INFLUENCE OF DEMINERALIZED DENTIN ON MARGINAL SEAL USING PRIMERLESS WET BONDING TECHNIQUE: *IN-VIVO* STUDY

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Prosthodontics
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อิทธิพลของเนื้อฟันที่สูญเสียแร่ธาตุต่อความแนบสนิทบริเวณขอบของวัสดุบูรณะด้วยเทคนิคไพรเมอร์เลสส์ เว็ตบอนด์ดิง โดยศึกษาในช่องปาก

น.ส.ธัญญรัตน์ เลิศตรีภพ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	INFLUENCE OF DEMINERALIZED DENTIN ON MARGINAL SEAL USING PRIMERLESS WET BONDING TECHNIQUE: *IN-VIVO* STUDY
By	Miss Thanyarat Lerttriphob
Field of Study	Prosthodontics
Thesis Advisor	Professor Morakot Piemjai, Ph.D.

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ธัญญรัตน์ เลิศตรีภพ : อิทธิพลของเนื้อฟันที่สูญเสียแร่ธาตุต่อความแนบสนิทบริเวณขอบของวัสดุบูรณะ ด้วยเทคนิคไพรเมอร์เลสส์ เว็ตบอนด์ดิง โดยศึกษาในช่องปาก. (INFLUENCE OF DEMINERALIZED DENTIN ON MARGINAL SEAL USING PRIMERLESS WET BONDING TECHNIQUE: *IN-VIVO* STUDY) อ.ที่ปรึกษาหลัก : ศ. ทญ. ดร.มรกต เปี่ยมใจ

การวิจัยนี้มีวัตถุประสงค์เพื่อปรียบเทียบการรั่วซึมของฟันที่บูรณะด้วยการอุดคลาสไฟฟ์โดยใช้ สารยึดเรซินชนิดโฟร์เมตาเอมเอมเอทีบีบี หรือสารยึดเรซินชนิดเอมเอมเอทีบีบี เมื่อทำการปรับสภาพผิวฟันด้วยสารละลายเฟอร์ริกคลอไรด์ร้อยละ1 ในกรดซิตริก ร้อยละ1 (1-1) เป็นเวลา 10 วินาที (กลุ่ม 1-1-10s) 30 วินาที(กลุ่ม 1-1-30s) และ 60 วินาที(กลุ่ม 1-1-60s) เป็นการศึกษาในช่องปาก วิธีทดสอบ แบ่งเป็น 6 กลุ่ม (กลุ่มละ 10 ตัวอย่าง) ทำในผู้ป่วยปริทันต์อักเสบที่จำเป็นต้องได้รับการถอนฟัน โดยเป็นฟันที่ยังมีชีวิต ฟันที่ไม่มีรอยผุ และรอยร้าว เตรียมโพรงฟันรูปคลาสไฟฟ์ที่ด้านแก้มและ/หรือด้านลิ้น ตำแหน่งเหนือ และใต้รอยต่อเคลือบฟันและเคลือบรากฟันอย่างละ 1 มม. ทำการปรับสภาพผิวฟันด้วยสารละลาย1-1 เป็นเวลา 10, 30 และ 60 วินาที(ตามกลุ่มตัวอย่างแต่ละกลุ่ม) ล้างออก 10 วินาที และซับแห้ง 10 วินาที จากนั้นอุดโพรงฟันด้วยเรซินคอมโพสิต โดยใช้สารยึดเรซิน 2 ชนิด ชนิดละ 3 กลุ่ม จากนั้นให้ผู้ป่วยกลับไปใช้งานตามปกติเป็นเวลา 7 วันก่อนถอนฟัน นำฟันที่ถอนมาแช่น้ำที่อุณหภูมิ 37 องศาเซลเซียสเป็นเวลา 24 ชั่วโมง ก่อนนำไปทดสอบการรั่วซึมโดยแช่ในสารละลายเบสิกฟุชซินร้อยละ 0.5 เป็นเวลา 24 ชั่วโมง จึงนำมาตัดผ่านกึ่งกลางวัสดุบูรณะในแนวดิ่ง และวัดการรั่วซึมภายใต้กล้องจุลทรรศน์สเตอริโอ ผลการทดลอง ไม่พบการรั่วซึมบริเวณรอยต่อสารยึดเรซินกับผิวเคลือบฟันและผิวเนื้อฟัน ของฟันในกลุ่มตัวอย่างทั้ง 6 กลุ่ม ยกเว้นกลุ่ม 1-1-60s ที่ใช้สารยึดชนิดเอมเอมเอทีบีบี พบการรั่วซึมของตัวอย่างจำนวน 1 ชิ้นระหว่างรอยต่อเนื้อฟันกับสารยึดเรซิน สรุปผลการทดลอง การเชื่อมผิวฟันด้วยสารยึดเรซินชนิดโฟร์เมตาเอมเอมเอทีบีบี หรือสารยึดเรซินชนิดเอมเอมเอทีบีบี โดยใช้สารละลาย (1-1) ปรับสภาพผิวฟันเป็นเวลา 10-60 วินาที ล้างออก ซับแห้ง สามารถสร้างชั้นไฮบริดที่สมบูรณ์ต่อเนื่อง ไม่เกิดการรั่วซึมได้ในช่องปาก ซึ่งช่วยป้องกันอาการเสียวฟัน การผุใต้วัสดุบูรณะ และ/หรือ การติดเชื้อในโพรงประสาทฟันได้

สาขาวิชา	ทันตกรรมประดิษฐ์		ลายมือชื่อนิสิต ....
ปีการศึกษา	2563		ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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KEYWORD: Primerless wet-bonding; Hybrid layer; 4-META/ MMA-TBB resin

Thanyarat Lerttriphob :
INFLUENCE OF DEMINERALIZED DENTIN ON MARGINAL SEAL USING PRIMERLESS WET BONDI NG TECHNIQUE: *IN-VIVO* STUDY. Advisor: Prof. Morakot Piemjai, Ph.D.

