การตรวจสอบจุดบกพร่องในเนื้อฟันที่ผ่านการกัดด้วยกรดฟอสฟอริกและบูรณะด้วยสารยึดแบบ๓ขั้นตอนที่ใช้ในปัจจุบัน

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DETECTION OF DEFECT ON PHOSPHRIC ETCHED DENTIN RESTORED WITH CONTEMPORARY 3-STEP ADHESIVES.

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Science Program in Prosthodontics

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Thesis Title	DETECTION OF DEFECT ON PHOSPHORIC ETCHED DENTIN RESTORED WITH CONTEMPORARY 3-STEP ADHESIVES.
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การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาเนื้อฟันที่ผ่านการกัดด้วยกรดฟอสฟอริกที่หลงเหลือภายหลังการบูรณะด้วยสารยึดแบบสามขั้นตอนจำนวน3 ชนิดโดยการทดสอบความทนแรงดึงของชิ้นตัวอย่างรูปมินิดัมเบลล์ และการประเมินลักษณะของรอยต่อระหว่างเนื้อฟันกับสารยึดภายหลังการแช่ชิ้นตัวอย่างในสารละลายกรดไฮโดรคลอริกและสารละลายโซเดียมไฮโปคลอไรท์สุ่มแบ่งฟันกรามมนุษย์ที่ไม่ผุ 36 ซี่เป็น 3 กลุ่ม กลุ่มละ 12 ซี่ ตัดแบ่งฟันให้ถึงชั้นเนื้อฟันด้วยเครื่องตัดฟันสร้างชั้นสเมียร์บนหน้าตัดฟันด้วยเข็มกรอเร็วกากเพชรรูปวงล้อที่มีน้ำหล่อตลอดเวลา ปรับสภาพเนื้อฟันด้วยกรดฟอสฟอริกความเข้มข้นร้อยละ32-37.5นาน 15 วินาที ล้างด้วยน้ำกลั่น เตรียมผิวฟันแบบชื้นและเชื่อมด้วยสารยึดแบบสามขั้นตอน 3 ชนิดได้แก่ ออลบอนด์ทรี ออบทิบอนด์เอฟแอล และสก๊อตบอนด์เอ็มพีในแต่ละกลุ่มตามขั้นตอนที่บริษัทผู้ผลิตแนะนำ จากนั้นบูรณะด้วยเรซินคอมโพสิต (เมทาฟิวซีเอ๊กซ์)โดยการอุดเป็นชั้นกรอชิ้นตัวอย่างเป็นรูปมินิดัมเบลล์เพื่อใช้ทดสอบความทนแรงดึง ตรวจสอบด้านตัดขวางของเนื้อฟันที่แตกหักด้วยกล้องจุลทรรศน์อิเลคตรอนชนิดส่องกราดเพื่อศึกษารูปแบบการแตกหัก เตรียมชิ้นตัวอย่างตามวิธีข้างต้นกลุ่มละ 4 ชิ้นเพื่อเปรียบเทียบลักษณะของรอยต่อระหว่างเนื้อฟันกับสารยึดภายใต้กล้องจุลทรรศน์อิเลคตรอนชนิดส่องกราดก่อนและหลังการแช่ในสารละลายกรดไฮโดรคลอริกความเข้มข้น6โมลต่อลิตรนาน30วินาทีและสารละลายโซเดียมไฮโปคลอไรท์ความเข้มข้นร้อยละ1 นาน 60นาทีการวิเคราะห์ผลด้วยสถิติชนิดวิเคราะห์ความแปรปรวนแบบจำแนกทางเดียวพบว่าค่าเฉลี่ยความทนแรงดึงของแต่ละกลุ่มไม่แตกต่างกัน (ประมาณ 11-12 เมกะปาสคาล) อย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ความหนาของรอยต่อระหว่างเนื้อฟันกับสารยึดของทุกกลุ่มลดลงอย่างชัดเจนภายหลังการแช่ชิ้นตัวอย่างในสารเคมีดังกล่าว ผลการศึกษาสรุปได้ว่าการปรับสภาพเนื้อฟันด้วยกรดฟอสฟอริกและบูรณะฟันด้วยสารยึดแบบสามขั้นตอนทั้งสามชนิดนี้ไม่สามารถสร้างชั้นรอยต่อระหว่างเนื้อฟันกับสารยึดที่สมบูรณ์ได้เพราะมีเนื้อฟันที่ถูกกรดกัดที่ไม่มีเรซินห่อหุ้ม (ดีมินเนอร์ไรซ์เดนทีน) หลงเหลืออยู่ซึ่งเป็นสาเหตุของการรั่วซึมภายหลังการบูรณะฟัน ซึ่งอาจทำให้เกิดอาการเสียวฟันและปวดฟันตามมา องค์ประกอบทางเคมีทั้งในสารไพรเมอร์และสารบอนด์ดิ้งที่ใช้ในการทดลองนี้ที่แตกต่างกันและวิธีที่ต่างกันในการทำให้เกิดสภาพชื้นภายหลังการปรับสภาพเนื้อฟันด้วยกรดฟอสฟอริก ส่งผลต่อการหลงเหลือของชั้นดีมินเนอร์ไรซ์เดนทีนไม่ต่างกันการเตรียมชิ้นตัวอย่างรูปมินิดัมเบลล์และเทคนิคการแช่ชิ้นตัวอย่างในสารเคมีเป็นวิธีที่มีประสิทธิภาพในการตรวจหาชั้นบกพร่องได้ภายใน 24 ชั่วโมง ซึ่งมีความสำคัญต่อการทำนายการคงอยู่ในระยะยาวของฟันที่บูรณะ

ภาควิชาทันตกรรมประดิษฐ์ลายมือชื่อนิสิต.....

สาขาวิชาทันตกรรมประดิษฐ์ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา2555

#5376140432: MAJORPROSTHODONTICS

KEYWORDS: DENTIN-RESIN INTERFACE / MINI-DUMBBELL SHAPED SPECIMEN / TENSILE BOND STRENGTH / PHOSPHORIC ACID ETCHED DENTIN / 3-STEP ADHESIVE

SUDARAT NUBDEE: DETECTION OF DEFECT ON PHOSPHORIC ETCHED DENTIN RESTORED WITH CONTEMPORARY 3-STEP ADHESIVES.

AVISOR: ASSOC.PROF. MORAKOT PIEMJAI, Ph.D., 69 pp.

The purpose of this study was toidentify the remaining of demineralized dentin in phosphoric etched dentin which was restored with three contemporary 3-step bonding adhesives using mini-dumbbell shaped tensile test and chemical challenge on dentin-resin interface. Thirty-six non-carious human molars were randomly divided into 3 groups of 12 specimens each. Each tooth was cross-sectioned to expose dentin using sectioning machine. The sectioned dentin which was wet-ground to create smear layerusing a diamond wheel-shaped bur with a high-speed handpiece was etchedwith 32-37.5% phosphoric acid for 15 seconds and rinsed off. Moist demineralized dentin was bonded either with All-Bond3®, OptiBond FL® orScotchbondMulti-Purpose® following the manufacturers' recommendations. Alight-cured resin composite(MetafilCX®) was restored using an incremental technique. All bonded samples were trimmed into mini-dumbbell shaped specimens for tensile bond strength test. The fractured surfaceswere examined underscanning electron microscope(SEM) to determine the failure mode. The characterizationof the dentin-resin interfacial layer of 4bonded specimensfor each adhesive before and afterimmersion in 6 mol/LHClfor 30 seconds and 1% NaOClfor 60 minuteswas evaluated using SEM.One-way ANOVA demonstrated no statistically significant difference ($p>0.05$) in tensile bond strength values (approximately11-12 MPa) among groups.The thickness of dentin-resin interfacewas degraded after chemical modification in all adhesive groups. The results of this study suggest that phosphoric acid etched dentin bonded with these threeadhesives cannot form the completedentin-resin interface, regardless of differences in chemical compositions in primer andbonding resinand/ortechniques to keepmoist. Microleakage taken place at theremainingpermeable demineralized dentin of the restoredtooth may consequently lead to the post-operative hypersensitivity and toothache. Thisdefective area was clearly identified by both mini-dumbbell shaped tensile testand chemical challenge which are the effective methodsto predict the durability of restored tooth in 24 hours.

Department:Prosthodontics Student's Signature .....

