhesive effect on the initial shear bond strength?
2. Does the type of enamel conditioners effect on the adhesive remaining after debonding the bracket?

Objectives

1. To compare the effect of different enamel conditioners on initial shear bond strength of resin-modified glass ionomer adhesive to enamel.
2. To compare the adhesive remaining among different enamel conditioners of resin-modified glass ionomer adhesive.
3. To observe the interface between enamel and orthodontic adhesive each type of enamel conditioners with a scanning electron microscope.

Research hypotheses

1. The type of enamel conditioners of resin-modified glass ionomer adhesive effect on initial shear bond strength.
2. The type of enamel conditioners effect on the adhesive remaining after debonding the bracket.

Assumptions

1. This research was an experimental study in vitro and a test in extracted human premolar teeth. These teeth were collected in the same solution which was 0.1% thymol solution. Moreover, a preparation of specimens was done by the same method and the same researcher. Therefore all specimens were the same characteristic.

2. This study imitated the bonding of the brackets with different types of conditioner in order to compare initial shear bond strength of resin-modified glass ionomer adhesive to enamel
3. This experiment compared the remaining adhesive on enamel after the shear bond strength test.
4. The conditioners were used in this study as below
    - 20% polyacrylic acid
    - 37% phosphoric acid
    - Self-etching primer
5. The adhesives were used in this study as below
    - Fuji ortho LC™ (RMGI)
    - Transbond XT™ (composite resin)
6. All brackets which were tested in this study were the premolar standard edgewise brackets with no tip and torque therefore these brackets can be used for all left or right and upper or lower premolar teeth. The brackets were stainless steel, 0.018"slot size, 10.38 mm^2 area of bracket base and produced by Ormco corporation.
7. The bonding procedure was done in the same scenario since it was done by the same researcher who has repeated practicing and strictly followed the instruction.
8. The shear bond strength test was implemented by Universal testing machine (Shimadzu Corp., Japan). 500-newton load and 1 mm/min speed was applied to the bracket.
9. Evaluating of adhesive remaining on enamel under the stereo microscope at x10 magnification. Adhesive remnant index (ARI scale), which was 1-5 scale, was used to determine remaining adhesive.

Limitations

The actual oral environment cannot be imitated due to the experiment in vitro such as temperature and humidity. Therefore a result of this study cannot refer to actual clinical result.

Operational definition

- Glass ionomer cement (GIC) is the generic for materials based on the reaction of glass powder and polyacrylic acid.
- Resin-modified glass ionomer (RMGIC) is the material which water-soluble methacrylate-based monomers have been used to replace part of liquid component of conventional GIC.

Expected benefit and application

To select the type of conditioner which provide the highest initial shear bond strength.

Research design

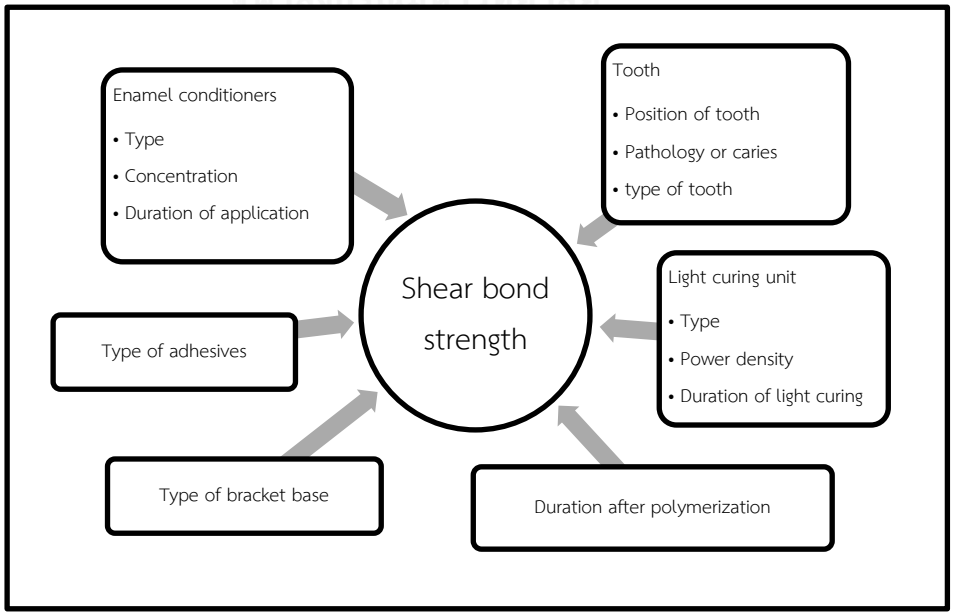
A experimental study in vitro

Obstacles and strategies

Error in shear bond strength test as below

- Tooth dislodged from acrylic resin during the test.
- The blade of the machine was not located parallel to the base of bracket.

Conceptual framework



Chapter II

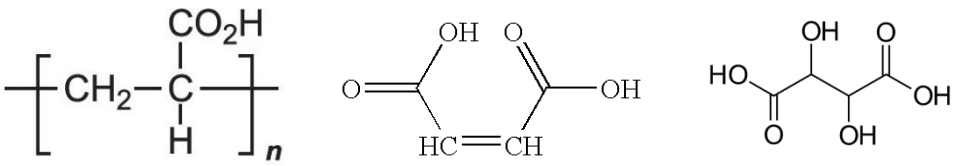
LITERATURE REVIEW

The development of resin-modified glass ionomer adhesive

Conventional glass ionomer cements (GIC) was developed by Wilson and Kent in 1972.[6] These materials were formed by the acid-base reaction of an aqueous polymeric acid and as ion leachable fluoroaluminosilicate (FAS) glass. The materials are supplied as two-part powder-liquid systems that require mixing and have the following essential components:

- Polycarboxylic acid
- Fluoroaluminosilicate (FAS) glass
- Water
- Tartaric acid

The polymeric matrix of most glass ionomers is a copolymer of acrylic acid and itaconic acid or maleic acid. Tartaric acid is added to control the working and setting characteristics of the material. The powder consists of an acid reactive comminuted FAS glass and has ion such as calcium, strontium and lanthanum. When powder and liquid are mixed, an acid-base setting reaction begins between the FAS glass and the polycarboxylic acid. An initial set is achieved within 3 to 4 minutes of mixing, but the ionic reaction continues until maturation. Maturation time has been improved in newer formulations to allow finishing after 15 minutes of placement of mix. The steps of ion extraction and complex formation are described in the scheme shown (Figure 2).



Polyacrylic acid Maleic acid Tartaric acid

Figure 1 The molecule of polyacrylic acid, maleic acid and tartaric acid

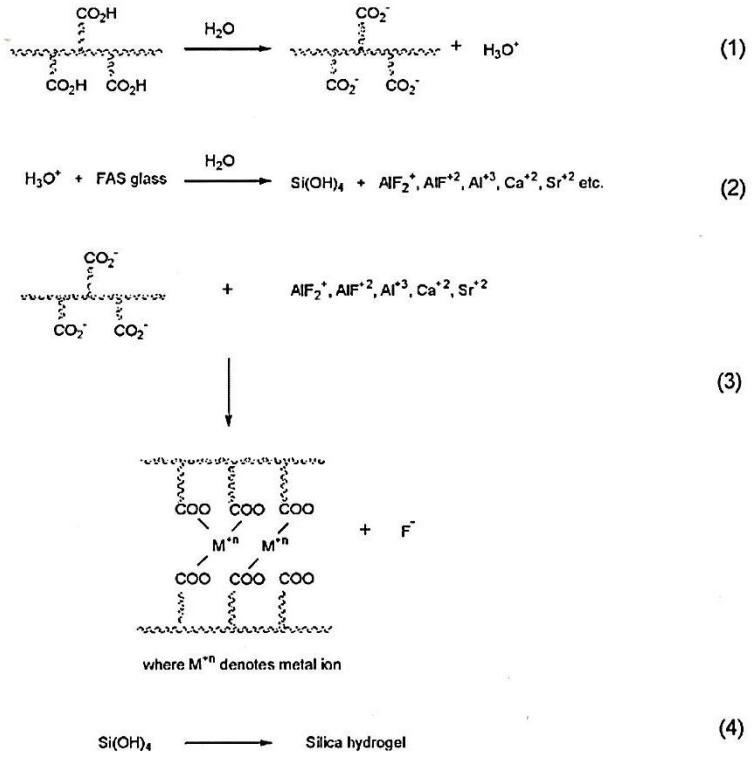


Figure 2 Setting mechanism of conventional glass ionomers

All carboxylic acids have a common organic functional group denoted by COOH. In the presence of water, the COOH group undergoes partial ionization to yield a carboxylate anion COO^- and a hydrated proton, $H3O^+$ (Fig 2, reaction 1). The hydrated proton attacks the surface of the glass particles releasing calcium and aluminum ions (Fig 2, reaction 2). The carboxylate ions from the polymer react these metallic ions to form a salt bridge (Fig 2, reaction 3), resulting in gelation and setting. During the initial setting, calcium ions are more rapidly bound to the polyacrylate chains; binding to the aluminum ions occurs at a later stage. The strength of the cement builds with time. Silicic acid is initially formed when the glass breaks down, but rapidly polymerizes to form silica hydrogel (Fig 2, reaction 4). A very important by-product of the setting reaction is the release of fluoride ions from the glass matrix. This fluoride release process is sustained and occurs over a long period of time. This fluoride ion release is a result of the setting reaction and the ion exchange

process. In this process the fluoride ion from the glass is being replaced hydrogen ions of carboxylic groups.

Water plays several important roles in overall setting. First, it provides for the ion transport needed for the acid-base setting reaction and fluoride release. Second, a portion of the water is also chemically bound in the set complex and provides stability to material.

The undissolved portion of glass particles is sheathed by silica-rich gel that is formed on the surface of the glass particles. Thus, the set cement consists of undissolved glass particles with a silica gel coating embedded in an amorphous matrix of hydrated calcium and aluminum polysalts containing fluoride. The stability of the matrix is given by an association of chain entanglement, weak ionic cross-linking and hydrogen bonding.

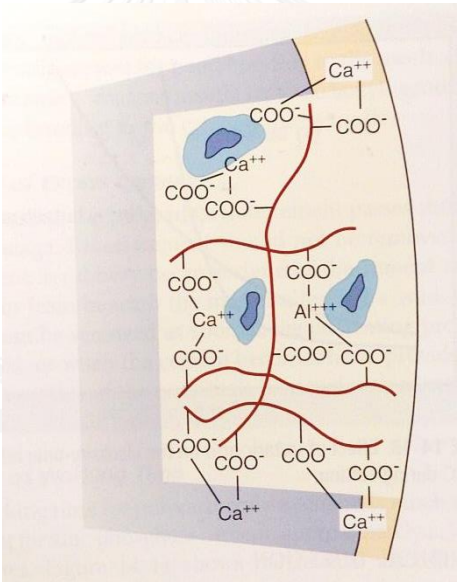


Figure 3 Diagram depicting the structure of GIC. The solid blue particles represent unreacted glass particles surrounded by the gel (light blue shaded structure) that form when Al^{3+} and Ca^{2+} ions are leached from the glass as a result of attack by polyacrylic acid. The Al^{3+} and Ca^{2+} ions form polysalts with the COO^- groups of the polyacrylic acid to form a cross-linked structure. The carboxyl groups react with the calcium in enamel.[7]

Resin-modified glass ionomer (RMGI) was introduced in the late 1980s. The essential components are similar to those in conventional glass ionomer and add

some polymerizable hydrophilic resins common in composite resin. Furthermore, there are some advantages over the conventional GIC for instance a longer working time, a control of the photochemical curing process by the clinician, and a rapid hardening of the surface of the cement. Hence, the photochemical reaction reduces the early sensitivity to moisture and the dehydration associated with the early stage of the acid–base setting reaction of the conventional GIC. Moreover, the RMGI present higher mechanical properties than the conventional GIC.[8] The essential components of true RMGI are as follows:

- Polycarboxylic acid polymer
- Fluoroaluminosilicate (FAS) glass
- Water
- Hydrophilic methacrylate monomer
- Free radical initiators

The RMGI contain some methacrylate components common in composite resin. There are two ways in which methacrylate components can be introduced. In first type, the polycarboxylic acid polymer chain is modified to contain a pendent methacrylate group. A common way of doing this is to react some of the carboxylic acid groups connected through the hydrolytically stable amide linkages. In addition to the methacrylate-modified carboxylic acid, the liquid portion contains a water-miscible methacrylate monomer, for example, hydroxyethyl methacrylate (HEMA) or glycerol dimethacrylate (GDMA). HEMA is a monovinyl monomer commonly used in adhesive chemistry as a hydrophilic primer and some component of adhesive resins. In RMGI, it makes the aqueous and the organic components miscible acting both as a co-solvent and polymerizable monomer.[9]

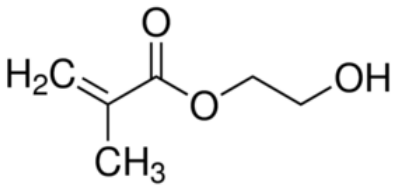


Figure 4 Hydroxyethyl methacrylate (HEMA) molecule

In another type of RMGI system, the polymer is unmodified carboxylic acid. The liquid is formulated with a mixture of hydrophilic methacrylate monomer and water. Generally, the water content of these materials is lower and the monomer content higher than for the first type. As a result, the coefficient of thermal expansion of these RMGI is high.