The purpose of this study was to compare microleakage distance between Class V restorations and tooth interface when using either 4-META-MMA/TBB or MMA/TBB resin adhesives by using 1 % ferric chloride in 1% citric acid (1-1) for conditioning periods of 10s (1-1-10s) 30s (1-1-30s) and 60s (1-1-60s) for *in-vivo* study. Material and methods: A total of 60 Class V box cavities were divided into 6 groups of 10 specimens (n= 10) on the caries-free, vital, and hopeless periodontally-compromised teeth, scheduled for extraction. A Class V cavity (3mm × 3mm × 1.5mm) was prepared on the buccal or lingual surface located at the cemento-enamel junction of each tooth providing margins in enamel and cementum/dentin. All cavities were conditioned with 1-1 aqueous conditioner for 10 s, 30s or 60s each group, rinsed off with water for 10 s, blot-dried for 10s and bonded with different resin adhesives (4-META/MMA-TBB or MMA-TBB) before coupled with light-cured resin composite. Restored teeth continued under function in oral cavity for 7 days before extraction. After extraction, all specimens were coated with nail varnish, except for restorations and 1 mm away from occlusal and cervical margins, then immersed in 0.5 % basic fuchsin dye solution for 24 h. All specimens were vertically sectioned. The margin and distance of dye penetration was investigated by a stereomicroscope. Results: There is no leakage at the tooth-resin interface at enamel and cementum/dentin margins either using 4META-MMA/TBB or MMA/TBB resin for all etching periods. Only 1 specimen of 60s etching and bonding with MMA/TBB resin has leakage distance at cementum/dentin margin of 0.13 mm. Conclusion: Impermeable hybridized interfaces were formed in all groups with 4META- MMA/TBB or MMA/TBB resin using (1-1) to condition tooth surface for 10-60 seconds. No leakage along tooth-adhesive interface was found in clinically. This suggests the ability of leakage prevention which help prevent tooth sensitivity, caries under the restorations and/or infection in the pulpal cavity predicting the long-term success of restored teeth.

Field of Study:	Prosthodontics	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....

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Thanyarat Lerttriphob

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CHAPTER 1

INTRODUCTION

Background and rationale

Studies(1-3) have shown that microleakage at the tooth-restoration interface is an important factor contributing to tooth-hypersensitivity, marginal staining, secondary caries, pulp inflammation and prosthesis dislodgement. Nowadays, newly developed bonding systems demonstrate better bonding between adhesive resins and tooth structure which may inhibit microleakage and maintain restorations under functions in long term. However, the complete seal of dentin in micro-level has not been shown in oral cavity. A vital ground dentin surface is moist with dentinal fluid flow after smear layer and plug removal, may cause disruption of resin-dentin bonds in some adhesive systems

Leakage-free dental restorative treatment can be achieved when complete hybridization of conditioned tooth substrates with diffusible resin adhesive occurs. The hybridized tooth-resin interface is demonstrated by the resistance of the penetration of HCl and NaOCl solutions.(4, 5) Complete hybridization of the tooth-resin interface depends on the permeability and thickness of the demineralized dentin and the ability of monomer to diffuse and fill in demineralized dentin. (6) Tooth conditioners and conditioning periods significantly influence on the complete hybridization of the tooth-resin interface. Conditioned dentin surface generated by some tooth conditioners, such as 37% phosphoric acid tends to collapse with strong air dried, and monomers are unable to entire diffuse into the collapsed demineralized dentin. This demineralized technique can lead to severe leakage.(4) Imperfect hybrid layer remaining under resin cement also results in the restoration microleakage after bonding procedure.(7)

In contrast, an aqueous mixture of 10% citric acid and 3% ferric chloride (10-3) that has been used as an efficient conditioner in clinical treatment to remove smear layer of both enamel and dentin can conditioned the surface to be easier for dehydration and permeable for 4-META and MMA impregnation after air drying.(8) However, prolonged 10-3 dentin conditioning (30–60 seconds) creates too deep demineralized layer to be completely impregnated by the monomers before polymerization starting, resulting in remaining demineralized dentin and subsequent marginal leakage.(4, 9)

Many *in vitro* studies have demonstrated bonding to dentin using 1% ferric chloride in 1% citric acid aqueous conditioner 1-1, a low concentration dentin conditioner, provide consistent hybridized layer of 1-2 μm after a wet bonding procedure. (10, 11) It suggested that the milder 1-1 conditioner prepares permeable demineralized dentin for complete resin impregnation in the long period of 10s to 60s.

Piemjai et.al.(12) investigated the remaining demineralized dentin when using primerless wet bonding with a total etching period of 10 to 60 seconds compared with contemporary resin adhesives. The result suggested that no leakage at the enamel- and dentin-resin interfaces was found for all etching periods of 1-1 primerless wet bonding specimens.

However, the clinical value of these results has not been conducted. And the outward fluid flow from the intrapulpal fluid pressure is believed to have an adverse effect on the formation of a resin-reinforced layer *in-vivo*. (14)

This study investigated the marginal seal when using 1-1 conditioner, primerless wet bonding, with MMA-TBB resin in comparison to 1-1 conditioner, primerless wet bonding using 4-META/MMA-TBB resin for Class V restorations in the *in-vivo* study. The microleakage after tooth bonding was analyzed in association with characteristics of the tooth-resin interfacial layer.

Research question

Can primerless wet bonding using 1-1 aqueous conditioner provide the complete hybrid layer with leakage-free margin in clinical operation?