Field of Study :Prosthodontics Advisor's Signature .....

Academic Year:2012 .....

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CHAPTER I

INTRODUCTION

Background and Significance of the problem

Dentin adhesives have been developed to provide retention for both direct and indirect restorations. Better dentin bonding via wet or moist system is achieved by removal of smear layer with acid demineralized agent and subsequent application of the hydrophilic primer promoted the wettability of the substrate and penetration of adhesive into demineralized intertubular dentin (Gwinnett et al., 1992a, Gwinnett et al., 1992b, Gwinnett et al., 1992c, Kanca et al., 1992). The conventional three-step total-etch adhesives produced higher bond strengths to dentin than that of adhesives with simplified application procedures, one-step self-etch adhesives, particularly when phosphoric acid was used as a dentin etching agent. High bond strength values obtained with microtensile bond strength testing of several contemporary phosphoric-etched dentin adhesives induced many researchers often used this testing to provide an estimation of the potential clinical performance of the treatment (Bouillaquet et al., 2001, De Munck et al., 2003, Inoue et al., 2001, Reis et al., 2010, Sarr et al., 2010). Unfortunately, these bond strength data are not the sole rationale for evaluating the reliability of true adhesion because post-operative hypersensitivity and toothache are still occurred. It has been reported that wet bonding using phosphoric acid conditioner of both 2- and 3-step total-etch adhesives has been shown the leakage of dye as well as silver nitrate solution at the dentin-resin interface under SEM (Piemjai et al., 2010, Piemjai et al., 2011a). These results indicated that phosphoric acid etched dentin

adhesives are unable to provide an impermeable interface which leads to ingress of oral pathogen or their products.

The defective layer which was not well hybridized by resin in dental treatment, called non-resin impregnation demineralized dentin, situated in demineralized dentin between the hybrid layer and the underlying intact mineralized dentin under SEM (Kato et al.,1996, Kato et al.,1998, Piemjai et al., 2010, Piemjai et al., 2011a) and TEM (Piemjai et al., 2004) has been reported. This permeable interface cannot resist the action of strong acid (HCl) and the longer immersion times in the alkaline (NaOCl) solution (Kato et al., 1996, Kato et al., 1998, Piemjai et al., 2001, Piemjai et al., 2010), resulting in decreased tensile bond strength to dentin (Kato et al., 1996, Kato et al., 1998, Nakabayashi et al., 2000, Piemjai et al., 2001) , induced microleakage (Piemjai et al., 2004, Piemjai et al., 2010, Piemjai et al., 2011a) and reduced long-term bond durability (Garcia-Godoy et al., 2006). It is important to be able to identify defects in substrates for the long lasting function of the restored tooth. The microtensile bond strength testing (Phrukkanon et al., 1998a, Phrukkanon et al., 1998b, Sano et al., 1994a, Sano et al., 1994b, Sano et al., 1995) and the traditional tensile bond strength testing according to ISO recommended (ISO TR 11405, 1994E) of specimens that are water-stored for just 24 hours yield little useful indication of this non-resin impregnated demineralized dentin. Cohesive resin failures of tested specimens were often observed under SEM investigations, leading to misinterpretation even though existing of an unfilled zone at the base of the hybrid layer. However the mini-dumbbell shaped specimens for establishing true adhesive tensile bond strength and identifying defective non-resin impregnated demineralized dentin of restored dentin in the short time has been reported by several studies (Nakabayashi et al., 1998b, Nakabayashi et al., 2000, Nakabayashi, 2004a, Piemjai et al., 2001).The shape of mini-dumbbell specimen was designed to

distribute equal tensile stress across its cross-sectional bonded area. Then, stresses are direct to the weakest region of the test specimens, non-resin impregnated demineralized dentin, so the fracture of specimens initiates at this area. It was therefore hypothesized that non-resin impregnated demineralized dentin obtained from contemporary 3-step phosphoric etched dentin adhesives which were clearly identified by the mini-dumbbell shaped specimen tensile bond strength test and chemical challenge would be the weakest parts in bonded dentins in terms of mechanical strength, regardless of differences in chemical compositions in primer and bonding resin and technique to keep moist on etched dentin.

The objective of this study is to identify the remaining of demineralized dentin, the defect, in dentin restored with three contemporary total-etch dentin adhesives in which phosphoric acid is used as an etchant. The mini-dumbbell specimens shaped tensile bond strength test and chemical challenge on dentin-resin interface will be used for this evaluation.

Research Questions

1. Does demineralized dentin obtained from phosphoric acid etching followed by various contemporary total-etch dentin adhesives application create complete resin impregnated dentin-resin interface without the existence of demineralized dentin?

2. Are mini-dumbbell tensile bond strength test together with chemical challenge on dentin-resin interface the standardized methods to clearly identify non-resin impregnated demineralized dentin, the defect , which is the weakest parts in terms of mechanical strength ?

3. Are there any relations in the mini-dumbbell shaped specimens tensile bond strength values and characteristics of dentin-resin interface before and after chemical challenge obtained from each type of contemporary total-etch dentin adhesives studied in which phosphoric acid is used as an etching agent?

Objectives

The purpose of this study is to

Identify the remaining of demineralized dentin, the defect, in dentin restored with three contemporary 3-step phosphoric etched dentin adhesives using mini-dumbbell shaped tensile test and chemical challenge on dentin-resin interface.

Research hypothesis

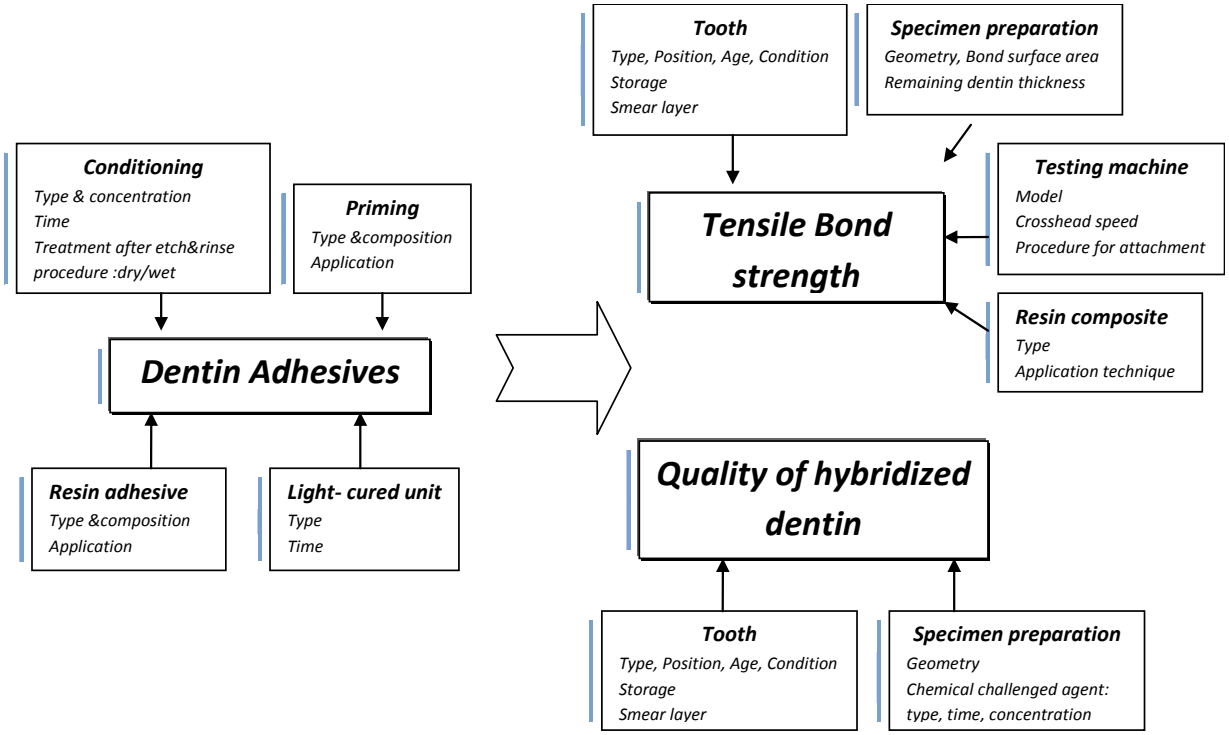
H_0: There are no differences in tensile bond strength values for the three contemporary total-etch dentin adhesives studied using the mini-dumbbell shaped specimens tensile bond strength test.