Furthermore, free radical initiators are added to trigger the curing of the methacrylate groups such as camphorquinone. Visible light initiators and/or self-cured redox initiators are employed to effect this curing and covalent cross-linking reaction.

RMGI was set by a dual reaction which was the acid-base reaction and free radical polymerization process. The acid-base reaction begins upon mixing and continues after polymerization at much slower rate than conventional glass ionomer because less water is present and the reaction is much slower in the solid phase than liquid phase.

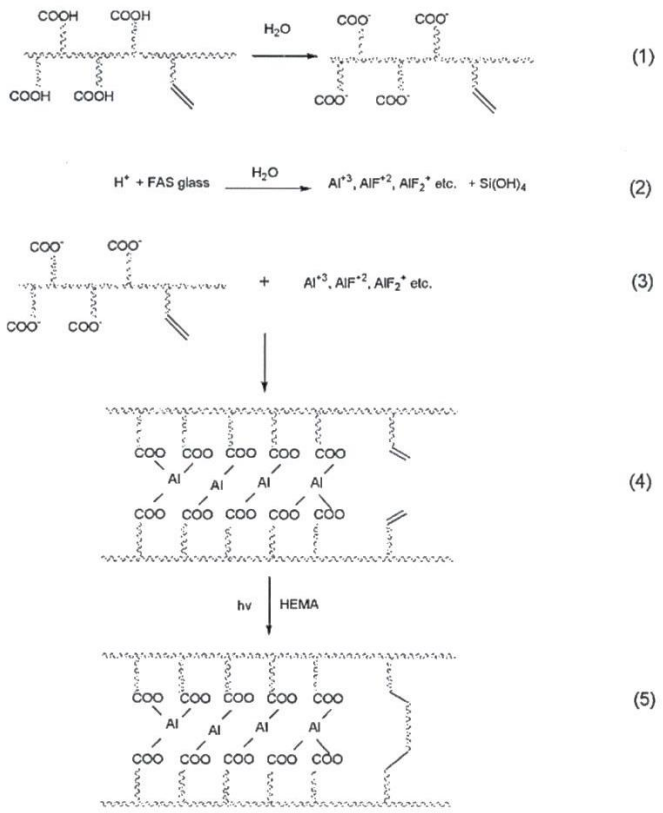


Figure 5 Setting mechanism of resin-modified glass ionomers

The bonding mechanism of RMGI to tooth structure is the same as that for conventional GIC. However, the use of polymerizable monomers in hybrid ionomers leads one to speculate that the bonding mechanism should also include the hybrid layer of the cement that infiltrates the tubules. Currently, no conclusive evidence exists of hybrid layer formation with RMGI. However higher bond strengths to teeth and resin based composites have been reported for RMGI than conventional glass ionomer, which is probably associated with enhanced micromechanical interlocking to the roughened tooth surface.[7, 10]

Enamel conditioner

Enamel conditioner is used to preparing the enamel surface for successful enamel adhesion to dental materials. The role of the conditioner for glass ionomer probably involves effective removal of the smear layer and provides for good wetting of the surface and an essential requirement for good bonding. However, as acidic materials, the conditioners may also produce microporosity in the enamel surface that could contribute to either increased surface area for chemical bonding or micromechanical bonding through polymer penetration. There are the different type of acids with various concentrations and/or etching times have been investigated in vitro to refine the acid-etching technique. Several types of conditioners were used as below.

Polyacylic acid

Polyacrylic acid is used as conditioning of the tooth surface prior to the application of GIC. The objective of conditioning is to remove surface contaminants and the smear layer, which may limit the bond of the GIC to tooth structure.[11] The mechanisms by which polyacrylic acid conditioning improves the bonding strength of GIC and enamel found in the work of Wilson et al.[6], who investigated the nature of chemical bonding between hydroxyapatite and polyacrylic acid. The reaction mechanism based on the exchange of calcium and phosphate ion versus carboxylic ions at the enamel surface.

Phosphoric acid

The modern bonding systems for resin-based materials are based on a micromechanical retention principle. Phosphoric acid is demonstrated to be the most effective in promoting enamel adhesion which used to clean the surface and dissolve the minerals. In addition, this acid removes the smear layer and about 10 microns of enamel to expose prisms of enamel rods to create a honeycomb-like and high energy retentive surface. The higher surface energy ensures that resin monomers will readily wet the surface, infiltrate into the micropores and polymerize to form resin tags. The resin tags are approximately 6 μm in diameter and 10 to 20 μm in length and lead to micromechanical interlocking.

Buonocore originally used an 85% phosphoric solution for 30 seconds for conditioning the enamel surface. When different concentrations of phosphoric acid were used in vitro, concentrations lower than 30% were concluded to be insufficient in producing enough enamel dissolution for bonding. Acid concentration higher than 50% presented less surface morphologic change. In general, concentration for optimal strength was between 35% and 50%. The manufacturers of various adhesive systems recommended 37% phosphoric acid concentration for 15-30 seconds.

Self-etching primer

Self-etching primer (SEP)[12] combines the conditioning and priming agents into a single acidic primer solution. These systems typically incorporate methacrylated phosphoric acid esters. After application to enamel, the phosphate group dissolves and removes calcium ions from hydroxyapatite, becoming incorporated in the network before the primer polymerizes and neutralizing the acid.

SEP showed much less etching ability because of their relatively higher pH as compared with phosphoric acid, thus minimizing the potential for iatrogenic damage of enamel. Therefore, SEP were used for bonding due to their benefits such as user-friendlier (shorter application time, less steps), improving adhesion procedures, reduction of enamel loss, prevention of saliva contamination and reduced sensitivity to moisture.

Shear bond strength (SBS)

A minimum bond strength of 6 – 8 MPa was adequate for most clinical orthodontic needs.[13] There are many factors that can potentially contribute to the bond strength between the enamel and the orthodontic bracket including type of enamel conditioner, acid concentration, duration of etching time, composition of adhesive, bracket base design, the bracket material, the oral environment as well as the skill of clinician.

Effect of altering type of enamel conditioner

Bishara et al.[14] studied the effects of changing the type of enamel conditioner on the SBS of a RMGI within half an hour after bonding the bracket to the tooth. Group 1, teeth were conditioned with 10% polyacrylic acid; group 2, teeth were conditioned with 20% polyacrylic; group 3, teeth were etched with 37% phosphoric acid. In group 1-3, the brackets were bonded with a RMGI adhesive; group 4, teeth were etched with 37% phosphoric acid and the brackets were bonded with a resin composite adhesive. The results of the SBS were significantly greater in the 2 groups etched with 37% phosphoric acid. This was true for both the RMGI (6.1 ± 2.7 MPa) and the composite resin (5.2 ± 2.9 MPa) adhesives. On the other hand, the SBS were significantly lower in the two groups conditioned with polyacrylic acid. The bond strength of the RMGI adhesive conditioned with 10% polyacrylic acid (0.4 ± 1.0 MPa) was significantly lower than the group conditioned with 20% polyacrylic acid (3.3 ± 2.6 MPa).

Cacciafesta et al.[15] studied the effects of 3 different enamel conditioners (10% polyacrylic acid, 37% phosphoric acid, and self-etching primer) on the SBS and site of bond failure of a RMGI (Fuji Ortho LC) bonded onto dry, water moistened, and saliva-moistened enamel. As a result, self-etching primer and RMGI application produced the highest shear bond strengths under all the different enamel surface conditions; these values were significantly higher than those achieved in the remaining groups, except when RMGI was used in combination with 37% phosphoric acid on dry enamel. The RMGI bonded without enamel conditioning produced the lowest shear bond strengths. The bond strength of the groups conditioned with 10%

polyacrylic acid was significantly lower than that of the groups conditioned with 37% phosphoric acid, except when both conditioners were used on enamel soaked with water.

Effect of the type of adhesive

Bishara et al.[5] studied SBS of orthodontic brackets bonded with one of three methods: (1) a glass ionomer adhesive used with a 20% polyacrylic acid enamel conditioner (2) a composite resin adhesive used with 37% phosphoric acid etchant and a conventional primer (3) the composite resin used with an acidic primer that combines the etchant with the primer in one application. The results indicated that the resin/phosphoric acid adhesive system (control group) provided the strongest shear bond strength (10.4 ± 2.8 MPa). The glass ionomer adhesive system provided significantly lower bond strength (6.5±1.9 MPa). The least shear bond strength was present when the acidic primer was used with an orthodontic adhesive (2.8±1.9 MPa).

Rix et al.[16] studied the bond strength of three adhesives: composite resin (Transbond XT), hybrid GIC (Fuji ortho LC), and glass-filled GIC (Assure). The bonded teeth were stored in deionized water at 37°C for 30 days and thermocycled for 24 hours before debonding. As a result, the bond strength of the composite resin control (20.19 MPa) was significantly greater than that of the adhesives in the other groups, clinically acceptable shear bond strengths were found for all adhesives (Fuji Ortho LC = 13.57 MPa, Assure-dry = 10.74 MPa, Assure-wet = 10.99 MPa). The bond strength for the Assure adhesive was not significantly affected by saliva contamination.

Chitnis et al.[17] studied the bond strength of 4 adhesives: a commercially available giomer material, a polyacid modified composite resin (PMCR), a resin-modified glass ionomer (RMGI), and a standard resin-based composite (RBC) adhesive after bonding 1 hour and 7 days . As a result, the RBC and the RMGI adhesives had significantly higher shear bond strength than the giomer and the PMCR materials at both 1 hour and 7 days. The chi-square test detected a significant difference in adhesive remnant index scores.

Effect of length of time after bonding

Bishara et al.[18] studied the effects of time on the SBS of a RMGI and a composite resin adhesive system specifically (1) within half an hour after bonding the bracket to the tooth and (2) at least 24 hours from the time of bonding when the adhesive has achieved most of its bond strength. As a result, the SBS were significantly greater in the 2 groups debonded after 24 hours. This was true for both the RMGI (8.8 ± 3.6 MPa) and the composite resin (10.4 ± 2.8 MPa) adhesives. On the other hand, the SBS were significantly lower in the 2 groups debonded within 30 minutes of their initial bonding. The bond strength of the RMGI (0.4 ± 1.0 MPa) was significantly lower than that for the composite resin (5.2 ± 2.9 MPa) adhesive. The present findings indicated that the RMGI adhesive has significantly lower initial bond strength but increased more than 20-fold within 24 hours. In comparison, the composite resin adhesive has a significantly larger initial bond strength that doubled within 24 hours.