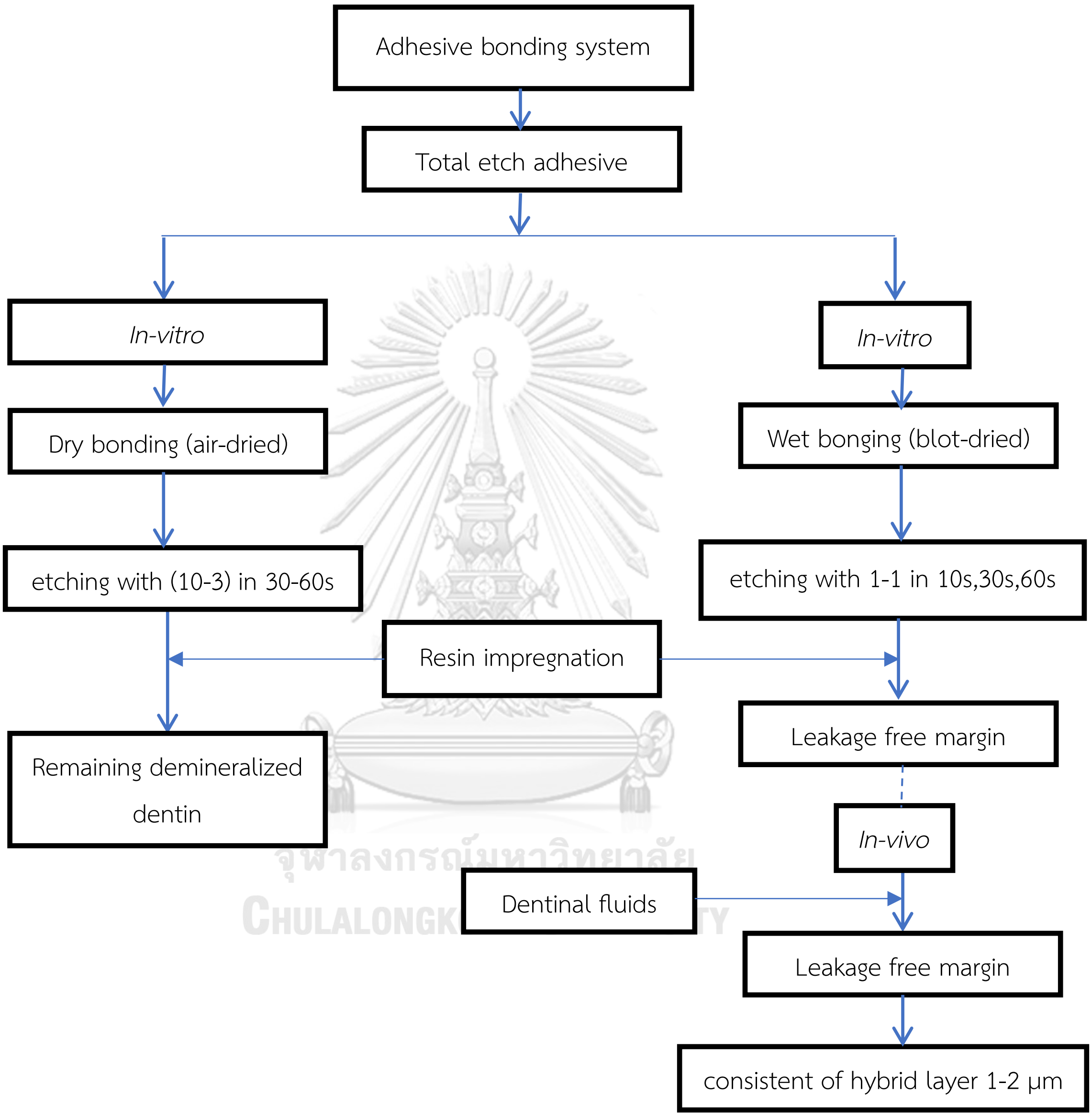
Research objectives

To compare microleakage distance between Class V restorations and tooth interface either when using a 1-1 primerless wet bonding of 4-META/MMA-TBB resin adhesive or using a 1-1 primerless wet bonding of MMA-TBB resin adhesive in 10s, 30s and 60s conditioning periods.

Research hypothesis

There is no different in microleakage distance between using primerless wet bonding with or without 4META in MMA-TBB resin in 10s, 30s and 60s in clinically

Conceptual framework



Limitation of research

Sample population, tooth selection from periodontal disease patients cannot represent all population due to biased from individual mannerism

Expected benefits

To identify the ability of primerless wet bonding using 1-1 conditioner with MMA-TBB could provide impermeable hybridized layer in oral compared to bonding using 1-1 conditioner with 4-META/MMA-TBB in 10s, 30s and 60s etching time.

Research design

Clinical trial research

Key words

Primerless wet-bonding; Hybrid layer; Marginal seal; 4-META/ MMA-TBB resin

CHAPTER 2

REVIEW LITERATURE

Tooth structure

Dental hard tissues consist of enamel, dentin, and cementum, which form a complex structure. 96% inorganic materials, mainly hydroxyapatite crystals, are made enamel the most highly mineralized tissue in the body. Other components of enamel are organic matrix and water.

Dentin is composed of more organic substances mainly collagen, and water. Non-collagenous macromolecules, proteoglycans(PG), glycosaminoglycan (GAG), and phosphoproteins (PP), have been identified in dentin and pre-dentin. Around 50% of dentin is hydroxyapatite minerals, 30 % is collagen and non-collagenous molecules, which 90% of the organic matrix in dentin is collagen type I and the fluids remained collectively form a rigid and durable mineral-rich biocomposite.(13)

Dentinal tubules contain dentinal fluid include proteins and proteoglycans. Encircled by a hypermineralized layer constituted highly mineralized composite material; phosphoproteins, proteoglycans, and glycosaminoglycans which lacks collagen fibrils called peritubular dentin. And the intertubular matrix is composed of collagen molecules, which contain type I fibrils, noncollagenous components, and water called intertubular dentin. (10-13)

Microleakage

Clinically undetectable leakage is usually referred to as microleakage which has been described as the passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material.(1) Microleakage at the tooth/restoration interface influenced the longevity of the dental restorations. It can lead to tooth hypersensitivity, tooth discoloration, staining at the margins of restorations, prosthesis or restoration detachment, secondary caries, and pulpal pathology.(2)

Demineralized dentin and/or smears remaining under cement can initiate leakage pathways. incomplete resin infiltration in the collagen network. It is speculated that the region is capable of hydrolytic degradation over a

period because of the exposed collagen fibrils, leading to a reduction in bond strength. (21, 22)

Hybridization

The concept of hybridization is to infiltrate tooth tissue with resin monomer to form a hybrid layer that is composed of both tooth tissue and resin. A complete hybrid layer is reached when complete hybridization of resin into the tooth-substrate occurs without any defects such as remaining demineralized tissue, smears, and water globules. Nakabayashi preferred to characterize hybridization as "molecular entanglement of dental components with resin copolymer chain in the hybrid layer".(6) It provides better retention between the tooth and resin interface than the resin-prosthesis interface and a leakage-free interface and margin. This layer can resist both acidic demineralization and proteolytic degradation so that the tooth structure enveloped or covered with this layer is protected from functional forces and chemical damage from the oral cavity.(5, 10, 13)

Bonding to etched enamel

In 1955 Buonocore found that increasing the tissue surface area at the micro level using phosphoric acid etching lead to increasing the duration of adhesion between acrylic resin material and the enamel surface.(14) Mostly hydroxyapatite crystallites were demineralized then methyl methacrylate (MMA) monomer could penetrate into the micro porosities of dried, etched enamel and form micro mechanical resin tags when polymerized.

Good bonding to enamel was achieve with monomers containing hydrophobic and hydrophilic groups because they could promote monomer impregnation into tooth substrates and provide high bond strength, suggesting that chemical bonding was not an important mechanism of adhesion to tooth substrate.

Ability to form a complete seal at the enamel-resin junction are key factors for long-term function of the restored tooth. Acids dissolve hydroxyapatite in enamel to create microscopic permeability for resin monomer diffusion to create an enamel hybrid layer.(15) The depth of acid penetration into acid-etched enamel depends on the type and concentration of acid and the etching periods.(6, 16)

Diffusivity of monomers into conditioned tooth structure depends on the diffusion rate which varies by size, concentration, viscosity and temperature of the monomers. Monomers that have both hydrophilic and hydrophobic groups such as 4-META (4-metha-cryloyloxyethyl trimellitate anhydride) helped promote the penetration of monomers into etched enamel.(6, 16)

The thickness of the enamel hybrid layer is dependent on the demineralization depth of enamel and the ability of adhesive monomers to penetrate the etched enamel. Thus, types of acids, acid concentration, etching time, types of monomers and diffusion time for monomers are the influencing factors in the characteristics of hybridized enamel. The milder acids usually provide less depth of penetration resulting in a thinner hybrid layer and shorter resin tags.