H_a: There are differences of tensile bond strength values for the three contemporary total-etch dentin adhesives studied using the mini-dumbbell shaped specimens tensile bond strength test.

H_o: There are no differences in thickness of dentin-resin interface between each of the contemporary total-etch dentin adhesive studied before and after chemical modification.

H_a: There are differences in thickness of dentin-resin interface of each contemporary total-etch dentin adhesive studied before and after chemical modification.

Conceptual framework



Assumption

The present study is an experimental research which was designed to measure the tensile bond strength values and determine the characterization of dentin-resin interface of phosphoric etched dentin restored with only three total-etch dentin adhesives (All-Bond 3®, OptiBond FL® and Scotchbond Multi-Purpose®).

Keywords

dentin-resin interface , mini-dumbbell shaped specimen, tensile bond strength, phosphoric acid etched dentin, 3-step adhesive

Research design

Experimental research

Limitations

1. This study uses experimental research methods, in which some factors such as temperature, humidity, and pulpal fluid flow cannot be controlled to be identical to the human oral cavity.

2. The research process, the collection of the data and the data analysis of this study were implemented by one researcher using the same apparatus throughout the entire study at the room temperature (25±3 ^{0}C).

Expected Benefits and Applications

The results of this study would confirm the effectiveness of the mini-dumbbell shaped specimen tensile bond strength test and the advantage of chemical challenge on dentin-resin interface which would be able to measure true tensile bond strength values and identify the presence of potentially defective non-resin-impregnated demineralized dentin regions which is the weakest zone that is susceptible to long-term degradation. Moreover, these results may provide valuable information in understanding of the characterization of wet demineralized dentin when phosphoric acid is used as an etching agent. Hence clinicians should realize how to prepare a good dentin substrate for forming a stable and reliable dentin-resin interface.

CHAPTER II

LITERATURE REVIEW

Concept, Theory and Relevant research

Micromechanical retention which is a reliable procedure for bonding resin to dentin is achieved by hybridization of dentin substrates. This procedure has been described by diffusion, impregnation and polymerization of adhesive monomer into the demineralized intertubular dentin, collagen-rich fibrous network, creating a zone of resin-impregnated dentin termed the “hybrid layer” or interdiffusion zone (Nakabayashi et al., 1982). Nakabayashi preferred to characterize hybridization as “molecular entanglement of dental components with resin copolymer chain in the hybrid layer”.

The existence of a hybrid layer along the resin-dentin interface has been demonstrated both in extracted dentin (Gwinnett et al., 1992c, Nakabayashi et al., 2000, Sarr et al., 2010, Tay et al., 1994) and in vital human dentin (Gwinnett et al., 1992b, Nakabayashi et al., 1992a, Tay et al., 1994) under both SEM and TEM. Intertubular resin permeation has appeared to be affected to a greater extent than intratubular permeation when the demineralized collagen network was desiccated prior to primer application, the reverse happened when dentinal tubules and the intertubular collagen network were saturated with water (Gwinnett, 1994, Tay et al., 1994c).

The properties of hybrid layer

There is no linear correlation between the thickness of the hybrid layer and the bond strength. It has been reported that the quality of hybridization, rather than the thickness of the hybrid layer per se has been suggested as a more significant

parameter in the success related with the use of contemporary adhesives that involve acid-conditioning of dentin surface (Kato et al., 1996, Kato et al., 1998). The attachment of resin to intertubular demineralized dentin plays more critical role in the development of overall bond strength (Gwinnett, 1992b, Gwinnett, 1994, Tay et al., 1994c). The quantitative contribution of resin infiltration of intertubular dentin to be approximately two thirds and the contribution of tubule tags to be approximate one third of the total shear bond strength (Gwinnett, 1993, Ferrari et al., 1996). The lateral branches which appear only in the first part of the tubules and were situated just beneath the hybrid layer may add to the bond strength (Ferrari et al., 1996). The acid-resistance of the "hybrid" layer (Nakabayashi et al., 1992b, Piemjai et al., 2001, Piemjai et al., 2010) reduced the incidence of secondary caries and post-operative hypersensitivity. Furthermore, the layer of closely packed collagen fibrils of hybridized dentin also eliminated microleakage (Piemjai et al., 2004, Piemjai et al., 2010, Piemjai et al., 2011a, Piemjai et al., 2011b) because of an inherent elastic buffering mechanism to compensate for the polymerization contraction of the restorative resin. These are thought to be essential in maintaining the durability of adhesive bonds to human dentin.

The essential factors for the hybridization of resin in the dental substrate

Bonding to dentin via the formation of hybridized dentin is significantly dependent on the monomer permeability of the substrate and the diffusion potential (diffusivity) of the monomers. The former is a characteristic of tooth substance which is altered by pretreatments an smear layer while, the later is a feature of dentin adhesive materials (Nakabayashi et al., 1982, Nakabayashi et al., 1998a).

1) The improvement of monomer permeability of the substrate

Maintenance of the structural integrity of the collagen fibrils and opening of the interfibrillar micro-channels within the demineralized network facilitated optimal permeation of adhesive monomers is important for systems that depend on the hybridization concept. First, the presence of cations such as Fe^{3+}, Ca^{2+} dissolved in pure acid tends to prevent the solubilization of noncollagenous polyelectrolytes during etching of dentin by either the cation forms insoluble salts of polyelectrolytes or that they crosslink the polyelectrolytes, then prevent the collapse of collagen following air drying (Iwasaki et al., 2004, Piemjai et al., 2003). Second, the use of HEMA to reverse or re-expand a collapsed demineralized dentin surface (Nakabayashi et al., 1992b, Nakabayashi et al., 2004b) has been reported for improving the penetrability and diffusivity of the demineralized dentin whose peptides were denatured. Lastly, maintaining wet substrates that had been etched with phosphoric acid to prevent the collapse of acid-etched dentin, then decreasing the potentially harmful water content of the acid demineralized dentin by applying an acetone-containing primer to promote the penetration of adhesive monomer, based on wet bonding protocol has been proposed (Gwinnett, 1992c, Kanca, 1992).

2) The improvement of diffusivity of monomer

To increase the wettability properties of the primer has been suggested for improving the diffusivity of monomer. For example, methacrylate group such as 4-META, phenyl-P in primer which contains both hydrophobic and hydrophilic groups on the ester molecule improves the adhesive strength of resin to teeth by promoting interpenetration, impregnation and entanglement of the hydrophilic methacrylate- based monomers into dentinal substrates and their polymerization therein.

The removal of smear layer

The smear layer which consists of pulverized dentin covers the dentin after cavity preparation. Two distinctly different approaches have been followed to bond restorative resin materials to dentin. The first preserves and/or modifies the smear layer, while the second advocates its removal. However studies on bond strength to enamel or dentin have showed that there was significant difference in bond strength exists between current generation bonding those system which preserved and / or modify smear layer and those which remove it prior to the placement of primers, bonding agent and composite (Bouillaquet et al., 2001, De Munck et al., 2003, Gwinnett et al., 1992b, Inoue et al., 2001, Sarr et al., 2010). The absence of the conditioning step failed to produce an acid resistant hybrid zone at the resin-dentin interface. It can be indicated that the smear layer interferes with adhesion to dentin via reducing dentin permeability. So, it is necessary to remove this layer to obtain more reliable and higher bond strengths.

Phosphoric acid has been widely used in restorative dentistry as an etching agent to remove smear layer for enamel (Buonocore, 1955) and later for dentin (Fusayama et al., 1979) because it is familiar to the clinicians, readily available and inexpensive to manufacture. Many studies reported higher bond strength when several hydrophilic primers were applied to dentins that had been acid etched with phosphoric acid (De Munck et al., 2003, Gwinnett et al., 1992b, Sarr et al., 2010). Etching dentin increases bond strength because it removes the smear layer, opens the dentinal tubules, increases the microporosities of intertubular dentin and lets the resin materials penetrate into the decalcified, forming “a hybrid layer” which contributes to adhesion. The used of phosphoric acid as a dentin-enamel conditioner in vitro (Gwinnett et al., 1992a, Gwinnett et al., 1992b) and in vivo (Ferrari et al., 1996, Gwinnett et al., 1992a, Walshaw et al., 1995) showed that there was no gap formation along the resin-dentin interface via a

layer of resin-impregnated demineralized dentin which was clearly revealed in the subsurface of a wet-bonded dentin substrate, essentially the same as the hybrid layer created by 4-META/MMA-TBB resin that described by Nakabayashi. A layer of resin-impregnated dentin along the resin-dentin interface measuring from 2 to 8 micrometers in width under the SEM of in vivo specimens formed a barrier with the potential of blocking the entrance of micro-organisms to the underlying dentin. Therefore, an effective seal following total etching was established. The use of phosphoric acid as dentin conditioner can be clinical indicated even in deep lesion because it demonstrated in preserving the biological and morphological integrity of the pulpo-dentinal complex (Gwinnett et al., 1992a, Tay et al., 1994). However when dentin is demineralized by pre-conditioning, the character and micromorphology of demineralized dentin has an important influence on penetration by subsequently applied adhesive monomer (Marshall et al., 1995). This variable nature of demineralized dentin makes bonding complicated.