Scanning electron microscope examination

The scanning electron microscope (SEM) is a useful device to observe action of the conditioner agents on enamel surface. Rogelio et al.[19] observed the enamel-adhesive interface of enamel etched with phosphoric acid and enamel conditioned with self- etch primer with SEM. The scanning electron micrographs showed that 37% phosphoric acid seemed to produce more enamel loss than self-etching primer. Moreover, the enamel–adhesive interface was more irregular when the enamel was etched with 37% phosphoric acid. A gentle etch pattern of self-etching primer on the enamel surface was also observed.

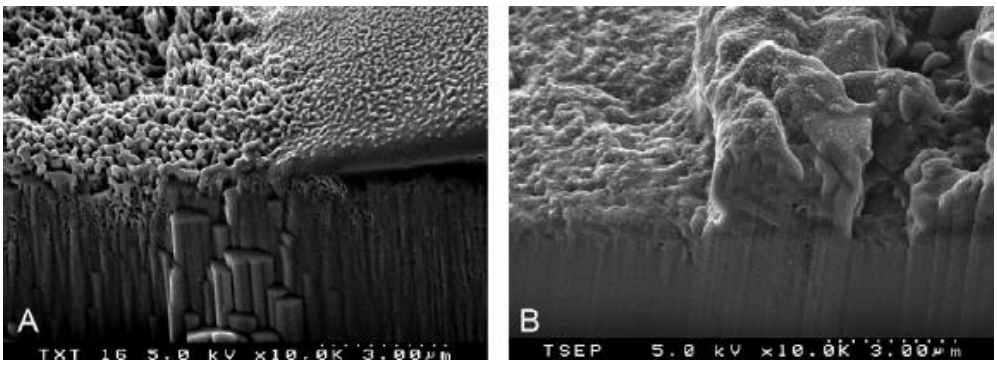


Figure 6 SEM images of enamel-adhesive interfaces.

A, Enamel conditioned with 37% phosphoric acid; B, enamel conditioned with SEP. Etch pattern with SEP appears to be more conservative.

Bishara et al.[14] observed the effect of enamel conditioners to enamel surface with SEM. The scanning electron micrographs showed that the phosphoric acid produced a much deeper etch (rough enamel surface) than polyacrylic acid conditioning whether a 10% or 20% concentration were used.

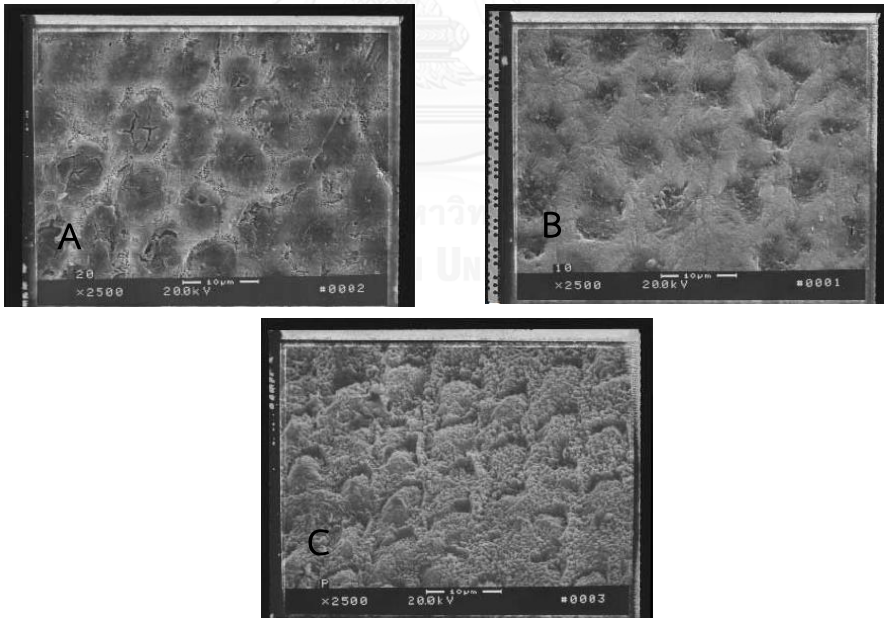


Figure 7 SEM images of enamel surfaces.

A, Enamel conditioned with 10% polyacrylic acid;

B, Enamel conditioned with 20% polyacrylic acid.

C, Enamel conditioned with 37% phosphoric acid

Es-Souni et al.[20] showed the SEM images of conditioned and non-conditioned enamel surfaces. It is seen that the surface treatment with 25% as well as 5% polyacrylic acid (PAA) lead to slight etching patterns on the enamel with regard to the reference surface, where only the grinding grooves dominate the micrograph. For comparison, the enamel which was etched with phosphoric acid showed the intense etching pattern.

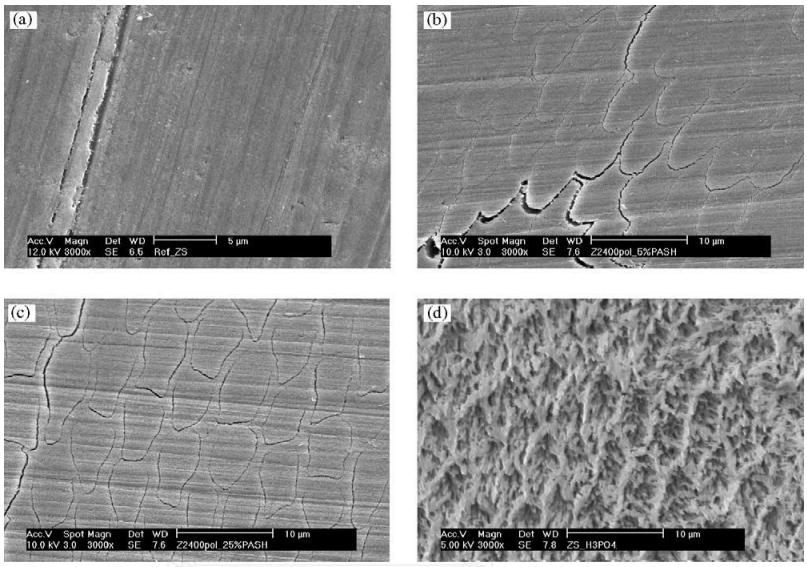
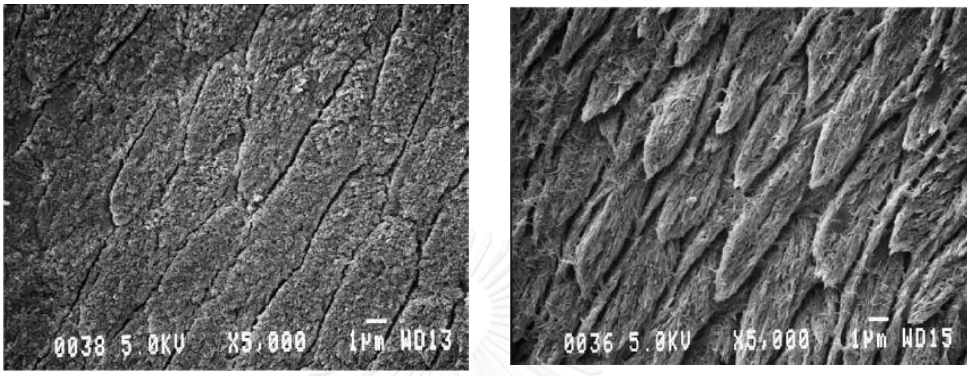


Figure 8 SEM micrographs of differently conditioned bovine enamel surfaces. (a) reference surface (b) 5% PAA (c) 25% PAA and (d) phosphoric acid

Glasspoole et al.[21] found that the etch pattern produced by 10% PAA conditioning was shallower than which produced by phosphoric acid and also exposed enamel rods and interrod porosity. With 35% phosphoric acid, a characteristic etch pattern showing enamel rods and deep interrod porosity was exposed.



A B

Figure 9 SEM images of the enamel etch pattern after conditioning (A) 10% polyacrylic acid for 20 sec. and (B) 35% phosphoric acid for 15 sec.

Chapter III

RESEARCH METHODOLOGY

Population

Extracted human permanent teeth from orthodontic patients.

Sample size estimation

- The formula below was used to estimate the sample size for testing mean of two independent populations.

$$n = \frac{(\sigma_1^2 + \sigma_2^2)\left(Z_\alpha + Z_\beta\right)^2}{(\mu_1 - \mu_2)^2}$$

- From Cacciafesta et al. (σ_1 = 2.87, σ_2 = 2.0, μ_1 = 15.47, μ_2 = 19.75), at 95% confidence level (α = 0.05) and statistical power of 0.80 (β = 0.20), the calculated sample size was 5.24.
- In this study, the sample size was set at 10 samples per group. Therefore, there were 50 samples for the shear bond strength test.

Sample

Fifty-five extracted permanent premolar teeth from orthodontic patients at dental clinics in Bangkok were selected. The criteria for tooth selection included intact buccal enamel without cracks, fillings, caries and previously conditioning the tooth surface. Furthermore, teeth were extracted from the patients within 6 months. Teeth were stored in a 0.1 % (weight/ volume) thymol solution. Teeth were divided into 2 experimental parts with randomly allocation. Therefore, fifty and five teeth were selected for shear bond strength test and scanning electron microscope examination, respectively.

Variables

- Independent variables : Enamel conditioners
- Dependent variables : Shear bond strength (MPa)

Research equipments

- Chemical agents and adhesives
  1. 0.1% thymol solution
  2. 20% polyacrylic acid
  3. 37% phosphoric acid
  4. Self-etching primer (3M Unitex)
  5. Fuji ortho LC (GC, Japan)
  6. Transbond XT (3M Unitex)
- Materials
  1. Standard bracket for premolar teeth (Mini-diamond, Standard Edgewise Bracket, Ormco, Orange CA, USA) : The area of bracket base was 10.38 mm^2.
  2. PVC pipe (21 mm. in diameter and 1 inch in length).
  3. Clear plastic sheets
  4. 0.018" x 0.025" rectangular stainless steel guide wire
  5. Elastomer
  6. Self-cured acrylic
- Instruments
  1. Amalgam carver
  2. Bracket holder
- Machines
  1. Low speed cutting machine (ISOMET 1000, Buchler, USA)
  2. LED light curing unit (Elipar S10, 3M ESPE, St.Paul, MN, USA) with the power density of 1200 mW/cm^2
  3. Universal testing machine (EZ-S Test Series, Shimadzu Corp., Japan) for shear bond strength test.

4. Stereo microscope (SZ61 Series, Olympus) for evaluation of the remaining adhesive
5. Scanning electron microscope (JSM-5410LV)
6. Stop watch

Methodology

This study was divided into 2 parts.

1. Shear bond strength test and evaluation of the remaining adhesive
2. Scanning electron microscope examination

Shear bond strength test

- Sample preparation

In order to have the buccal surface paralleled to the force during the shear bond test, the teeth were prepared as follows:

1. The index A was prepared according to Padungvarasart and Techalertpaisam[22] method. Then, attached the two brackets to the border of PVC pipe (21 mm. in diameter and 0.5 centimeters in length) and used a guide wire (0.018" x 0.025" rectangular stainless steel) for guiding bracket position.

Figure 10 The index A

2. The roots of the teeth were cut 3 millimeters from cementoenamel junction by low speed cutting machine (Buchler, USA).
3. The buccal surfaces of the teeth were wrapped by a thin, clear plastic sheet.
4. The standard brackets (Mini-diamond, Standard Edgewise Bracket, Ormco, Orange CA, USA) were bonded on the clear plastic sheet at the middle third of buccal surface of the teeth with composite adhesive (Transbond XT, 3M

Unitex) without enamel conditioner and liquid resin, then light cured 10 seconds each at occlusal and ginigival aspects.

5. Attaching a guide wire of the index A to the bracket with O-ring. Then embedding the teeth in self-cured acrylic which was placed in a PVC pipes (21 mm. in diameter and 1 inch in length).
6. After acrylic was set, the plastic sheet and the bracket were removed.
7. Teeth were cleaned and polished by using non-fluoridated pumice and rubber prophylactic cups for 10 seconds and then rinsed with water. At the end, teeth were randomly divided into 5 groups. Each group consisted of 10 teeth.