A mixture of 10% citric acid and 3% ferric chloride applied as an aqueous conditioner (10-3) on enamel for 10 s and bonded with 4-META/MMA-TBB resin cement provided a 2-3 µm constant thickness hybrid layer after immersion in HCl and NaOCl solutions. The milder acid than10-3, 1-1, etched on ground enamel 10 - 60 s, rinsed, blot-dried for 10 s and bonded with 4-META/MMA-TBB resin without primer provided hybridized enamel of 1-4 µm with the fewer resin tags.(11, 12) Hybridized enamel exhibits leakage-free margins and interfaces for both direct restorations and fixed prostheses.

Bonding to etched dentin

Infiltration of resin into dentin forms hybridized dentin which is a molecular-level mixture of collagen and resin polymers located in the subsurface of conditioned dentin.(16) The bonding process is more complicated than enamel cause dentin is more complex structure, composed of more organic substance, mainly collagen and water

Hybridization dentin is the process of creating a hybrid layer within the subsurface by demineralization of the surface, followed by infiltration of monomers and their subsequent polymerization. The mechanism of dentin bonding depends monomer permeability of the substrate and the ability of monomer to diffuse into the demineralized dentin.(6)

Conditioning of dentin with 10% citric acid and 3% ferric chloride (10-3) for 10s, 30s, and applying 4-META/MMA-TBB resin on dried demineralized human dentin provided hybridized dentin(16) The 4-META adhesive resin can diffuse and impregnate demineralized dentin, mainly exposed collagen bundles to create a hybrid layer.

The ferric chloride in 10-3 conditioner prevents the collapse of demineralized dentin when air-dried.(16) Ferric chloride in acid conditioners has function to provide a higher permeability and faster dehydration of demineralized dentin.(8) The mechanism is based on a reaction between ferric ions and polyelectrolytes in demineralized dentin. Ferric ion can minimize the amount of dissolved polyelectrolytes and maintain the spaces where the hydroxyapatite had been dissloved even after air-drying. Monomers can penetrate this permeable demineralized dentin and form hybridized dentin after their polymerization. It has been reported that after dentin etching, the solubility of collagen peptides is increased due to the denaturing of collagen fibrils. These solubilized peptides may contribute to the collapse of the matrix during air drying so that interfibrillar spaces between exposed collagen fibrils were eliminated.

Dentin conditioned with an aqueous mixture of 10% citric acid and 3% ferric chloride (10-3) creates chemical aggregation of water-soluble glycosaminoglycans (GAGs) with cationic ion such as Fe ions. Hydrophilic agents and cationic ion such as Fe ions added to the citric acid, calcium ions dissolved in 10% citric acid easily are attracted by these polyelectrolytes distributed along the collagen fibrils and make them insoluble then, decrease the dissolved concentration in the demineralized dentin resulting in minimizing the shrinkage of etched dentin. (8, 13) The permeability of demineralized dentin for facilitating optimal permeation of adhesive monomers and increasing the rate of dehydration with the acetone primer consequently improved good hybridized dentin with higher resin content was the result. This can provide higher permeability to 4-META and MMA for their impregnation after air-drying. However, longer periods of 10-3 conditioning (30–60 seconds) on dentin demineralized it too deeply for the monomers to complete infiltration, resulting in remaining demineralized dentin which permits marginal leakage. Besides, demineralized dentin could be hydrolyzed easily in the mouth, which can induce detachment of restorations from prepared dentin. (6, 10)

A 1% ferric chloride in 1% citric acid aqueous conditioner (1-1), a less concentration conditioner than 10-3, can produce impermeable hybridized dentin in a wet bonding procedure with conditioning periods varying between 10 and 60 seconds. The thin hybridized dentin layer (1-2 μm) suggested that the milder 1-1 conditioner did not demineralize the prepared dentin too deeply for monomers to completely impregnate into the demineralized dentin. (6, 10)

Microleakage testing method

Microleakage cannot visualize intra-orally. More often patients can feel tooth-hypersensitivity represent microleakage after restoration placement. Light microscopy and scanning electron microscopy have been used widely to detect or compare the quantity and/or quality of microleakage values in the laboratory. *In-vitro* studies of restorations in extracted tooth are much simpler than making restorations *in-vivo* and allowing them to age. *In-vitro* microleakage tests do not simulate pulp pressure, dentinal fluid flow, and the presence of oral fluid including acids. However, microleakage results comparing tests done *in-vitro* and *in-vivo* show conflicting results: microleakage *in-vivo* less than that *in-vitro*, with little difference and microleakage *in-vivo* higher than that *in-vitro*. Nonetheless, many laboratory techniques have been widely used to investigate leakage at the margin and tooth-restoration interfaces.

Tracer penetration

By immersing the specimens in the dye solution for an interval of time, after some time the specimen is removed, washed, and sectioned to measure the distance of tracer penetration under the microscope. The most common and oldest tracers used to detect microleakage are organic dyes.(1)

Dye solutions (0.5%-20%) i.e., basic fuchsin, methylene blue, fluorescein and eosin have been used in the laboratory as well as the clinic because they are easily detectable and prepared, inexpensive, and nontoxic.(1) The leakage distance can be measured by combining light microscopy with grid and image software that can calculate the length on the magnified image.

CHAPTER 3
RESEARCH METHODOLOGY

Materials and Methods

Patient Selection and cavity preparation

Patients who came for the dental care at the surgical clinic, prosthodontics clinic or periodontal clinic, Faculty of Dentistry, Chulalongkorn University during the Year 2018-2020, were informed the study procedures, alternative treatments available, and the risks involved with the study. Patients were asked to voluntarily participate and provided written informed consent.

Inclusion criteria	Exclusion criteria
1. Patients who have had caries-free, vital, and hopeless periodontally-compromised teeth scheduled for extraction	Patients who have known allergy or intolerance to anesthesia or resin adhesives
2. Patients who agreed to participate in the study and gave their informed consent	

Study Sample

Thai patients who passed all of the inclusion criteria and without exclusion criteria. Patients who accepted ethical issues: informed consent, treatment plan, before experimental.

Cavity preparation

The vitality test of all teeth was determined by using the electric pulp tester. Following local anesthesia and rubber dam application, sixty cavities were prepared on the buccal and/or lingual surface of each tooth. Prepared Class V box cavity with buttjoint cavosurface margins, 3 mm wide, 3 mm height and 1.5 mm depth located at the cemento-enamel junction with one horizontal margin in enamel and the other in cementum using a diamond bur (204, Intensiv, Montagnola, Switzerland) in a high-speed handpiece under air-water spray. All cavities randomly divided into 6 groups, with 10 restorations each for the application of two different bonding agents (4-META/MMA-TBB resin or MMA-TBB resin) at the etching period of 10s, 30s or 60s.