On the other hand, several SEM examinations of cross-sectional sample showed that applied monomers did not diffuse into dentin substrates pre-treated with the “pure” acid either phosphoric or citric acid (Marshall et al., 1995, Pashley et al., 1993, Nakabayashi et al., 2000). The dentin-resin interfaces obtained from phosphoric etched moist dentins restored with various total-etch adhesives cannot resist the action of strong acid (HCl) and the longer immersion times in the alkaline (NaOCl) solution (Piemjai et al., 2010). The tensile bond strength decreased significantly while cohesive failures in demineralized dentin increased in the phosphoric etched moist dentin specimens which soaked in water for 1 month, 6 months and 1 year of water immersion even 4-META in acetone primer was employed (Kato et al., 1998). The severe microleakage accessed by penetration of dye and silver nitrate solution at the dentin-resin interface of phosphoric

etched dentins restored with total-etch adhesives such as All-Bond 2 (3-step) , Single-Bond 2 (2-step) and Variolink II cement even wet bonding followed by direct and indirect resin composite restoration, respectively were reported (Piemjai et al., 2010, Piemjai et al., 2011a). These results suggested that phosphoric etched-dentin adhesives are unable to provide an impermeable interface that serves as a defect in the adhesive interface. The explanation is that the channels between the phosphoric-denatuerd collagen fibers were difficult to preserve so adhesive monomer impregnation of these substrates was impaired. Consequently some demineralized dentin did not converted to hybridized dentin, an exposed collagenous, demineralized dentin remained between hybridized dentin and the unaltered, still mineralized dentin causing defects in the bonded specimens. TEM examination demonstrated stainable exposed collagen fibrils in the remaining demineralized dentin which correlations with zones of incompletely infiltrated demineralized dentin (Piemjai et al., 2004). The bonding durability might be expected to be short-lived due to hydrolytic degradation of the exposed and non-resin-impregnated collagen (Gracia-Godoy et al., 2006).

It has been reported that after dentin etching, the solubility of collagen peptides are increased due to the denaturing of collagen fibrils (Asmussen et al., 1993). These solubilized peptides may contribute to collapse of the matrix during air drying so that interfibrillar spaces between exposed collagen fibrils were eliminated. Another reason is that phosphoric acid etching may increase the concentration of dissolved dentinal substances such as the polyelectrotyes, as it dissolves the denatured collagen. However these dissolved dentinal polyelectrolytes which stay around collagen fiber are too large to be rinsed out from the demineralized regions before their degradation. This might be the reason why the demineralized dentin shows a hydrophilic character (Iwasaki et al., 2004, Piemjai et al., 2003). Hydrophilic agents and cationic ion such as

Fe ions added to the citric acid and 10% phosphoric acid, calcium ions dissolved in 10% phosphoric 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 under AFM observation (Iwasaki et al., 2004, Nakabayashi et al., 2004b). The permeability of demineralized dentin for facilitating optimal permeation of adhesive monomers and increasing the rate of dehydration with the acetone primer consequently, improved TBS and good hybridized dentin with higher resin content were result (Nakabayashi et al., 2004b).

Adhesives system

A number of new adhesive systems have been developed in an attempt to reduce the steps and simplify clinical bonding procedure. Two major adhesives systems can be classified according to management of smear layer and numbers of clinical steps. The first which utilizes the total-etching technique to simultaneously remove the smear layers from both enamel and dentin surfaces is called “total-etch adhesive” They can be employ two steps or a single one, depending on the way the acidic primer and bonding resin are provided by the manufacturer. Traditional total-etch bonding system are three-step system (eg. All-Bond 3, OptiBond FL, Scotchbond Multi-Purpose). First, acids or calcium chelating solutions are used to completely remove the smear layer and partially demineralize the surface layer of the tooth substrates. This must be followed by application of a hydrophilic primer (acetone- or alcohol- containing primer resins) and adhesive resin on wet dentin. During priming, hydrophilic monomers that diffuse across the demineralized dentin stabilize the hydrated collagen network and displace water with polymerizable monomers. Lastly, the adhesive resins are applied to the primed dentin and polymerized to form a hybrid layer for micromechanical retention. While, two-step total-etch adhesives combine the primer and adhesive resin into one application. The second approach which is based upon the simultaneous etching and

priming of the smear–covered dentin using an acidic primer followed by the application of an adhesive resin is called “Self-etching adhesives”.

3-step total-etch adhesive system

Many studies reported that the highest microtensile bond strength was obtained with the conventional three-step etch&rinse adhesive, OptiBond FL, and the lowest with one-step self-etch adhesive (Adper prompt L-pop). Pretesting failures also occurred with the one-step self-etch adhesive Adper Prompt L-pop and NRC/Prime&Bond NT (De Munck et al., 2003, Inoue et al., 2001, Sarr et al., 2010). There is no difference in bonding effectiveness of two- and three-step etch&rinse adhesives when tested to flat surfaces and in the short-term. However three-step etch&rinse adhesives easily outperform their two-step equivalents in complex cavities or in the long-term (De Munck et al., 2003).

Nevertheless, there are several concerns of all etch&rinse adhesive. First, the risk of over-etching dentin due to the rather aggressive acid-etching procedure (commonly 30-40% phosphoric acid). Excessive etching of the dentin produces weak bonding because collagen fibers at the base of the demineralized dentin are not completely impregnated by the resin (Marshall et al., 1995, Pashley et al., 1993). Second, the requirement of a post-conditioning rinse phase, this is a very technique-sensitive step, where the operator should take care not to “under-“ or “over-dry” the dentin surface as both are known to result in impaired bonding effectiveness (Gwinnett, 1992c, Tay et al., 1996b, Tay et al., 1996c). Third, a risk upon surface contamination, especially if no rubber-dam is used. Finally, the three-step bonding procedures described so far may take up to 2 minutes, it is therefore that the so-called one bottle system (two-step bonding systems, primer-adhesive) which combine the functions of the primer and the adhesive have become very popular today.

2-step total-etch adhesive (Self-priming adhesive)

Two-step total-etch adhesives combine the primer (hydrophilic substances such as HEMA or PENTA) and unfilled or lightly filled low-viscosity adhesive resin in order to reduce the number of clinical application steps from three to two, thus a simplification of the clinical procedure. Even though these materials are called 'single-bottle' or 'one-step' adhesives, they require a separate conditioning step. This technique has been advocated as a safe and effective method to achieve significant adhesion to dentin. Gap-free restorations (Gwinnett et al., 1992a, Gwinnett, 1992c, Tay et al., 1995) and high dentin bond strengths (Gwinnett, 1992c, Kanca, 1992) have been reported for this system. Nevertheless, one major reason of the inferior effectiveness of two-step total-etch adhesives compared with conventional three-step total-etch adhesive might be the lack of sufficient resin thickness provided with one-bottle adhesive due to containing significantly more solvent. Thus, one must take care that the combined primer/adhesive resin is copiously applied, producing a sufficiently thick and glossy film without "dry" spot. Then elastic stress absorber is created to partially compensate for polymerization shrinkage (Inoue et al., 2001). Addition of fillers for increasing the viscosity helps clinicians would not over thin the adhesive and radiopacity can also be improved.