Conditioners and adhesive usage

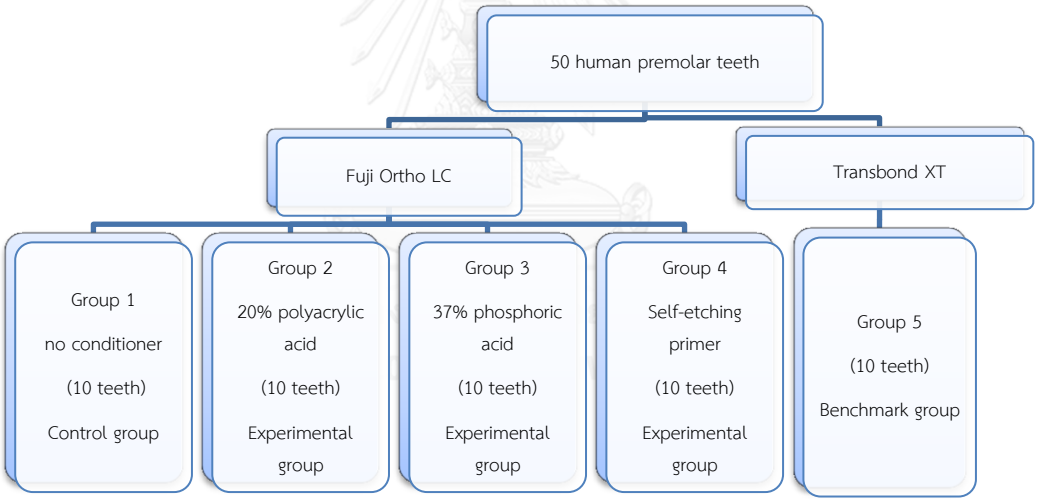


Figure 11 The conditioner and the adhesive of each group

Group 1 without conditioner was a control group. Group 2-4 were experimental group. In group 1-4, the brackets were bonded to the teeth with the conditioners according to the protocol of each group and bonded with RMGI adhesive (Fuji Ortho LC, GC, Japan) while group 5 (a benchmark group) was bonded with the conventional composite resin adhesive (Transbond XT).

Group 1: No conditioner, the excessive water was removed with a wet cotton roll.

Group 2: 20% polyacrylic acid was applied for 20 seconds, the tooth was then thoroughly rinsed with water for 20 seconds. The excessive water was removed with the wet cotton roll.

Group 3: 37% phosphoric acid was applied for 15 seconds, the tooth was then thoroughly rinsed with water for 20 seconds. The excessive water was removed with the wet cotton roll.

Group 4: Self-etching primer (Transbond plus SEP, 3M) was applied on the tooth surface for 3 seconds, and then gently evaporated with an air flow from a triple syringe.

For groups 1-4, the brackets were bonded with RMGI adhesive, mixing power and liquid (3:1 w/w) for 10 seconds. After that, adhesive was applied to the bracket base and brackets were placed at the middle third of buccal surface of teeth.

Group 5: 37% phosphoric acid was applied for 15 seconds, the tooth was then thoroughly rinsed with water and dried with air flow from a triple syringe. Then, liquid resin (Transbond XT primer) was applied on the tooth. After that, Transbond XT adhesive was applied to the bracket base and the bracket was placed at the middle third of the buccal surface. Lastly, the excessive adhesive was removed by an amalgam carver.

Then, all brackets were photopolymerized with a LED light curing unit (Elipar S10, 3M ESPE, St. Paul, MN, USA) for 10 seconds on occlusal aspect and 10 seconds on gingival aspect of the bracket with the power density of 1200 mW/cm^2.

Debonding procedure

After the brackets were bonded to the teeth for 5 minutes, Shear bond strength test was implemented by Universal testing machine (EZ-s Series, Shimadzu Corp., Japan). Then, an occlusogingival load with 1 mm/min speed was applied to the bracket. The required load for debonding was recorded in newton and was converted into megapascal as a ratio of newton to surface area of the bracket. The area of bracket base was 10.38 mm^2.

Evaluation of the residual adhesive

After bond failure, the buccal enamel surfaces were examined by the same operator under the stereo microscope at x10 magnification. The adhesive remnant index (ARI) was used to assess the amount of adhesive left on the enamel surface. The ARI scale was scaled between 1 and 5

Scale 1 indicates all of the adhesive remained on the enamel surface

Scale 2 indicates more than 90% of adhesive remained

Scale 3 indicates more than 10% but less than 90% of adhesive remained

Scale 4 indicates less than 10% of adhesive remained

Scale 5 indicates no adhesive remained on the enamel surface.

All samples were examined twice, 2 weeks apart, by the same investigator. If the area of adhesive remnant cannot be indicated to the scale, the area of adhesive remnant will be measured by Image J version 1.47.

Scanning electron microscope examination

Five premolar teeth were prepared for examination. The roots of the teeth were cut 3 millimeters from cementoenamel junction by a low speed cutting machine. Teeth were cleaned and polished with non-fluoridated pumice and rubber prophylactic cups for 10 seconds and rinsed with water. Then, the teeth were randomly divided into 5 groups which the same as in shear bond test and the brackets were bonded. The bonded teeth were embedded with acrylic resin. Then, the teeth were longitudinally sectioned into halves in the buccal-palatal direction, through the center of the orthodontic bracket, with a water-cooled and double-sided diamond disk. All specimens were soaked in 2 mole/L HCL for 20 seconds.[23] The cross-sections were then dried and their surfaces were sputtered with gold using a Quick Auto Coater. These specimens were studies under the SEM (scanning electron microscope: JSM-5410LV).

The microhardness test was in appendix section.

Statistical Analysis

- Shear bond strength test

1. Descriptive statistics including mean, standard deviation, minimum and maximum values were calculated for all five tested groups.
2. The data were tested to normality by the Kolmogorov-Smirnov method and were tested to homogeneity of variances by Levene's test**.**
    a. If the data were normal distribution and equal variances, the 1-way analysis of variance (ANOVA) was used to determine significant differences among the various groups. If the significant differences were found, the LSD test was tested to identify which groups were different.
    b. If the data were normal distribution and unequal variances, the Welch ANOVA was used to determine significant differences among the various groups. If the significant differences were found, the Games-Howell test was tested to identify which groups were different.
    b. If the data were not represented as a normal distribution, the median test was used to determine significant differences among the various groups. If significant differences were found, the non-parametric t-test was used to identify which groups were different.

- Evaluation of the residual adhesive

The chi-square test was used to determine significant differences in the ARI scores among the different groups. Significance for all statistical tests was predetermined at $p<.05$.

Ethical consideration

In this study, the teeth were consented from dentists who were the dental clinic owners. The Human Research Ethics Committee of the faculty of Dentistry, Chulalongkorn University has approved this study to be carried out according to the protocol and patient/participant information sheet dated and/or amended in compliance with the ICH/GCP with exemption.

Chapter IV
RESULTS

Shear bond strength test

The descriptive statistics for the shear bond strength of the five groups including means, standard deviations, minimum and maximum values were shown in Table 1. The Kolmogorov-Smirnov test (Table 5) showed normal distribution in each group. The Levene's test (Table 6) showed unequal variances. The Welch ANOVA (Table 7) showed statistically significant differences in the shear bond strength among the 5 groups ($p<0.05$). From Table 8, the Games-Howell test showed that the shear bond strength of group 3 was conditioned with phosphoric acid and group 4 was conditioned with self-etching primer were not statistically significantly different ($p=0.328$). Significant differences were found between group 1 and 2 ($p=0.020$), group 1 and 3 ($p<0.05$), group 1 and 4 ($p<0.05$), group 1 and 5 ($p<0.05$), group 2 and 3 ($p=0.004$), group 2 and 4 ($p=0.001$), group 2 and 5 ($p<0.05$), group 3 and 5 ($p=0.010$), group 4 and 5 ($p=0.001$).

The adhesive remnant index scale for the 5 groups is shown in Table 2. The chi-square test (Table 9) results showed significant differences among the various groups ($p<0.05$). Group 1, which there was no conditioner, showed higher frequency of ARI scale 5 which indicated that no adhesive remained on the enamel surface. Group 2, which the teeth were conditioned with polyacrylic acid, showed higher frequency of ARI scale 4 which indicated that less than 10% of adhesive remained on the enamel surface. Group 3, which the teeth conditioned with phosphoric acid, showed higher frequency of ARI scale 1. This indicated that all adhesive remained on the enamel surface. Group 4, which the teeth were conditioned with self-etching primer showed higher frequency of ARI scale 2 which indicates more than 90% of adhesive remained on the enamel surface. Group 5 (control group) showed higher frequency of ARI scale 1 the same as group 3.

Table 1 Descriptive statistics in megapascal (MPa) of shear bond strength in each group.

Group	N	Mean	SD.	Min	Max	LSD*
1 no conditioner + Fuji ortho LC	10	8.4	2.0	5.6	11.1	a
2 Polyacrylic + Fuji ortho LC	10	10.8	0.7	9.5	11.7	b
3 Phosphoric + Fuji ortho LC	10	14.6	3.0	9.1	18.2	c
4 SEP + Fuji ortho LC	10	13.7	1.7	12.2	17.0	c
5 Phosphoric + Transbond XT	10	21.5	4.0	16.9	28.2	d

*LSD grouping. Means with same letter are not significantly different.

Table 2 Adhesive remnant index after debonding

Group	Adhesive Remnant Index (ARI) Scale*					N
	1	2	3	4	5	
1 no conditioner / Fuji ortho LC	-	-	1	1	8	10
2 Polyacrylic acid 20% / Fuji ortho LC	-	-	2	6	2	10
3 Phosphoric acid 37% / Fuji ortho LC	5	1	1	3	-	10
4 Self-etching primer / Fuji ortho LC	4	5	-	1	-	10
5 Phosphoric acid 37% / Transbond XT	6	3	1	-	-	10

*The ARI scale ranges from 1 to 5,

with 5 indicating that no adhesive remained on the enamel;

4, less than 10% of adhesive remained on the tooth surface;

3, more than 10% but less than 90% of the adhesive remained on the tooth;

2, more than 90% of the adhesive remained; and

1, all of the adhesive remained on the tooth

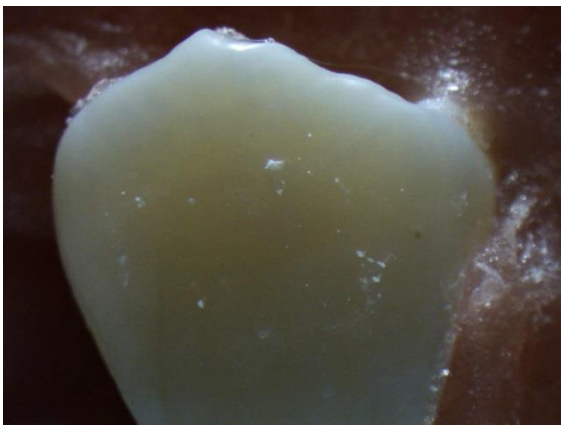


Figure 12 Scale 5 indicates that no adhesive remained on the enamel

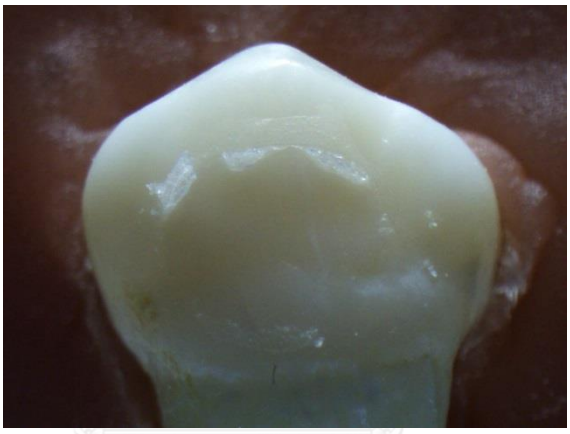


Figure 13 Scale 4 indicates that less than 10% of adhesive remained on the tooth surface