Adhesive bonding system and Resin composite restoration

The 1% citric acid in 1% ferric chloride aqueous conditioner 1-1 was applied to the cavity walls either for 10s, 30s or 60s in each individual group. Each cavity was rinsed with water for 10 s, the excess water was blot-dried with absorbent paper (KIMTECH SCIENCE * KIMWIPES Wipers Delicate Task Wipers, NSW, Australia), applied cavity with 4-META/MMA-TBB resin (Sun Medical, shiga, Japan) or MMA-TBB resin (Sigma-aldrich, Saint louis, USA) and PMMA powder (Sun Medical, shiga, Japan) as the bonding agents using brush-dip technique. After tooth bonding, all cavities were filled with resin composite (Metafil CXa, Sun Medical, shiga, Japan) using a bulk-filled technique, and light-cured (3M ESPE, MN, USA) for 30s as manufacturers' instruction. The restorations were polished by using a fine diamond burs (5205L, Intensiv, Montagnola, Switzerland) and left under function in oral cavity for 7 days. Then, the restored teeth were extracted by using atraumatic technique under local anesthesia.

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Staining for microleakage test

Extracted teeth were immediately cleaned under running tap water. All tooth surfaces, except for the restoration and 1 mm away from the occlusal (enamel) and gingival (cementum/dentin) margins were coated twice with nail varnish (Ten Ten Nail Colour, Bangkok, Thailand). All the root apex for all teeth were sealed with sticky wax. Whole tooth except the root apex were immersed in 0.5% basic fuchsin solution (Sigma-aldrich, Saint louis, USA) for 24 hours, then rinsed under running tap water, before sectioned vertically through the midline of each restoration with a diamond disc (270D, Intensiv, Montagnola, Switzerland) and a slow speed handpiece. The sectioned specimens were sequentially polished with silicon carbide grit Paper No.400, 600, 800, 1000, and 1200 (TOA, Samut Pragan, Thailand) under wet condition with Polishing Machine (MINITECH 233, PRESI, France). The margin and the distance of dye penetration on sectioned specimens were investigated by a stereomicroscope (Olympus, SZ61, JAPAN) and taken photographs of dye penetration using attached digital camera (DP21, OLYMPUS, JAPAN) at x50 to x200 magnifications. All Images were analysed by an 'Image pro plus' program.

Evaluation of hybridized dentin

Two restored specimens for each group were prepared as previously described to investigate the tooth-resin interfacial area. Each bonded specimen was vertically cross-sectioned into two 1-mm-thick specimens. The surface was smoothed with 600-grit to 1200-grit abrasive paper, polished with 0.05 micron aluminum oxide and finally cleaned with ultrasonic cleaner (VGT-1990QTD, China) for 15 minutes. One sectioned specimen from each restoration was immersed in 6 mol/L hydrochloric acid (HCI) for 30 s, rinsed with water, and then soaked in 1wt% sodium hypochtorite (NaOCl) for 60 mm and totally rinsed off with water. The polished and chemically soaked specimens were dehydrated in a desiccator (Quorum, East Sussex, United Kingdom) for at least 48 h prior for gold sputtering using Mudular Coater System

(Model Q150R, Quorum, East Sussex, United Kingdom) for scanning electron microscope (SEM) investigation. The thickness of hybridized dentin was measured on scanning electron micrographs at x2000 magnification.

Statistical analysis

The statistical significance in the mean leakage distance was set at $P< 0.05$, using ONE-WAY ANOVA and analyze DATA with SPSS program V.22

CHAPTER 4
RESULTS

No leakage at the tooth-resin interface at occlusal and gingival margins was found in 1-1 primerless wet bonding of 4-META/MMA-TBB resin adhesive in 10s (1-1/10 seconds, Fig. 1), 30s (1-1/30 seconds, Fig. 2) and 60s (1-1/60 seconds, Fig. 3) conditioning periods, which are summarized in Table 1.

No leakage at the tooth-resin interface was found in specimens of 1-1 primerless wet bonding of MMA-TBB resin adhesive (Figs. 4-6) except 1 specimen (10%) in 60s conditioning period (1-1/60 seconds, Fig. 7) had leakage at the dentin-resin interface, which are summarized in Table 2.

Table 1. Mean ± SD of leakage distance (mm) and numbers of specimens leaking at tooth-resin interface for 1-1 primerless wet bonding of 4-META/MMA-TBB resin adhesive groups.

Groups (n=10)	Leakage distance at the interfaces (mm) (Number of leaked specimens)	
	Enamel-resin	Dentin-resin
1-1 10 seconds	0(0)	0(0)
1-1 30 seconds	0(0)	0(0)
1-1 60 seconds	0(0)	0(0)

Table 2. Mean ± SD of leakage distance (mm) and numbers of specimens leaking at tooth-resin interface for 1-1 primerless wet bonding of MMA-TBB resin adhesive groups.

Groups (n=10)	Leakage distance at the interfaces (mm) (Number of leaked specimens)	
	Enamel-resin	Dentin-resin
1-1 10 seconds	0(0)	0(0)
1-1 30 seconds	0(0)	0(0)
1-1 60 seconds	0(0)	0.13 (1)

0 = No microleakage

Leakage values connected by the straight line are not significantly different

(P = 0.05)