Filled versus Unfilled adhesive resin

The importance of filler particles in some adhesive is somewhat controversial. Incorporation of filler particles into the bonding resin not only may promote the formation of adhesive films with appropriate thickness but also reduce the shrinkage of the adhesive resulting in less microleakage. It has been proposed that the high μTBS of OptiBond FL, compared with unfilled resin as Scotchbond Multi-Purpose, should probably also be attributed to the high filler loading of the adhesive resin, when applied in sufficient thickness. The highly glass-filled adhesive resin of the OptiBond FL provides an elastic buffer zone beneath the restorative materials that may offer the resin

dentin interface a sufficient strain capacity to accommodate tensions generated by the shrinking stresses created during polymerization of the composite using a light-activation mode (Sarr et al., 2010). This also better distributes stresses induced by thermal changes, water sorption, and occlusal loads across the interface (Cadoso et al., 2001, Nunes et al., 2001). Additionally, adhesives with high densities and viscosities can also show high wettability, low contact angles and adequate infiltration of monomer into dentin (Toledano et al., 2003). Nanofiller present in Prime&Bond NT does not increase the adhesive viscosity or accumulate on the surface but may be able to infiltrate the tubules and the demineralized intertubular dentin, especially the spaces between collagen fibrils and increase the strength of the adhesive joint (Nunes et al., 2001).

On the contrary, the study of Nunes showed that the two-step total-etch dentin adhesives tested, Single Bond, did not provide higher bond strength with the addition of filler particles. This result reinforces the hypothesis that filler in the adhesive resin might decrease the wetting of the primed dentin surface because of the higher viscosity of filled resin. This would decrease the penetration of resin monomers resulting in the existence of internal voids (defective hybrid layer) thus reducing the shear bond strength (Miyazaki et al., 1995, Nunes et al., 2001). Only 10% filler content which was therefore necessary to increase the bond strength and improve the mechanical properties of bonding agents was suggested (Miyazaki et al., 1995). Furthermore, during long periods of storage, it is possible that a filled resin may convert to a higher degree of conversion than an unfilled resin (Cardoso et al., 2001).

Bond strength Tests

In vitro bond strength test is the most popular screening method of quantitative bonding effectiveness measurements used to compare dentin bonding systems. The difference in testing assembly for measuring adhesion which has been proposed may contribute to that variability. Besides that, inherent characteristics of dentin such as density of tubules, inorganic content, moisture condition and surface preparation may also explain the variation in bond strengths values.

Microtensile bond strength test

Microtensile testing takes advantage of the fact that there is an inverse relationship between dentin and enamel μTBS and cross-sectional area. This relationship explained that the tensile strength characteristics of homogenous brittle materials were affected by the quantity of defects within the system. When the specimen is loaded, stress concentration occurs at the defects and initiates crack formation. The small cross sectional bonded area has led to improved stress distribution and to materials failure at forces that more closely approach their ultimate strength. Sano recommended testing a surface are of 1.6-1.8 mm^2which corresponds to the bonding area size reported by Phrukkanon. (Phrukkanon et al., 1998a, Phrukkanon et al., 1998b, Sano et al., 1994a, Sano et al., 1994b, Sano et al., 1995). The strength valves obtained using microtensile testing technique were significantly higher than those obtained with shear bond technique, resulting from fewer flaw such as air bubbles, water blisters, or region of resin-solvent phase separations which can serve as stress concentrators during bond testing in small specimens. However the rectangular cross-sectional shape of these specimens is not ideal for testing bond strengths because of the unequal distribution of stress across the adhesive interface. Moreover, the rectangular constriction at the interface of these micro-specimens prepared by hand using a dental handpiece is not

only laborious, but it also largely depends on the skills of the operator, thereby introducing a defect at the interface. This may lead to lower bond strength values or even pre-testing failures (Nunes et al., 2001).

Later, the preparation of cylindrical bond area was introduced because of better stress distribution at the interface area. Using finite element analysis, an hour-glass cylindrical shaped tensile specimen helped the loading stress concentrate at the narrowest part of the specimen, namely the bonded interface. The stress from tensile loading will concentrate at the circumference of the specimen and will be distributed in an even pattern though to the center of the bonded interface (Phrukkanon et al., 1998b). Another reason is the ease of specimen processing which compensates for dentin irregularities and potential internal interface defects causing pre-testing failures.

Microtensile testing permitted multiple specimens be prepared from a single tooth, resulting in better controlled substrate variables (Sano et al., 1994a, Sano et al., 1994b). Small surface area bonding can be used to investigate different parts of the tooth or teeth which were affected by disease such as dental caries. However the yield rate to harvest the test specimens is important, as the size of the tested specimen is too small to trim for the microtensile test. Therefore zero (spontaneously debonded pre-test) bond strength data which gives valuable information and serves as a pointer for improving bonding conditions.

Miniaturized dumbbell and Mini-dumbbell shaped specimen tensile test

The recommended method which measures bond strength to tooth substrates in ISO (ISO TR 11405, 1994E) was not good enough because it does not control the size of substrates as the cross-sectional area of substrates is wider than that of bonded interface which is not suitable to identify defect created in the substrate created during bonding procedures. Cohesive failure within the resin occurred more easily due to stress concentration in the resin near the adhesive interface, even if such defect existed.

Nakabayashi proposed miniaturized dumbbell specimens (Nakabayashi et al., 1998b), cross-section of 7x2 mm^2 at the rod part, based on a Japanese Industrial standard (JIS) K-6911 (1994), Specifications of American Society for testing and Materials (ASTM) D-638-95 (1995) and International Organization for Standardization (ISO) 527-1 (1993) or thermosetting plastics to measure TBS to human dentin. The cross-section of these dumbbell specimens is smallest at the adhesive interface and stresses are directed to it so that fracture of the specimens initiates at the weakest region of the tested interface. This method eliminates most of the cohesive resin and dentin fractures seen in more traditional tensile strength test procedures that are due to non-uniform stress concentrations. Both TBS and SEM examinations of many studies confirmed the superiority of using dumbbell specimens to clearly reveal defects within bonded specimen (Nakabayashi et al., 2000, Piemjai et al., 2001). Comparison of the TBS data for specimens acid-etched for 10 seconds versus 60 seconds with 10-3 solution showed that TBS of 60s group was only 12 MPa and specimen failure clearly occurred within the region of non-resin-impregnated demineralized dentin. The stainable collagen fibrils which are visible in the demineralized dentin between the hybrid layer and the underlying intact mineralized dentin under TEM examination was more extensive in 60s than that in 10s specimens. While, the 10s group showed cohesive fracture in the

cured resin with high TBS. These results demonstrate the sensitivity of the test methodology to the presence of non-resin-impregnated demineralized dentin which is the weakest point in terms of mechanical strength (Nakabayashi et al., 1998b). Specimens in the dumbbell shape reduce the effect of a defect within the specimens that could create weak points so that specimen fracture must take place at that weak site. The cutting and trimming procedures necessary to fashion miniaturized dumbbell-shaped specimens have not an adverse effect on the TBS of prepared specimens, because this would not tend to lower bond strength. TBS determination of miniaturized dumbbell-shaped specimens is very good for demonstrating adhesive interface defects in the short time. And it is also a simple and reproducible test procedure (Nakabayashi et al., 1998b, Nakabayashi, 2004a).

Unfortunately, fabrication of 7x2 miniaturized dumbbell shaped specimen out of bonded human dentin was too difficult as the widespread availability of such large teeth was poor, the smaller sized specimens (3x2mm^2), making a mini-dumbbell with a 3x2x1.2 mm^3 rod whilst keeping the shape of the curves might be more convenient for the measurement of TBS using this technique. The differences of specimen size and shape between the mini-dumbbell of bovine and the miniaturized dumbbell of human dentin did not show a statistically significant influence on the TBS (Piemjai et al., 2003).

CHAPTER III

METHODOLOGY

1. Tensile bond strength test

1.1 Tooth preparation

Thirty-six non-carious human molars used within 6 months after extraction were cleaned and stored in distilled water at -4 ^{0}C.These teeth were randomly divided into 3 groups of 12 specimens each. Two millimeters below the central groove of the coronal part of tested molars were sectioned off with an Isomet low-speed diamond saw (Isomet 1000, Buchler, Lake Bluff, IL, USA) at a speed of 450 cycles/min under water cooling to expose dentin surfaces (Figure 1). A uniform smear layer was created using a diamond wheel-shaped 100 microns bur (111 Intensive, Grancia Switzerland) with a high-speed handpiece which was attached by a dental surveyor under copious air-water spray (Figure 2). A rectangular polymethyl methacrylate (PMMA) frame with inner dimension of 3x7x1.5 mm was use to standardize the bonding area of dentin.