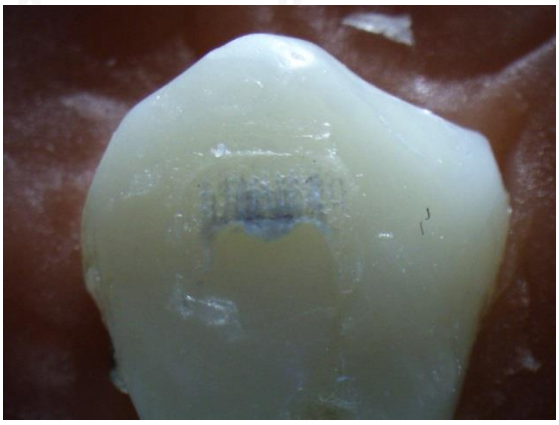


Figure 14 Scale 3 indicates that more than 10% but less than 90% of the adhesive remained on the tooth surface

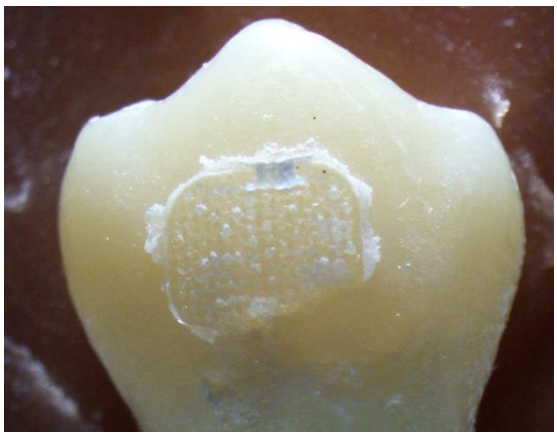


Figure 15 Scale 2 indicate that more than 90% of the adhesive remained; and

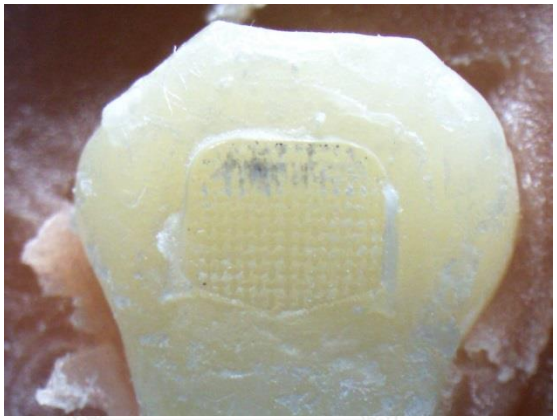


Figure 16 Scale 1 indicates that all of the adhesive remained on the tooth

Scanning electron microscope examination

SEM images of junction between the enamel and adhesive were showed in Fig 17. Group 1, which was without conditioner, showed the enamel-adhesive interface without resin tag (Fig 17, A). Group 2, which the teeth were conditioned with 20% polyacrylic acid, showed smaller amount of resin tags and less penetrated into the enamel (Fig 17, B). Group 3, which the teeth were conditioned with 37% phosphoric acid, showed larger amount of resin tags (Fig 17, C). The shape of these resin tags were thinner than they were found in group 4 which the teeth were conditioned with self-etching primer. In group 4 (Fig 17, D), the shape of resin tags were bigger and thicker than they were found in the other experimental groups. Group 5 (Fig 17, E) which the teeth were conditioned with 37% phosphoric acid , applied liquid resin and bonded with composite resin, showed the most amount of

resin tags. These tags were the longer and bigger than all experimental groups which were bonded with RMGI.

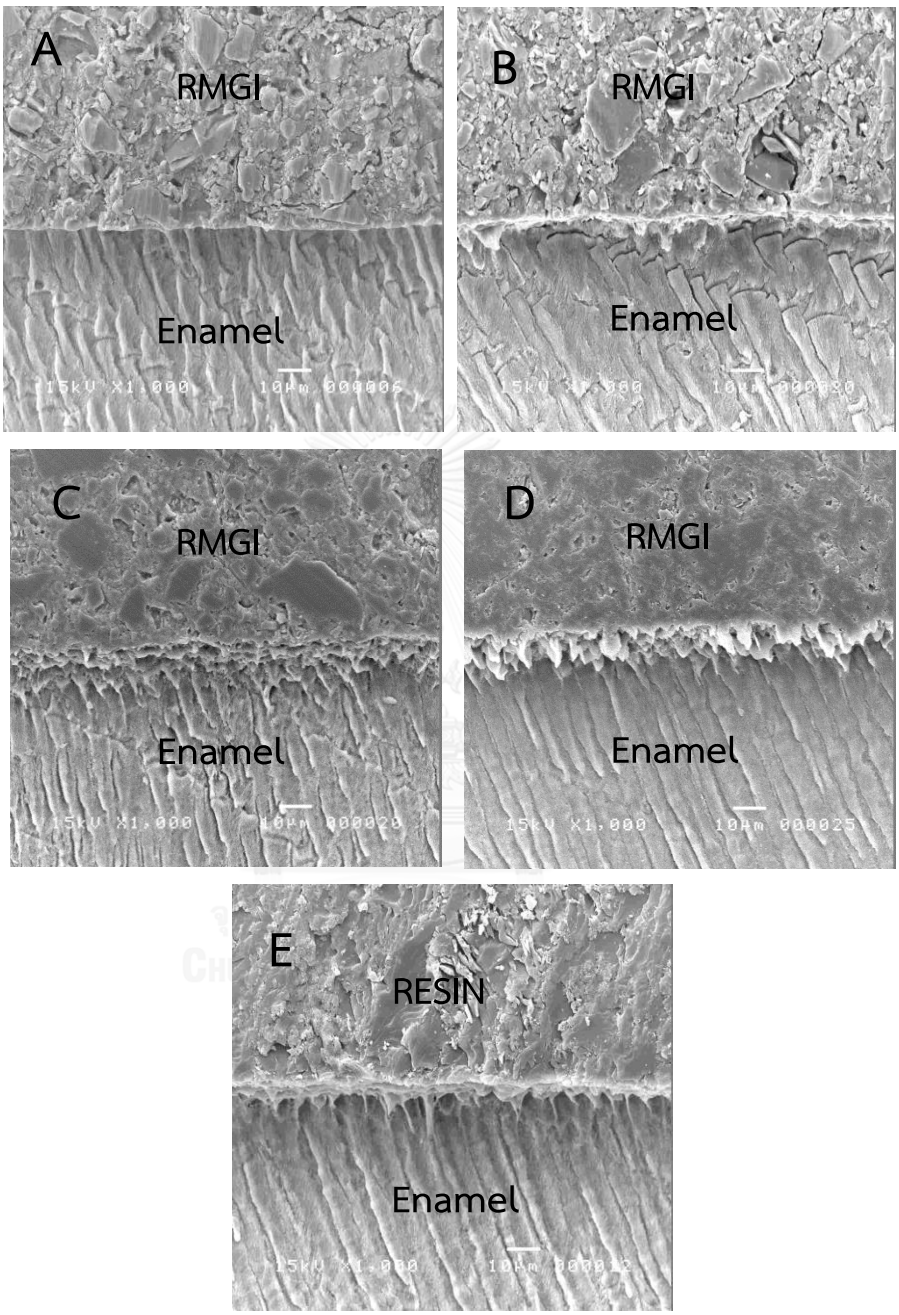


Figure 17 Scanning electron micrographs of the specimens from experimental groups (A-D) conditioned with (A) no conditioner, (B) 20% polyacrylic acid (C) 37% phosphoric acid and (D) self-etching primer, the brackets bonded RMGI adhesive and the control group (E) conditioned with 37% phosphoric acid and the bracket bonded with composite resin.

Chapter V
DISCUSSION AND CONCLUSION

Discussion

Resin-modified glass ionomer can release fluoride over a long-term for reducing white spot lesion.[24-26] The amount of fluoride releasing in RMGI was greater than in compomer, fluoride-releasing composite resin, and conventional composite resin, respectively.[25, 27, 28] Fluoride releasing adhesives increased plaque fluoride concentration but did not altered salivary or urinary fluoride concentration. Therefore fluoride released is likely to exert a local and not a systemic effect.[29] Furthermore, fluoride recharge treatments such as 1000 ppm NaF solution or acidulated phosphate fluoride gel (APF) increased amount of fluoride ion released for 2 days and then returned to the levels that was found before fluoride application was taken. However, the amount of fluoride ion released after topical fluoride treatment was proportional to the amount of fluoride ion previously released from the adhesives.[30] Fluoride-releasing materials showed a "burst-effect" fluoride release pattern. For instance, the greatest amount of fluoride was released within the first few days. With a rapid decline to lower levels, it is important to examine the usefulness of these materials as fluoride reservoirs during the average orthodontic treatment time of 2 to 3 years by determining the fluoride-rerelease pattern after exposure to additional fluoride[31]. In addition, using RMGI showed a better reduction of enamel demineralization around orthodontic bracket than using composite resin. The demineralization was assessed by microhardness test and polarized light microscope examination.[4, 32, 33] The manufacturer recommended that the conditioner for Fuji Ortho LC is polyacrylic acid which could remove surface contaminants and increased wettability of material. However, Bishara et al.[14] found that the bond strength of RMGI was lower than the minimum bond strength (6-8 MPa) which was adequate for clinical orthodontic needs.[13]

In this study, we changed the enamel conditioners to increase the initial shear bond strength of RMGI. The results of each group showed that any conditioner was

adequate for clinical orthodontic needs.[13] In group 1, which was no conditioner, showed the lowest shear bond strengths (8.4 ± 2 MPa). This represented the chemical bond between the carboxyl groups (COO^-) of a glass ionomer adhesives and calcium of hydroxyapatite. In group 2, which the teeth were conditioned by 20% polyacrylic acid, showed significantly increased the shear bond strengths (10.8 ± 0.7 MPa). In group 3 and 4 which the teeth were conditioned by 37% phosphoric acid and self-etching primer respectively, showed significantly increased the value of shear bond strengths (14.6 ± 3 and 13.7 ± 1.7 MPa). Therefore, the conditioners from last two groups gave an effective result as represented in the shear bond strength values.

When the results of shear bond strength (SBS) were compared to previous studies, SBS value of polyacrylic group was higher than the results of Bishara et al.[14] (3.3 ± 2.6 MPa) at 30 minutes, Newman et al.[34] (7.9 ± 2.3 MPa) at 14 days and Reicheneder et al.[35] (6.8 ± 1.3 MPa) at 24 hours but lower than Cacciafesta et al. [36] (13.1 ± 2.6 MPa) at 24 hours. SBS value of phosphoric group was close to the results of Cacciafesta et al. [36] (15.5 ± 2.9 MPa) but higher than the results of Bishara et al.[14] (6.1 ± 2.7 MPa), Chung et al.[37] (5.3 ± 2.5 MPa) at 24 hours and J Godoy-Bezerra et al.[38] (3.88 ± 0.54 MPa) at 250 minutes. In-vitro bond strength test showed a variable result as it was depended on test conditions such as enamel origin (bovine vs human)[39], substrate storage (artificial saliva or water)[40, 41], crosshead speed[40], intensity of light device[42, 43], polymerization time[44], thermocycling[45] and type of bracket.[46] From above studies, SBS value of phosphoric acid and self-etching primer group were higher than polyacrylic acid group.

The SBS value of self-etching primer group (13.7 ± 1.7 MPa) was slightly lower than phosphoric group (14.6 ± 3 MPa). From previous studies, the results of self-etching primer group were lower than phosphoric group in composite resin adhesives.[47-49] However, group 5 (21.5 ± 4 MPa), which the teeth were conditioned by phosphoric acid and attached with composite resin adhesive, was the highest SBS. This showed that the bond strength value from composite resin adhesive was higher than from RMGI adhesive.

The manufacturer recommended that the tooth surface which was attached with RMGI should be moist to improve the acid dissociation during acid-base reaction. Cacciafesta et al.[36] found that the dry or moist enamel surface influenced the SBS value. In no conditioner group, SBS value in moist enamel was higher than dry enamel significantly. In phosphoric acid group, SBS value in dry enamel was higher than moist enamel significantly. In polyacrylic and self-etching primer group, the dry or moist enamel surface did not influence SBS value. Therefore, the dentist should consider the type of conditioner before selecting moist or dry enamel surface.