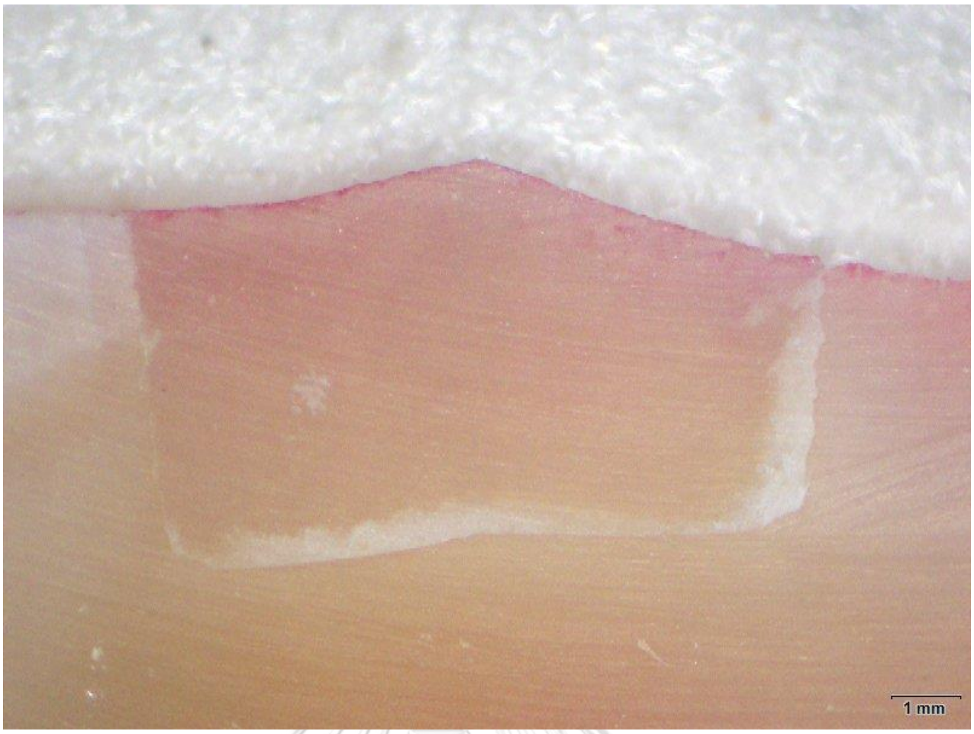

Figure 1 No leakage at tooth-resin interface (arrowed) of 1-1 primerless wet bonding of 4-META/MMA-TBB resin adhesive in 1-1 10 seconds group

Figure 2 No leakage at tooth-resin interface (arrowed) of 1-1 primerless wet bonding of 4-META/MMA-TBB resin adhesive in 1-1 30 seconds group

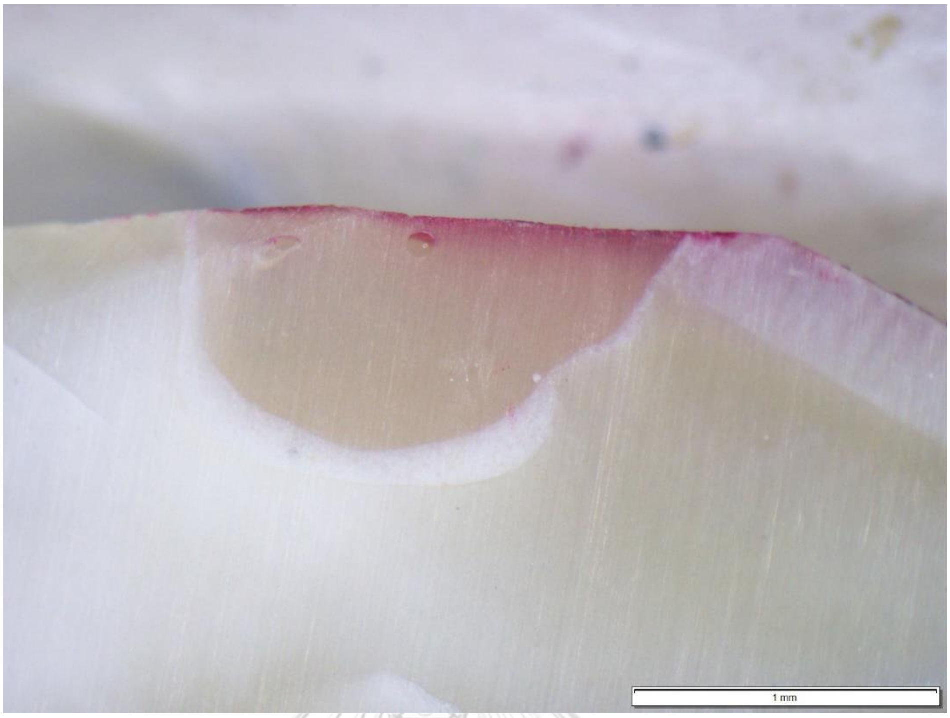

Figure 3 No leakage at tooth-resin interface of 1-1 primerless wet bonding of 4-META/MMA-TBB resin adhesive in 1-1 60 seconds group

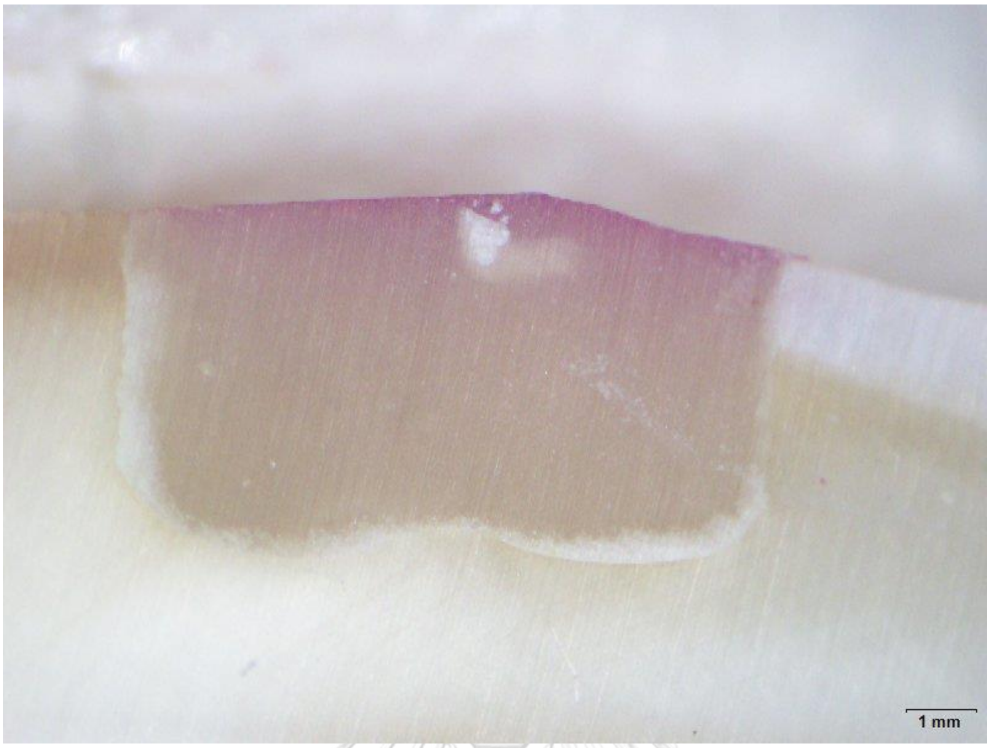

Figure 4 No leakage at tooth-resin interface (arrowed) of 1-1 primerless wet bonding of MMA-TBB resin adhesive in 1-1 10 seconds group