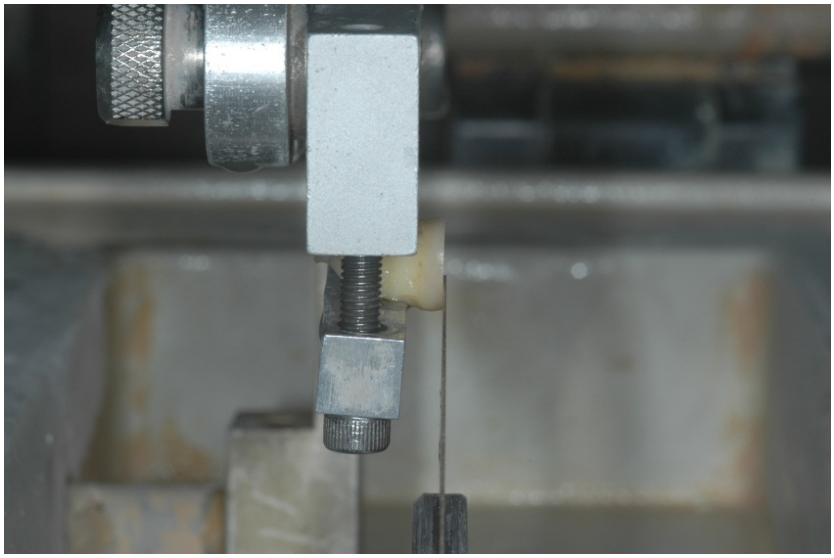


Figure 1. Sectioning method on coronal half of a tested molar to expose dentin

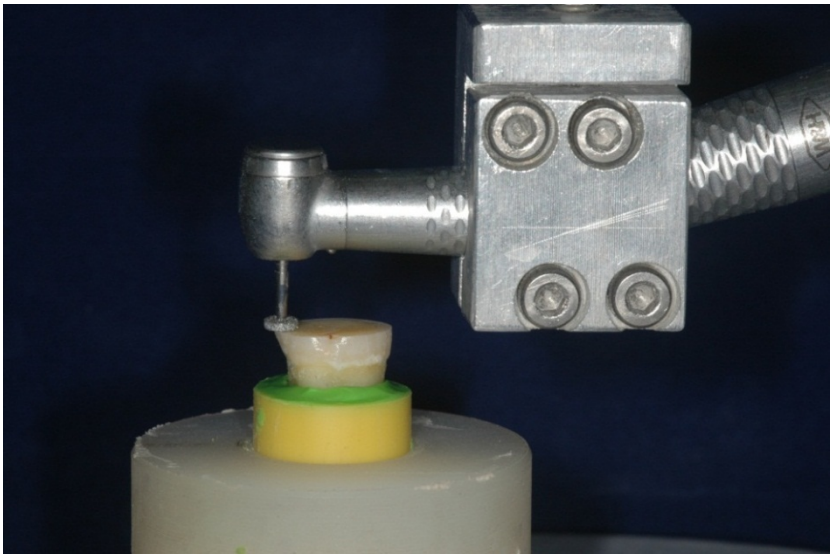


Figure 2. Preparation of a uniform smear layer using a diamond wheel-shaped bur with a high-speed handpiece

1.2 Bonding procedure

Three total-etch dentin adhesives which are All-Bond 3® (BISCO, Inc, Schaumburg, IL, USA), OptiBond FL® (Kerr, Orange, CA, USA) and Scotchbond Multi-Purpose® (3M ESPE, Seefeld, Germany) were tested. Twelve teeth were used for each adhesive. The adhesives were applied on the dentin substrates, according to the manufacturers' recommendations (Table 1). Then, a resin composite (Metafil CX®, Sun medical Co, Ltd, Kyoto, Japan) was built up using incremental technique. Each layer (except third layer) was polymerized with light-cured for 40 seconds with a 3M Curing Light XL 3000 (3M dental Product MN, USA). A rectangular PMMA rod (7x7x2 deep mm) which was polished with 180 grit paper disc for 30 seconds, grooved with diamond inverted shaped bur (Intensive, Grancia Switzerland) and cleaned with an ultrasonic cleaner (5210, Bransonic Germany) was bonded to the third layer of uncured-resin composite by 4-META/MMA-TBB resin (SuperBond C&B®, Sun medical Co, Ltd, Kyoto, Japan) using a brush dip technique and then 40 seconds light-curing (Figure 3).

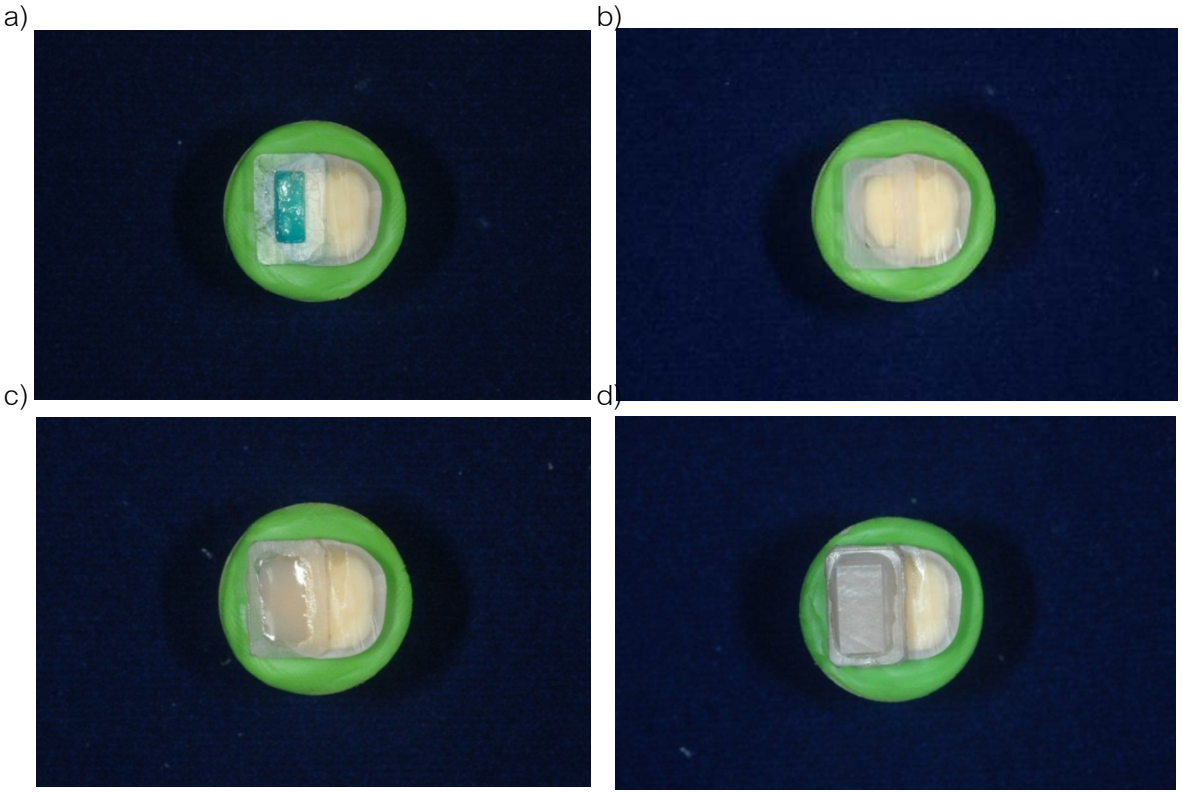


Figure 3. Bonding procedure of Scotchbond Multi-Purpose® a) conditioning with 35% phosphoric acid b) Priming and bonding following the manufacturers' recommendation c) restoring with light-cured resin composite using an incremental technique d) bonding to PMMA rectangular rod with 4-META/MMA-TBB resin

Table1. Composition and instruction of the used dental adhesives recommended by the manufacturers