In orthodontic bond strength test, the shear bond strength (SBS) has been widely used and more popular than the tensile bond strength (TBS) because SBS was a vertical force which was similar to the applied force during chewing the foods. On the other hand, the TBS was similar to the debonding force during removing the brackets or was studied the bond strength of adhesive and self-ligating bracket. Reicheneder et al.[35] found that SBS value of RMGI showed lower than TBS insignificantly. Jassem et al.[50] found that TBS value versus SBS value were not significantly different. Cheng et al.[51] found that TBS value of phosphoric group showed 13.2 ± 5.9 MPa which was resemble to the SBS value of this study (14.6 ± 3 MPa).

From the past studies, SBS was “Macro” bond strength. These tests were performed in specimens with relatively large bonded area, which was usually 7-28 mm^2. The advantages in shear tests include easy performing, minimal equipment requirement and specimen preparation. After that, “Micro” bond strength was widely used for elimination of tooth dependency through balanced designed and showed reduced test variance. The surface area of specimens was usually very small, approximately 1 mm^2, in order to reduce cohesive failures and increase adhesive failures. The versatility of this method to evaluate clinically relevant sites and substrates was comprehensively detailed and critiqued.[52, 53] In addition, micro bond strength tended to be much higher than that of macro bond strength because the defect concentration in the small cross-sectional interfacial areas was lower.[54] However, micro bond strength cannot test for orthodontic adhesive since brackets

could not be cut into very small pieces. Therefore, this method was not popular for orthodontic study.

It was found that enamel loss during acid conditioning depended on the type of conditioner. Enamel loss showed greater in phosphoric acid group, less in self-etching primer group and the lowest in polyacrylic acid group. Es-Souni et al.[20] found that the roughness of enamel in polyacrylic group was between 11 and 13 nm. Enamel loss was between 10-30 μm in phosphoric acid group.[55, 56] Hosein et al. [57] found that enamel loss was 1.1-4.6 μm and 0.03-0.7 μm in phosphoric acid group and self-etching primer group, respectively. Van Meerbeek et al.[58] found that self-etching primer penetrated to enamel for 5 μm. This related to SEM examination that enamel loss in phosphoric acid group greater than in self-etching primer group.[19] Bishara et al.[14] found that the phosphoric acid etch produced a much deeper etch (rougher enamel surface) than the polyacrylic acid conditioning. Moreover, Phosphoric acid reduced surface hardness of enamel significantly.[4]

Altering type of enamel conditioners related to ARI scale. The experiment was to monitor the remaining adhesive on the enamel surface after debonding. The results of ARI was similar to the previous study.[36] In group without conditioner, greater incidence of no adhesive on enamel surface indicated that the bond strength value at enamel-adhesive interface was weaker than bond strength at bracket-adhesive interface. Moreover, the adhesive remaining on enamel showed greater in polyacrylic acid group, self-etching primer group and phosphoric acid group, respectively which related to the increased of SBS value.

When adhesive remnant scales were compared between types of adhesive, the remaining adhesive in RMGI group lesser than in composite resin group. These result was similar to the study of Lee et al.[59] They found that the amount of adhesive remaining in RMGI group (0.6 ± 0.6 mm^3) was smaller than in composite resin group (1.4 ± 0.9 mm^3). Similarly, Shamsi et al.[60] found that the remaining adhesive thickness in RMGI group (31.2 ± 26.5 μm) was thinner than in composite resin group (102.7 ± 79.7 μm). Therefore, the smaller amount of adhesive remained on enamel in RMGI group was an advantage since it was implied that time

consumption for cleaning the teeth by the operator was decreased and it could minimize the chair time.

From the SEM examination, the shapes of resin tags of self-etching primer group were larger and greater amount than the other experimental groups. In contrast, the SBS value of self-etching primer group was not higher than phosphoric group. This result was similar to the study of Shinchi et al.[23] which found that the length of the resin tags contributed little to the tensile bond strength of the test specimens. Therefore, the length of resin tags was not the major factor which influenced the bond strength between adhesive and enamel. However, Fjeld and Ogaard[61] found that the enamel surfaces exposed to 10% polyacrylic acid and RMGI adhesive showed no visible tag. The enamel surface after etching with phosphoric acid and bonding, the SEM images showed many long and thick resin tags that had penetrated into the enamel with most tags' length about 10 to 20 μm. The surfaces treated with the self-etching primer and bonding showed thin resin tags, but they were fewer and shorter than those seen when the teeth were exposed to phosphoric acid. Most tags' length was 5 to 10 μm. In this study, the lengths of resin tags of in phosphoric group were shorter than in SEP group because RMGI adhesive did not apply the liquid resin after conditioning. When this procedure was absence, the resin penetration was worse because the liquid resin had a low viscosity that provided high penetration properties. Dongpaiboon and Techalertpaisarn[62] found that the enamel-adhesive interface of groups without liquid resin represented less penetration of resin than those with liquid resin. But there was no difference between SBS of the liquid resin group and that of no liquid resin group. Their study also confirmed the less importance of the length of resin tags.

Conditioning with SEP showed higher SBS value than conditioning with polyacrylic acid according to manufacturer's instruction significantly. Advantage of SEP is to have less amount of enamel loss compared to phosphoric acid. It also showed that SBS value was closely to phosphoric acid. Furthermore, debonding process after using phosphoric acid will increase the risk of enamel damages such as enamel cracks, fractures, splits and cusp fractures.[63] However, SEP provided a higher

enamel loss than polyacrylic acid. Therefore, SEP was a good choice for operator to choose before bonding orthodontic bracket with RMGI adhesive.

Conclusion

1. The types of conditioner give an effect on the shear bond strength. Fuji Ortho LC used after conditioning with SEP and 37% phosphoric acid showed the higher shear bond strength than 20% polyacrylic acid group and group without enamel conditioning.
2. The SBS value of negative control group (Transbond XT) was significantly higher than the other experimental groups (Fuji ortho LC).
3. The ARI study showed that the predominant bracket failure interface for group without enamel conditioner and 20% polyacrylic acid group was at the enamel-adhesive interface and for SEP and 37% phosphoric acid was at the bracket-adhesive interface.
4. The SEM study showed that conditioning with SEP on enamel produced many and larger resin tags than the other experimental groups.

Clinical implication

From the results of this study, it revealed that self-etching primer and phosphoric acid was capable for improving initial shear bond strength of RMGI. The dentist should consider this conditioners and adhesive as another option for bonding the bracket in poor oral hygiene patient.

Suggestion

- The results of this conditioner experiment only affected to one adhesive, therefore it could not refer to other adhesives which should do a further study.
- The residual adhesive on enamel was examined by the stereo microscope which could not measure the amount or the thickness of adhesive. For the

future studies, the researcher should implement the test on three-dimensional measurement.

- As for a further study of shear bond strength, the method of sample preparation should be modified by adding the second bonding brackets. The index A should be placed for the same brackets position as the first bonding the brackets.

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APPENDICES

Appendix A

Microhardness Test

The advantages of using proper conditioner for RMGI are not only to provide high initial shear bond strength, but also decrease enamel loss. Therefore, the pilot study of microhardness is tested for determine the effects of the conditioners on hardness of enamel.

Literature Review

Microhardness was one of the most important physical characteristics for a comparative study of dental materials and was used for evaluating enamel demineralization. Pascotto et al.[64] studied *in vivo* effect of the RMGI adhesive on reducing enamel demineralization around orthodontic brackets. Orthodontic patients were divided into 2 groups which were bonded with composite resin adhesive (control group) and RMGI (experimental group). After bonding the brackets for 30 days, the teeth were extracted and microhardness test was used to evaluate enamel demineralization. As a result, the RMGI group was statistically more efficient for reducing enamel demineralization than the composite resin group.

Moura et al.[32] studied *in vivo* effects of fluoridated antiplaque dentifrice and bonding material on enamel demineralization adjacent to orthodontic appliances. Orthodontic patients were divided into 2 groups. One groups brushed 3 times a day with a fluoridated dentifrice and the other group used an experimental antiplaque fluoridated formation. The bracket were randomly bonded with RMGI or composite resin to buccal surface of the maxillary right and left premolars in each subject. After 28 days of dentifrice use, the teeth were extracted, and enamel loss in enamel adjacent to the bonded material was assessed by cross-sectional microhardness. As a result, mineral loss in the enamel surrounding the bond with the RMGI cement was lower than with the composite resin adhesive.

Uysal et al.[65] studied the effect of an antibacterial monomer-containing self-etching adhesive in reducing enamel demineralization around orthodontic brackets *in vivo* and compared it with the conventional adhesive. Orthodontic patients were divided into 2 groups which were bonded with Clearfil Protect Bond (experimental group) and Transbond XT (control group). Demineralization was assessed by cross-sectional microhardness. Determinations were made at the bracket edge cementing limits at occlusal and cervical points, which were 100 and 200 microns away from the edge. At all of these positions, 6 indentations were made at depths of 10 to 90 microns from the enamel surface. The results indicated that using antibacterial monomer-containing adhesive for bonding orthodontic brackets successfully inhibited caries *in vivo*. This cariostatic effect was localized at the area around the brackets and was significant after 30 days.

Method of microhardness test

Hardness test is done by applying a standardized force or weight to an indenter. This produces a symmetrically shaped indentation that can be measured under microscope for area or width of the indentation produced. The indentation dimensions are related to hardness value. With a fixed load applied to a standardized indenter, the dimensions of the indentation vary inversely with the resistance of penetration of the tested material.

Microhardness test has two indenter types, i.e. Knoop indenter and Vickers indenter. Both indenters are suitable for surface hardness testing of non-metallic materials. Knoop indenter is rhomboid-shaped with an approximate ratio between long and short diagonals of 7:1 (only the long diagonal is used for hardness value calculations). The Vickers indenter is square-shaped (both diagonals are used in the calculation of hardness values), whereas Lippert and Lynch [58] reported that there was indifferences between the test results of Knoop and Vickers indenters on surface microhardness in their ability to measure enamel demineralization. Therefore, this research selected Knoop indenter for testing the microhardness of conditioned enamel.

A B

Figure 18 Knoop indentor (A) and Vicker indentor (B)

With Knoop indenter method, the load can be varied to test such as their indentation area and the nature of the materials. The advantage of this method is that materials with a great range of hardness can be tested simply by varying the test load. The main disadvantages of the method are the need for highly polished and precisely flat test specimen and the time required to complete the test operation, which is considerably greater than that required for some other less precisely controlled methods.[10]

Objectives

1. To compare the effect of different enamel conditioners on microhardness of enamel.
2. To compare microhardness of enamel in baseline and post-conditioning.

Research Questions

1. Does the type of enamel conditioners effect on the microhardness value of enamel?
2. Is the microhardness of enamel different in baseline and post-conditioning?

Research Hypotheses

1. The type of enamel conditioners of resin-modified glass ionomer adhesive effect on the microhardness.
2. The microhardness of enamel between baseline and post-conditioning is different.