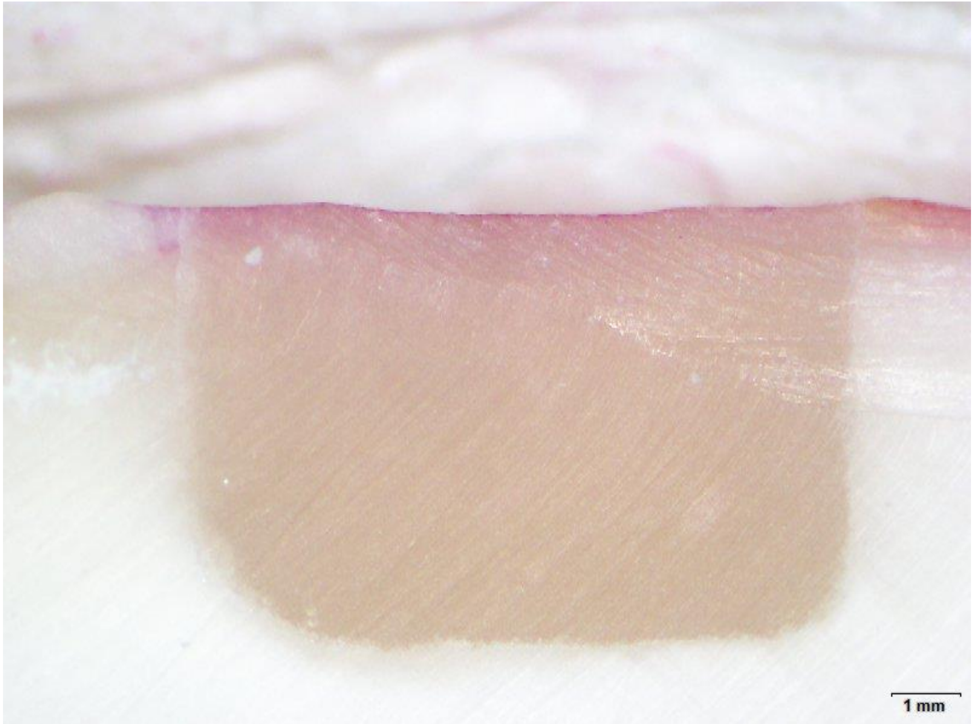

Figure 5 No leakage at tooth-resin interface of 1-1 primerless wet bonding of MMA-TBB resin adhesive in 1-1 30 seconds group

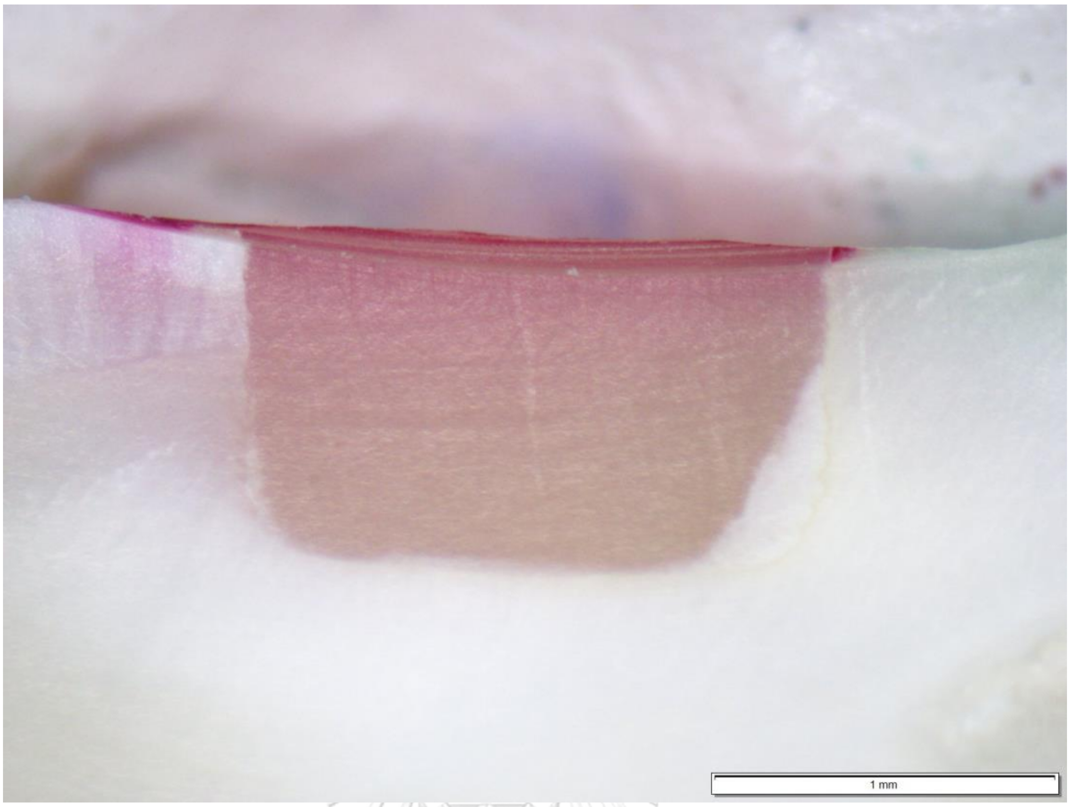

Figure 6 No leakage at tooth-resin interface of 1-1 primerless wet bonding of MMA-TBB resin adhesive in 1-1 60 seconds group

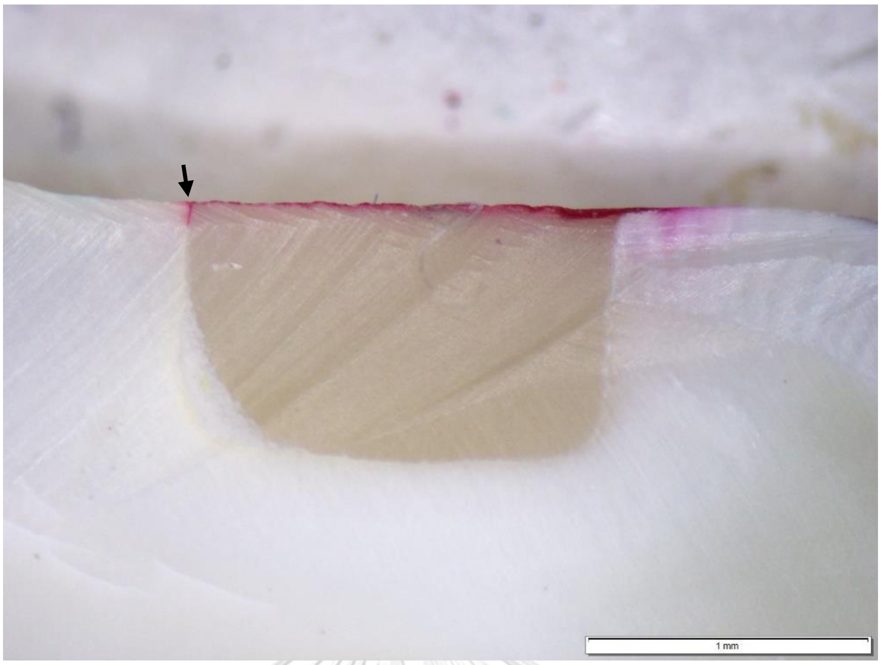

Figure 7 leakage at tooth-resin interface at gingival margin (arrowed) of one specimen in 1-1 60 seconds group of 1-1 primerless wet bonding of MMA-TBB resin adhesive