Adhesives	Category	Composition	Instructions for use
All-Bond 3 (BISCO,Inc)	Three-step Etch&rinse	Conditioner: 32% phosphoric acid, benzalkonium chloride Primer part A: Ethanol and glycine-glycidyl methacrylate salt Primer part B: Bis-GMA, BPDM, HEMA Adhesive: Bis-GMA, UDMA, TEGDMA, glass frit	Apply the conditioner to the dentin surface for 15 seconds. Rinse for 15 seconds, remove excess water using a foam pellet, leaving a visible moist. Mixing primer part A and part B (1:1) until the achievement of an uniform color for 5 seconds. Application of 1-2 coats of the primer. Air dry gently until there is no visible movement of material and light-cure for 10 seconds. Apply one thin coat of the adhesive resin and light cure for 10 seconds.
OptiBond FL (Kerr, Orange, CA, USA)	Three-step Etch&rinse	Conditioner: 37.5% phosphoric acid Primer: HEMA, GPDM, MMEP, CQ, ethanol, water, BHT Adhesive: Bis-GMA, HEMA, GDMA, filler (Barium aluminoborosilicate, silica), CQ	Apply the conditioner to the dentin surface for 15 seconds. Rinse for 15 seconds. Gently air dry for a few seconds being careful not to desiccate dentin. Scrub the surface for 15 seconds with the primer. Air dry gently for 5 seconds. Apply a thin coat of the adhesive with scrubbing motion for 15 seconds and light cure for 20 seconds.
Scotchbond MP (3 M ESPE, Seefeld, Germany)	Three-step Etch&rinse	Conditioner: 35% phosphoric acid, water, silica Primer: water, HEMA, polyalkenoic acid polymer Adhesive: BisGMA, HEMA, CQ, EDMAB, DHEPT.	Apply the conditioner to the dentin surface for 15 seconds. Rinse for 15 seconds and gently dry for 5 seconds. Leave moist. Apply the primer to etched dentin. Dry gently for 5 seconds. Apply the adhesive and light cure for 10 seconds.

Abbreviations BHT: butylhydroxytoluene, Bis-GMA: bisphenol "A" diglycidyl methacrylate, BPDM:biphenyl dimethacrylate, CQ: camphorquinone, DHEPT: N,N-di-(2-hydroxyethyl)-4-toluidine, EDMAB: ethyl 4-dimethyl amino benzoate, GPDM: glycerol phosphate dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, MMEP: mono-2-methacryloyloxyethyl phthalate, TEGDMA: triethylene glycol dimethacrylate UDMA:urethane dimethacrylate

1.3 Tensile bond strength testing on mini-dumbbell specimens

All bonded samples were allowed to stand at room temperature for 15 min and were then serially sectioned vertically using an Isomet low-speed diamond saw at a speed of 450 cycles/min under cooling water to make a 2.0 mm thick bonded dentin slab (Figure 4). All bonded slabs (36 slabs) were trimmed into mini-dumbbell shaped specimens (cross-sectional area of 3x2mm^2 at the bonded interface) using diamond fissure burs (Intensive, Grancia, Switzerland) with a high-speed handpiece under copious air-water spray. One mini-dumbbell specimen was harvested from one tooth (Figure 5). All specimens were stored in water at 37 ^{0}C for 24 hours and allowed to dry at room temperature before tensile testing. The PMMA jigs were affixed with 4-META/MMA-TBB resin to the dentin end of prepared mini-dumbbell specimens, while the PMMA rod end of prepared mini-dumbbell specimens were affixed with self-cured acrylic resin (Unifast, Gc Corporation, Tokyo, Japan) (Figure 6). The assembled specimens were then tensile stressed to failure using a Shimadzu Compact Table-Top Universal Testing Machine (model EZ TEST) at a crosshead speed of 1 mm/min (Figure 7). The cross-sectional area at the bonded interface of the fractured specimens was re-measured with a digital caliper (Digimatic caliper series 500, Mitutoyo, Tokyo, Japan). The maximum tensile breaking forces in Newtons were recorded and calculated in MPa.

$$\text{Tensile bond strength (MPa)} = \frac{\text{The maximum tensile breaking forces (Newtons)}}{\text{The cross – sectional area at the bonded interface of the fractured specimens}}$$

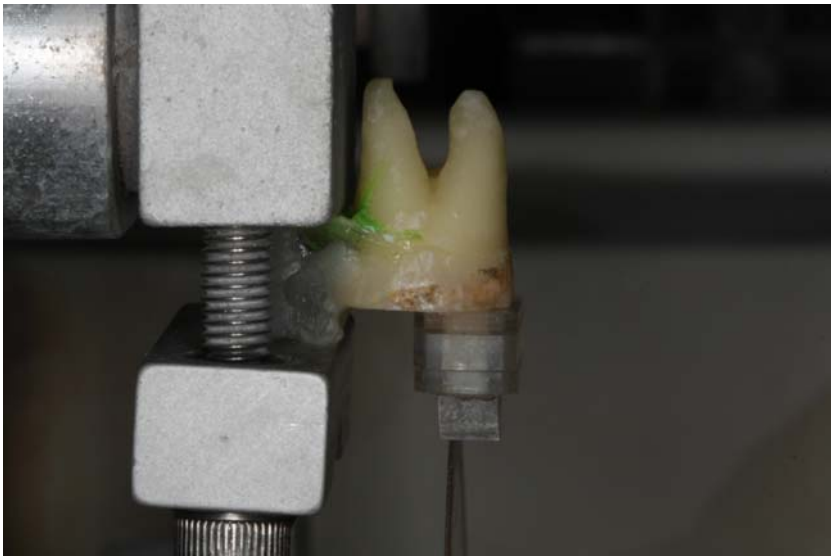
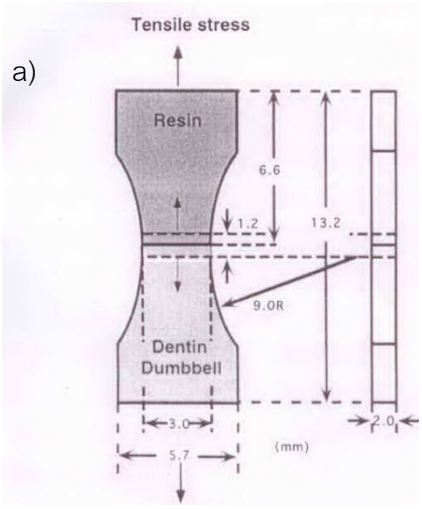


Figure 4. Vertical section to create a 2.0 mm thick bonded dentin slab

a)



b)

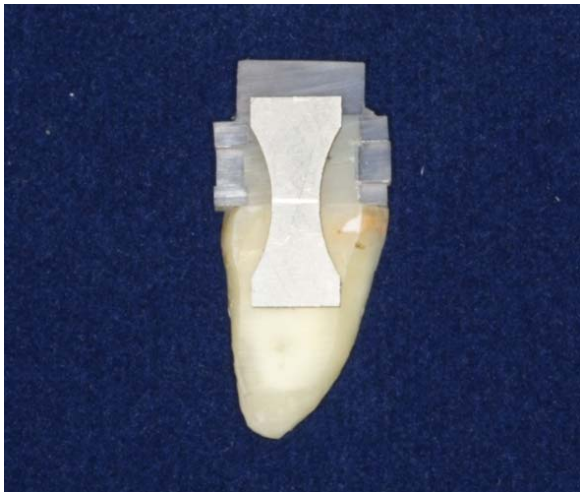


Figure 5. a) Schematic illustration of mini-dumbbell specimen (Nakabayashi, 2004a) b) Using a mini-dumbbell shaped jig to create a uniform tested specimen