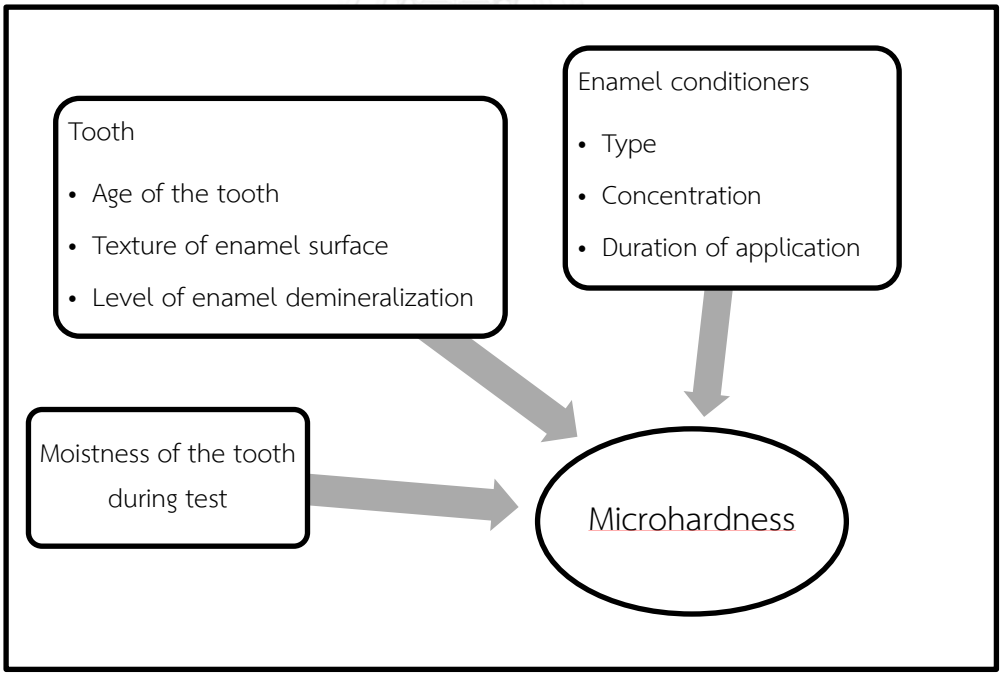
Sample size

Sample size estimation formula for testing mean of two independent population was calculated as below.

$$n = \frac{(\sigma_1^2 + \sigma_2^2)\left(Z_\alpha + Z_\beta\right)^2}{(\mu_1 - \mu_2)^2}$$

- Using data from study of Pascotto et al. (σ_1 = 15.6, σ_2 = 13.1, μ_1 = 358.3, μ_2 = 348.9) at a 95% confidence level (α = 0.05) and statistical power of 0.80 (β = 0.20), the calculated sample size is 6.37.
- In this study, The sample size is set at 8 samples per group. With 4 conditioners, 32 samples are required for the microhardness test.

Conceptual Framework



Research Methodology

Population

Human permanent premolar teeth.

Sample

Eight extracted permanent premolar teeth from orthodontic patients who are 11-16 years old were selected for the test. The criteria for tooth selection included intact buccal enamel without cracks, fillings and caries. Teeth were stored in a 0.1 % (weight/ volume) thymol solution.

Material and methods

The root of the tooth was cut by 3 millimeters from cementoenamel junction with a low speed cutting machine. Each tooth was divided into 4 sections in buccolingual direction with water-cooled, double-sided diamond disk. Each section of the tooth was distributed into each group (Group 1-4) in clockwise rotation as described by Figure 19. These sections were polished with 3 grades of abrasive paper discs (320, 600 and 1200 grit) and final polishing was done with a 1-μm diamond spray and a polishing cloth disc. All samples were kept in distilled water at room temperature prior to testing.

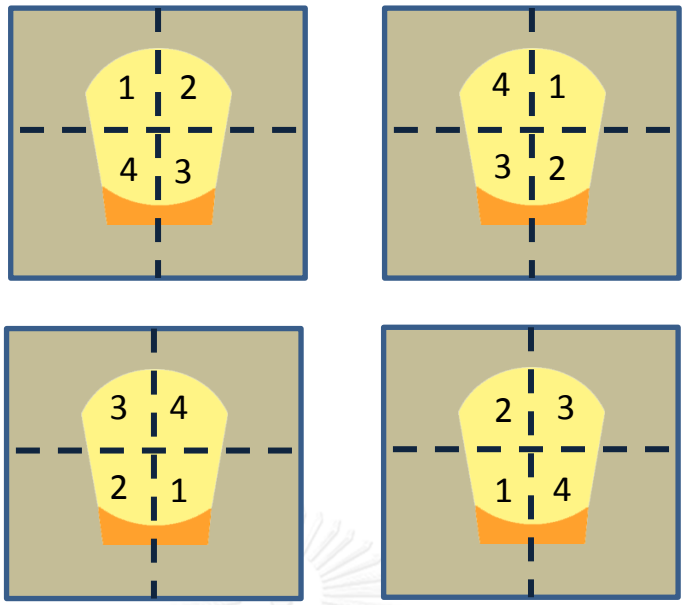


Figure 19 Each tooth was divided into 4 sections
and each section was distributed to each test group in a clockwise rotation.

Baseline microhardness measurements were performed by using Knoop indentor on microhardness tester (FM 700e TYPE D Future-tech, Japan). Before each test, the samples were dried with air flow from triple syringe for one minute. The indentation load was 50 grams for 5 seconds dwell time. Three indentations were made at 10, 20 and 30 microns from the external surface of enamel, near center point of buccal crown, according to figure 20.

After that, teeth were cleaned and polished with non–fluoridated pumice and rubber prophylactic cups for 10 seconds and rinsed with water. Conditioners according protocol of each group as below

Group 1: No conditioner

Group 2: 20% polyacrylic acid was applied for 20 seconds, the tooth was then thoroughly rinsed with water for 20 seconds.

Group 3: 37% phosphoric acid was applied for 15 seconds, the tooth was then thoroughly rinsed with water for 20 seconds.

Group 4: Self-etching primer (Transbond plus SEP, 3M) was applied on the tooth surface for 3 seconds, and then gently evaporated with an air flow.

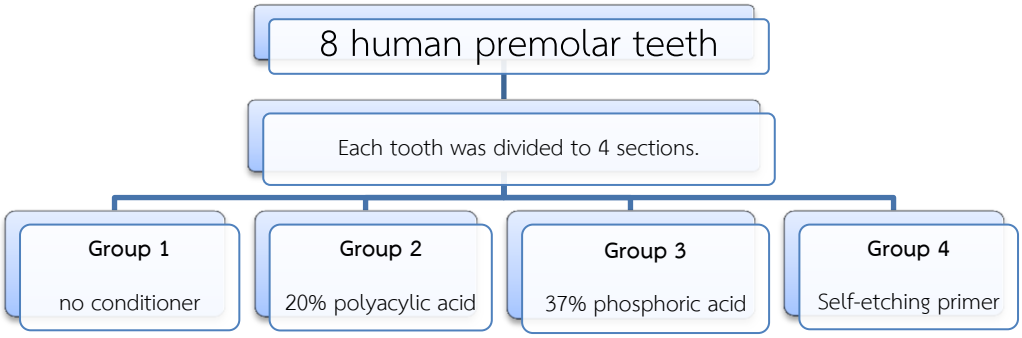


Figure 20 The type of conditioners was used for each group.

After conditioning the buccal surface of samples, each sample was dried with a triple syringe for one minute. The post-conditioning microhardness measurement was measured at 10, 20 and 30 microns from external surface of enamel and 300 microns far from baseline indentation vertically, according to figure 21.

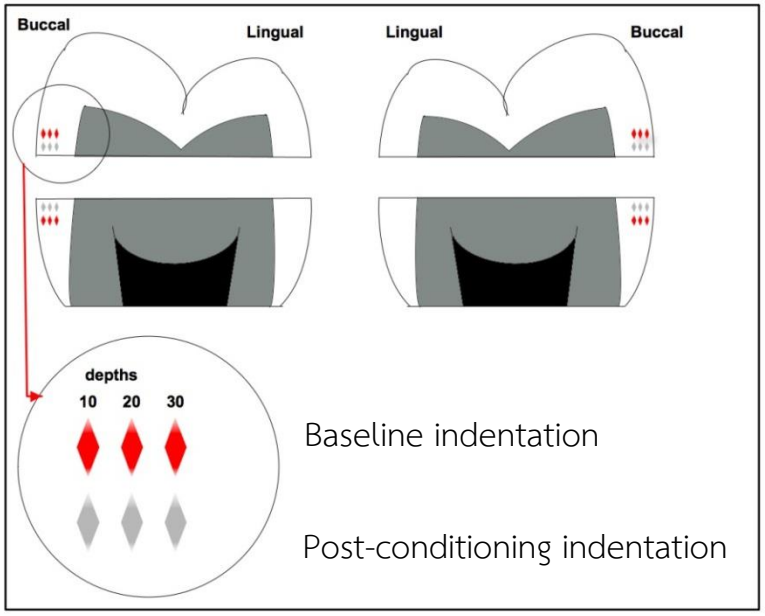


Figure 21 Baseline indentation and post-conditioning indentation were made at 10, 20 and 30 microns from the external surface of enamel

Statistical Analysis

- Descriptive statistics including mean, standard deviation and minimum and maximum values were calculated for all groups.
- The post-conditioning data were tested to normality by the Kolmogorov-Smirnov method and were tested to homogeneity of variances by Levene's test**.**
    c. If the data were normal distribution and equal variances, the 1-way analysis of variance (ANOVA) was used to determine significant differences among the various groups. If the significant differences were found, the LSD test was tested to identify which groups were different.
    d. If the data were normal distribution and unequal variances, the Welch ANOVA was used to determine significant differences among the various groups. If the significant differences were found, the Games-Howell test was tested to identify which groups were different.
    e. If the data were not normal distribution, the median test was used to determine significant differences among the various groups. If a statistically significant was found, the non-parametric t-test was used to identify which of the groups were different.
- Paired t-test was used to evaluate the effect of conditioners before and after conditioning the enamel based on different depth values from the enamel surface (10, 20 and 30 μm). Significance for all statistical tests was predetermined at $p < .05$.

Results

The descriptive statistics for Knoop Hardness Number (KHN) of the four groups including means, standard deviation, minimum and maximum values were shown in Table 3. The Kolmogorov-Smirnov test (Table 10) showed normal distribution in each group. From post-conditioning data, Levene's test showed equal variance in 20 μm groups and unequal variances in 10 μm and 30 μm groups. Analysis of variance (One-

way ANOVA) (Table 12) showed no statistically significant differences in KHN among the 4 groups in 20 μm groups (p=0.905) and Welch ANOVA showed no statistically significant differences in 10 μm (p=0.57) and 30 μm (p=0.291) groups.

Paired t-test (Table 13) showed significant differences in KHN between baseline and after conditioning in group 3 at 30 μm (p=0.043) and group 4 at 10 μm (p=0.042), and were not significant differences in the other groups.

Table 3 Mean and standard deviation of Knoop Hardness Number (KHN)

Knoop Hardness Number (KHN)

		Baseline			Post conditioning		
Group		10 μm	20 μm	30 μm	10 μm	20 μm	30 μm
1	Mean	174.9	257.8	308.0	204.1	275.3	297.7
	SD.	52.0	59.8	89.0	102.5	106.9	114.0
	Minimum	72.6	188.2	204.9	83.5	158.7	136.2
	Maximum	227.8	365.3	435.9	369.3	447.1	444.9
2	Mean	128.8	318.5	363.4	158.8	312.2	352.0
	SD.	67.3	102.5	72.4	92.2	59.8	54.1
	Minimum	73.9	200.1	273.0	60.9	201.4	262.9
	Maximum	267.0	476.8	504.0	303.2	402.7	417.5
3	Mean	214.7	291.5	324.3*	184.0	287.1	282.9*
	SD.	116.6	89.0	72.7	91.6	104.7	83.7
	Minimum	64.2	148.3	203.6	57.8	80.1	147.8
	Maximum	364.2	464.2	420.2	299.7	455.1	414.5
4	Mean	139.0*	306.7	329.2	267.2*	278.8	333.8
	SD.	89.9	126.1	82.9	200.5	148.1	140.6
	Minimum	35.7	80.4	206.5	62.5	145.9	143.5
	Maximum	278.9	475.8	422.4	564.5	548.7	504.3

* represent that baseline and post conditioning value are significantly different.

Discussion

Enamel microhardness can be indirect indicator of minerals (calcium and phosphate) in enamel. Knoop hardness number has been correlated with volume percent mineral of enamel. The Knoop indenter with 50 g load used in the present study is similar to a previous study[4, 32] where surface hardness measurement was used to identify the effect of RMGI adhesive on enamel demineralization around orthodontic bracket. In addition, this load was selected because of the appropriate size of indentations for accurate measurement with the available equipment.

In this study, Baseline microhardness values for enamel were measured between 35.7 and 504 KHN. These values are different to the studies of Tantbirojn et al.[66] which microhardness value of enamel was ranged between 244 and 337 KHN and the studies of Wongkhantee et al. [67] which microhardness value of enamel were ranged between 260 and 279. In each group, the standard deviations were higher than the previous studies because the specimen preparation was not ideal or other factors influence to the test results such as the moistness of the teeth.