CHAPTER 5

DISCUSSION

No leakage at the tooth-resin interface at occlusal and gingival margins found both using 1-1 for 10s, 30s, 60s conditioning, primerless wet bonding with 4-META and without 4-META in MMA-TBB resin in oral cavity suggests that using 1-1 conditioner without primer and blot-dried on conditioned surface are able to provide the permeability of demineralized tissue for complete impregnation of both 4-META/MMA-TBB and MMA-TBB resin to form an impermeable hybrid layer.

The dual immersion in HCL and NaOCL solutions demineralized any mineral or organic components within the hybrid layer that was not protected by resin infiltration and solubilized all non-protected demineralized dentin matrix beneath the hybrid layer, respectively (13). The present study also found that all the hybrid layer at dentin-resin interfaces of bonded specimens were resistant to this dual chemical challenge in all depths under SEM. This indicates that monomers can completely infiltrated the whole demineralized dentin created using 1-1 etched for 10-60 seconds.

The resistant to dual challenge of dentin-resin interfaces of all tested specimens suggests that 1-1 etched dentin and blot-dried substrate had high permeability for monomer's impregnation to form a complete resin infiltrated collagen rich fibrous network, even without 4-META monomers promoter. This infers that the permeability characteristics of dentin substrate after acid etching has more influence on preparing a perfect resin-impregnated dentin described as hybridized dentin than do by way of the improvement of chemical ingredients of the contemporary dental adhesives. The possible explanations why 1-1 etched dentin adhesives produced the perfect hybrid layer at dentin-resin interfaces intra-orally for current study.

Conditioning dentin by less aggressive 1-1 conditioner for 10 to 60 seconds did not demineralize dentin too deeply (1-2 μm)(9,10) compared with 10-3 conditioner for 10 seconds (3-4 μm)(13), this allowed the penetration of monomers through inter- and intra-tubular demineralized dentin for complete impregnation. There is no effect on the adhesion of either 4-META/MMA-TBB or MMA-TBB resin to *in-vivo* 1-1 demineralized dentin substrates.

Wet bonding technique in which the tooth surface is left moist help to preserve channels between the collagen fibers for adhesive monomer penetration and impregnation. The 4-META has been introduced to adhesive dentistry as not only an excellent adhesion-promoting monomer by both increasing the penetrability and diffusivity of the demineralized dentin substrates(15) but also it is good for supplying water due to its hydrophilicity. The ferric chloride in citric acid conditioner has been used as a chemical stabilizer of exposed collagen fibrils by means of strong cationic ion interfering the collapse of deminerlized dentin(4, 8) and maintaining a higher monomer permeability in blot-dried dentin substrates. Ferric chloride increased the water permeability of the demineralized dentin thus resulting in smoother dehydration in both the dry and wet demineralized dentin, leading to a positive influence on hybridization and bonding.

Cations such as Fe^{3+}, Ca^{2+} dissolved in acid are easily attracted by polyelectrolytes (PG, GAGs, PP) distributed along the collagen fibrils of demineralized dentin and make them insoluble in the demineralized dentin, improving the dehydration rate of the thin demineralized dentin(7), which can eliminate the need for 4-META either for primers or bonding agent in clinical treatment as shown in this study.

This results of Fe^{3+} in increasing the permeability of demineralized dentin to acid during etching, increasing the rate of dehydration and enabling the resin monomer to diffuse and impregnate in the collagen fibrils before the initiation of polymerization(8) promote the complete resin-impregnation in conditioned dentin without defect or existing demineralized dentin leads to the high resin content hybrid layer with higher tensile bond strength and leakage free interface(11) which are essential to obtain reliable dentin bonding.

The decreasing of dissolved concentration of polyelectrolytes could minimize the shrinkage of demineralized dentin even when it is air dried. Therefore when an acidic conditioner like 1-1 is available, blot-dried bonding is beneficial in minimizing the water contamination compared to wet bonding technique(9,12). The permeability of 1-1 etched dentin to water is high enough to dehydrate wet substrate so that blot drying for 10 seconds could provide the permeability of 1-2 μm demineralized human dentin for complete impregnation of monomers even without the use of an acetone primer.

All 1-1 primerless wet bonding of MMA-TBB and 4-META/MMA-TBB adhesives used in this study could provide complete hybridization of resin into 1-1 etched dentin, leaving no remaining demineralized dentin resulting in impermeable hybrid layer of dentin-resin interface. As the completed hybrid layer was created, suggests no demineralized dentin or water in bonded specimens was remained for dye to penetrate through. This could provide a reliable interface to protect dentin and pulp from external stimuli in the oral cavity.

Only 1 specimen of 60s etching time in MMA-TBB group leaked at resin-dentin margin (0.13 mm), this might be from monomers did not completely replace the remaining water within the interfibrillar spaces of the demineralized dentin. Non-resin impregnated collagen network of etched dentin (residual demineralized dentin) and the entrapped resin-water blister or primer globules described as overwet phenomenon(17-19) or water treeing(20) are the weakest in terms of mechanical strength in the boned specimen(5, 16) and leading to microleakage and secondary caries.(12, 21)

This study showed that MMA-TBB resin may have a better ability to wet the resin composite, which provides better adaptation of the composite to the bonding adhesive surface. This is because MMA on slowly polymerized adhesive initiated by

TBB can diffuse deeper into the composite and copolymerize with fillers which have unpolymerized- pendant-methacrylate groups in the Metafil resin composite. Thus, covalent bonding between this hybridized dentin and resin-composite is possible.(21)

Further studies on how to safely prepare impermeable hybridized dentin in clinical treatment are needed as there are more factors in dentin and pulp, other than collagen, controlling the quality of hybridized dentin.

CHAPTER 6
CONCLUSION

There was no leakage at the tooth-resin interface at enamel and cementum/dentin margins using 1-1 primer-less wet bonding with 4-META/MMA-TBB or MMA-TBB resin of 10s, 30s and 60s conditioning periods in oral cavity. Only 1 specimen of 60s etching using MMA-TBB bonding had leakage distance at resin-dentin margin of 0.13 mm.

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