Meredith et al. found that the Knoop hardness value of enamel decreased from the outer surface to the dentinoenamel junction. In their studied, the outmost indentation was 300 micron from the outer enamel surface and adjacent indentations were made at 300 micron apart from the previous indentation. In constrast to this study, baseline microhardness values at 10 micron were lesser than at 20 and 30 micron. Therefore, the KHN of outer enamel surface was lesser than this value of inner enamel surface. The reasons for this contrast may from the first indentation was very closely to outer enamel surface. Besides, microhardness values vary with tooth side (buccal, central, and lingual or occlusal and gingival). Therefore, in this study, the indentations of each tooth were test closely to the center of buccal surface in order to get same baseline value.

The microhardness measurement was inaccurate because some indentations resulted in cracking and enamel chipping. These damage was presented due to the indentations were very closely to outer surface of enamel. In addition, the plane of surface were slightly inclined so the indentation were not symmetry, the base of

specimens were not in the same plane so the specimen were tilt during the test and the surface were not highly polish so the indentation were not clear.

Conclusions

- The microhardness of enamel was not differed by the altering types of enamel conditioner.

Limitation

Microhardness tests can be performed only on precisely flat, highly polished surfaces. This necessitates grinding and polishing the enamel surface before testing. In the specimens prepared process, researcher intended to have the same baseline microhardness value in all group, but when the samples were polished so that it would be smaller than the original size. Therefore, the baseline value were not the closely value for each tooth.

Obstacles and strategies

- Humidity of sample effect on the hardness therefore all sample should be dried by air flow for one minute before the test.
- Level of demineralization effect on the hardness therefore this study select the teeth from the orthodontic patients who are 11-16 years old.

Expected Benefit and Application

To select the type of conditioner which provides high shear bond strength and less enamel loss.

Appendix B

Figure 22 Research equipment: Chemical agents and adhesives were used in this study

20% polyacrylic acid

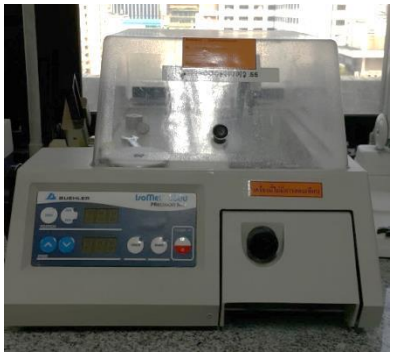
37% Phosphoric acid

Transbond Plus self-etching primer

Fuji ortho LC

Transbond XT

Figure 23 Research equipment: Machines were used in this study



Low speed cutting machine (ISOMET 1000, Buchler, USA)

LED light curing unit (Elipar S10, 3M ESPE,St.Paul, MN, USA)

Universal testing machine (Shimadzu Corp., Japan) for shear bond strength test

Stereo microscope (SZ61 Seires, Olympus,) for evaluation of the residual adhesive

Appendix C

Table 4 Research equipment: Chemical agents and adhesives were used in this study

Material	Components	Ingredients
20% polyacrylic acid (ortho conditioner)	Conditioner	20% polyacrylic acid
37% Phosphoric acid	Conditioner	37% Phosphoric acid
Transbond Plus self-etching primer (3M)	Primer and bond	Primer: fluoride, no filler, bond: methacrylate ester derivative
Fuji ortho LC (GC Corporation, Tokyo, Japan)	RMGI light-cured adhesive	Powder Alumino-silicate glass: 100% Liquid Polyacrylic acid: 20%-22% 2-hydroxyethyl methacrylate: 35%-40% Proprietary ingredient: 5%-15% 2,2,4, Trimethyl hexamethylene dicarbonate: 5%-7% Triethylene glycol dimethacrylate: 4%-6%
Transbond XT (3M ESPE, St Paul, Minn)	Composite light-cured adhesive	Silane-treated quartz filler: 70%-80% Bis-GMA: 10%-20% Bisphenol A Bis (2-hydroxyethyl ether) dimethacrylate: 5%-10% Dichlorodimethylsilane reaction product with silica by weight: < 2%

Shear bond test

Table 5 The normality test of shear bond strength by Kolmogorov-Smirnov method

One-Sample Kolmogorov-Smirnov Test

		Group 1	Group 2	Group 3	Group 4	Group 5
N		10	10	10	10	10
Normal Parameters	Mean	8.42	10.78	14.59	13.69	21.48
	Std. Deviation	1.98	.73	3.04	1.68	3.98
Most Extreme Differences	Absolute	.187	.137	.158	.251	.163
	Positive	.187	.103	.129	.251	.163
	Negative	-.145	-.137	-.158	-.182	-.123
Kolmogorov-Smirnov Z		.590	.432	.500	.794	.515
Asymp. Sig. (2-tailed)		.877	.992	.964	.554	.954

Table 6 Test of homogeneity of Variances

Levene Statistic	df1	df2	Sig.
7.027	4	45	.000

Table 7 Welch ANOVA

Robust Tests of Equality of Means

	Statistic[a]	df1	df2	Sig.
Welch	32.744	4	18.504	.000
Brown-Forsythe	44.013	4	23.432	.000

Table 8 Multiple comparison by Games-Howell

Multiple Comparisons

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-2.5523924*	.662	.020	-4.720	-.385
	3	-7.0631289*	1.039	.000	-10.280	-3.847
	4	-5.1936149*	.756	.000	-7.541	-2.846
	5	-12.6039124*	1.292	.000	-16.703	-8.505
2	1	2.5523924*	.662	.020	.385	4.720
	3	-4.5107365*	.868	.004	-7.411	-1.611
	4	-2.6412224*	.494	.001	-4.209	-1.073
	5	-10.0515200*	1.158	.000	-13.978	-6.126
3	1	7.0631289*	1.039	.000	3.847	10.280
	2	4.5107365*	.868	.004	1.611	7.411
	4	1.87	.941	.328	-1.130	4.869
	5	-5.5407836*	1.409	.010	-9.902	-1.180
4	1	5.1936149*	.756	.000	2.846	7.541
	2	2.6412224*	.494	.001	1.073	4.209
	3	-1.87	.941	.328	-4.869	1.130
	5	-7.4102976*	1.215	.001	-11.386	-3.435
5	1	12.6039124*	1.292	.000	8.505	16.703
	2	10.0515200*	1.158	.000	6.126	13.978
	3	5.5407836*	1.409	.010	1.180	9.902
	4	7.4102976*	1.215	.001	3.435	11.386

Table 9 Adhesive remnant index

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	57.475a	16	.000
Likelihood Ratio	63.533	16	.000
Linear-by-Linear Association	30.526	1	.000
N of Valid Cases	50		

a. 25 cells (100.0%) have expected count less than 5. The minimum expected count is 1.00.

Microhardness test

Table 10 The normality test of microhardness value by Kolmogorov-Smirnov method

One-Sample Kolmogorov-Smirnov Test

		Group 1						Group 2					
		Baseline			Post conditioning			Baseline			Post conditioning		
		10 µm	20 µm	30 µm	10 µm	20 µm	30 µm	10 µm	20 µm	30 µm	10 µm	20 µm	30 µm
N		8	8	8	8	8	8	8	8	8	8	8	8
Normal Parameters	Mean	174.9	257.8	308.0	204.1	275.3	297.7	128.8	318.5	363.4	158.8	312.2	352.0
	SD.	52.0	59.8	89.0	102.5	106.9	114.0	67.3	102.5	72.4	92.2	59.8	54.1
Most Extreme Differences	Absolute	.227	.149	.206	.288	.212	.208	.244	.169	.191	.236	.159	.167
	Positive	.154	.149	.206	.288	.212	.208	.244	.169	.191	.236	.116	.143
	Negative	-.227	-.122	-.179	-.174	-.166	-.183	-.207	-.132	-.106	-.165	-.159	-.167
Kolmogorov-Smirnov Z		.642	.420	.582	.815	.600	.588	.691	.478	.541	.669	.449	.471
Asymp. Sig. (2-tailed)		.805	.994	.888	.521	.865	.880	.726	.976	.932	.762	.988	.980

		Group 3						Group 4					
		Baseline			Post conditioning			Baseline			Post conditioning		
		10 µm	20 µm	30 µm	10 µm	20 µm	30 µm	10 µm	20 µm	30 µm	10 µm	20 µm	30 µm
N		8	8	8	8	8	8	8	8	8	8	8	8
Normal Parameters	Mean	214.7	291.5	324.3	184.0	287.1	282.9	139.0	306.7	329.2	267.2	278.8	333.8
	SD.	116.6	89.0	72.7	91.6	104.7	83.7	89.9	126.1	82.9	200.5	148.1	140.6
Most Extreme Differences	Absolute	.156	.221	.266	.215	.264	.177	.227	.159	.317	.197	.268	.232
	Positive	.150	.221	.158	.154	.199	.158	.227	.134	.173	.197	.268	.181
	Negative	-.156	-.197	-.266	-.215	-.264	-.177	-.161	-.159	-.317	-.188	-.185	-.232
Kolmogorov-Smirnov Z		.440	.625	.751	.607	.747	.499	.641	.450	.897	.557	.757	.656
Asymp. Sig. (2-tailed)		.990	.830	.625	.854	.633	.964	.806	.987	.397	.916	.615	.783

Table 11 Test of homogeneity of Variances

Test of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.
10 µm	5.567	3	28	.004
20 µm	2.886	3	28	.053
30 µm	3.658	3	28	.024

Table 12 ANOVA

ANOVA

Post-conditioning

		Sum of Squares	df	Mean Square	F	Sig.
20 µm	Between Groups	6660.8	3.0	2220.3	.185	.905
	Within Groups	335278.5	28.0	11974.2		
	Total	341939.3	31.0			

Robust Tests of Equality of Means

Post-conditioning

		Statistic[a]	df1	df2	Sig.
10 µm	Welch	.693	3	15.243	.570
	Brown-Forsythe	1.015	3	17.113	.410
30 µm	Welch	1.368	3	14.651	.291
	Brown-Forsythe	.760	3	20.675	.529

Table 13 Paired-t test (before VS after conditioning)

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Group 1	HNb10gr1 - HNp10gr1	-29.25	91.03	32.18	-105.35	46.85	-.909	7	.394
	HNb20gr1 - HNp20gr1	-17.51	60.29	21.32	-67.92	32.89	-.822	7	.438
	HNb30gr1 - HNp30gr1	10.30	45.13	15.96	-27.43	48.03	.645	7	.539
Group 2	HNb10gr2 - HNp10gr2	-30.03	77.52	27.41	-94.84	34.79	-1.095	7	.310
	HNb20gr2 - HNp20gr2	6.26	110.95	39.23	-86.50	99.02	.160	7	.878
	HNb30gr2 - HNp30gr2	11.35	40.54	14.33	-22.54	45.24	.792	7	.454
Group 3	HNb10gr3 - HNp10gr3	30.66	51.93	18.36	-12.75	74.08	1.670	7	.139
	HNb20gr3 - HNp20gr3	4.42	76.56	27.07	-59.58	68.43	.163	7	.875
	HNb30gr3 - HNp3ogr3	41.41	47.49	16.79	1.71	81.11	2.467	7	.043
Group 4	HNb10gr4 - HNp10gr4	-128.21	145.91	51.59	-250.20	-6.23	-2.485	7	.042
	HNb20gr4 - HNp20gr4	27.85	199.33	70.47	-138.80	194.50	.395	7	.704
	HNb30gr4 - HNp30gr4	-4.59	146.15	51.67	-126.77	117.60	-.089	7	.932

VITA

Miss Nattaporn Laotaveerungrueng was born on 22 April 1984. She graduated her Doctor of Dental Surgery from Chulalongkorn University in 2008. After graduation, she worked at Soi-dao hospital as a general practitioner for 2 years and at Thongphapoom hospital for 1 year. After that, she worked at a private dental clinic for 1 year. In 2013, she started her Master degree at Chulalongkorn University and continued ever since.