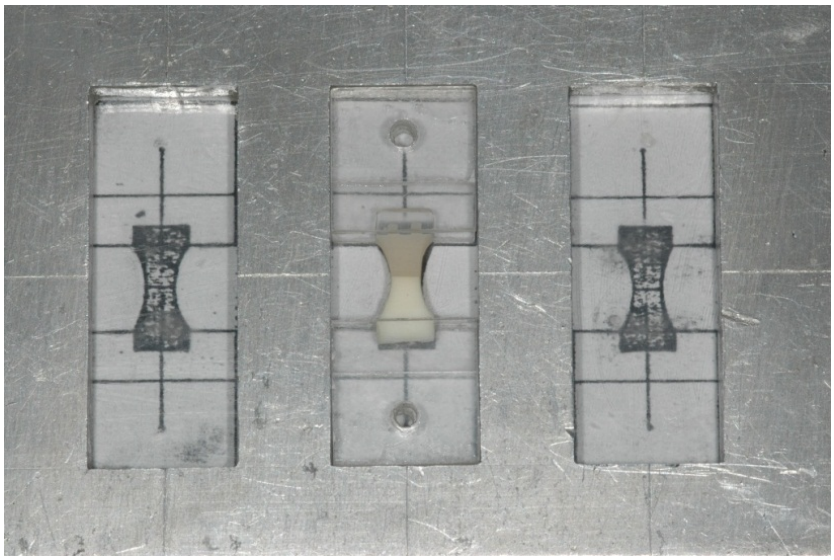


Figure 6. Technique to assemble a mini-dumbbell bonded specimen to PMMA jigs

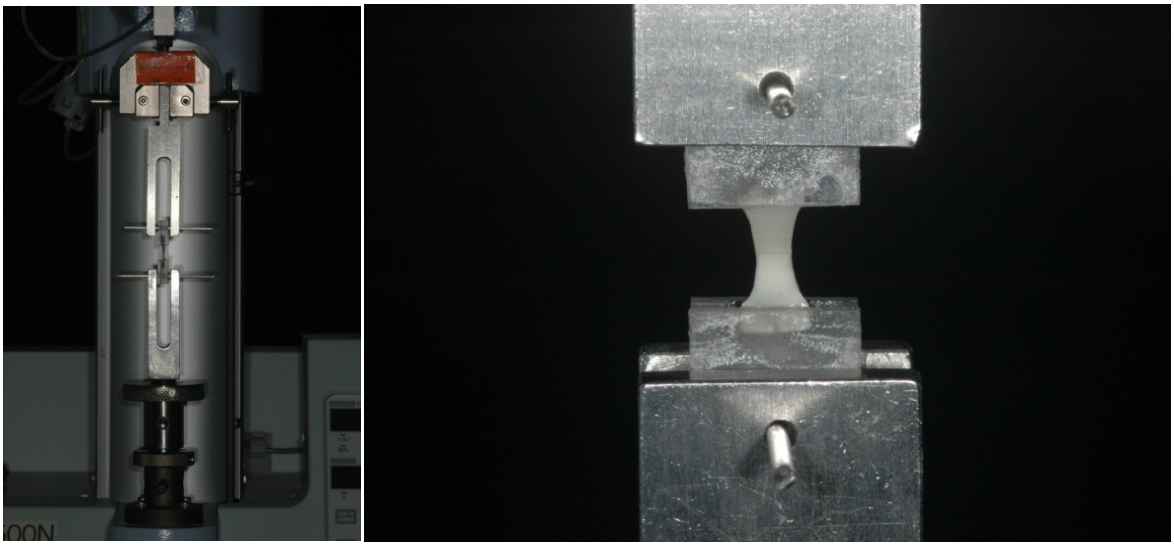


Figure 7. Mounting the assembled mini-dumbbell bonded specimen on a universal testing machine for tensile bond strength test

$$\frac{\text{The maximum tensile breaking forces (Newtons)}}{\text{The cross – sectional area at the bonded interface of the fractured specimens}}$$

All fractured surfaces resulting from tensile loading of mini-dumbbell specimens were gold-sputtered and examined under a scanning electron microscope (JSM 6400, JEOL company, Tokyo, Japan) at 35x and 1500x magnification to determine mode of failure.

2. Determination of quality of dentin-resin interface

Four dentin bonded slabs of each group (n=12) were prepared with the same procedures for tensile testing except only one layer of 2 mm thickness of resin composite was constructed. Each slab was perpendicularly sectioned to create two cross-sectional surface specimens. The cross-sectional surfaces of all specimens were sequentially smoothed by 600-1200 grit paper disk, polished with 0.05 μm alumina paste (Figure 8) and finally cleaned with an ultrasonic cleaner for 15 min. One specimen from each sample was then immersed in 6 mol/L HCL for 30 seconds, rinsed with water and challenged with 1% NaOCL for 60 min and totally rinsed off with water. All polished and challenged samples were slowly dehydrated in desiccators for at least 24 hours prior to scanning electron microscope investigation at 500x, 2000x and 5000x magnification to determine morphological appearances of dentin-resin interface and also measure its thickness before and after chemical modification.

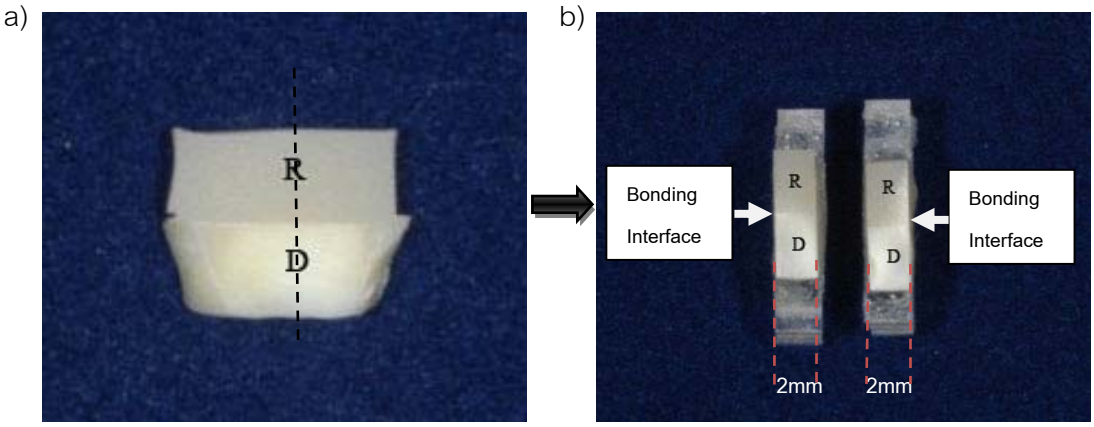


Figure 8. Preparation of specimens for determination of characterization of dentin resin interface: A 2.0×7.0 mm^2 bonding area of dentin bonded slab was vertically sectioned (a) to prepare 2 bonding interfaces (b) for chemical challenge test

3. Data collection and Statistical analysis

3.1 Data collection

3.1.1 Tensile bond strength test: the maximum tensile breaking forces in Newtons were calculated in MPa.

3.1.2 Determination of quality of dentin-resin interface: the thicknesses of dentin-resin interfaces before and after chemical modification were recorded under scanning electron microscope at 500x, 2000x and 5000x magnification.

3.2 Statistical analysis

3.2.1 Descriptive statistics: the mean values and standard deviations (SD) of tensile bond strength for all adhesives were calculated.

3.2.2 Inferential statistics: As the populations from which the samples were obtained are normally distributed (One-Sample Kolmogorov-Smirnov Test) and the variances of the populations are homogenous (Levene Test for Equality of Variances), one-way analysis of variance (one-way ANOVA) is used to analyze for significant differences. Then if the decision is to reject the null hypothesis, *Post hoc* multiple comparisons test is performed with Turkey HSD test with Statistic Package for the Social Science version 17.0 at the 95% confidence level to tell where the difference lies. However if the data do not fit the assumptions of an ANOVA, do a Kruskal-Wallis test instead of a one-way ANOVA.

CHAPTER IV

RESEARCH RESULT

The overall means and standard deviations (MPa) of tensile bond strength (TBS) of bonded mini-dumbbell shaped specimens and the fracture mode due to tensile loading are summarized in Table 2. One-way ANOVA demonstrated that there were no significant differences in tensile bond strengths among three groups.

Table2. Means ± standard deviations (MPa) of tensile bond strength and failure mode for all groups

Adhesives	Number of specimens	TBS(MPa)[a)]	Mode of failure[b)]
All-Bond 3	12	11.6 ±1.6	A+R+DD
OptiBond FL	12	12.3 ± 2.1	A+R+DD
Scotchbond MP	12	11.5 ± 1.9	A+R+DD

a) Values connected by vertical line are not significantly different at $p > 0.05$

b) A = Adhesive failure, R= Cohesive failure in the cured resin, DD= cohesive failure in remaining demineralized dentin, + = mixed failure of A, R and DD

SEM examination of the fractured dentin surfaces after tensile stressing is shown in Figure 9 to 11. Mixed adhesive failure and cohesive failure within the cured resin and remaining demineralized dentin were identified in all groups. Higher magnification of the adhesive failure area showed that adhesive failure occurred at both inter- and intra-tubular demineralized dentin (Figure 9a, 9b, 10a, 10b, 11a, 11b).

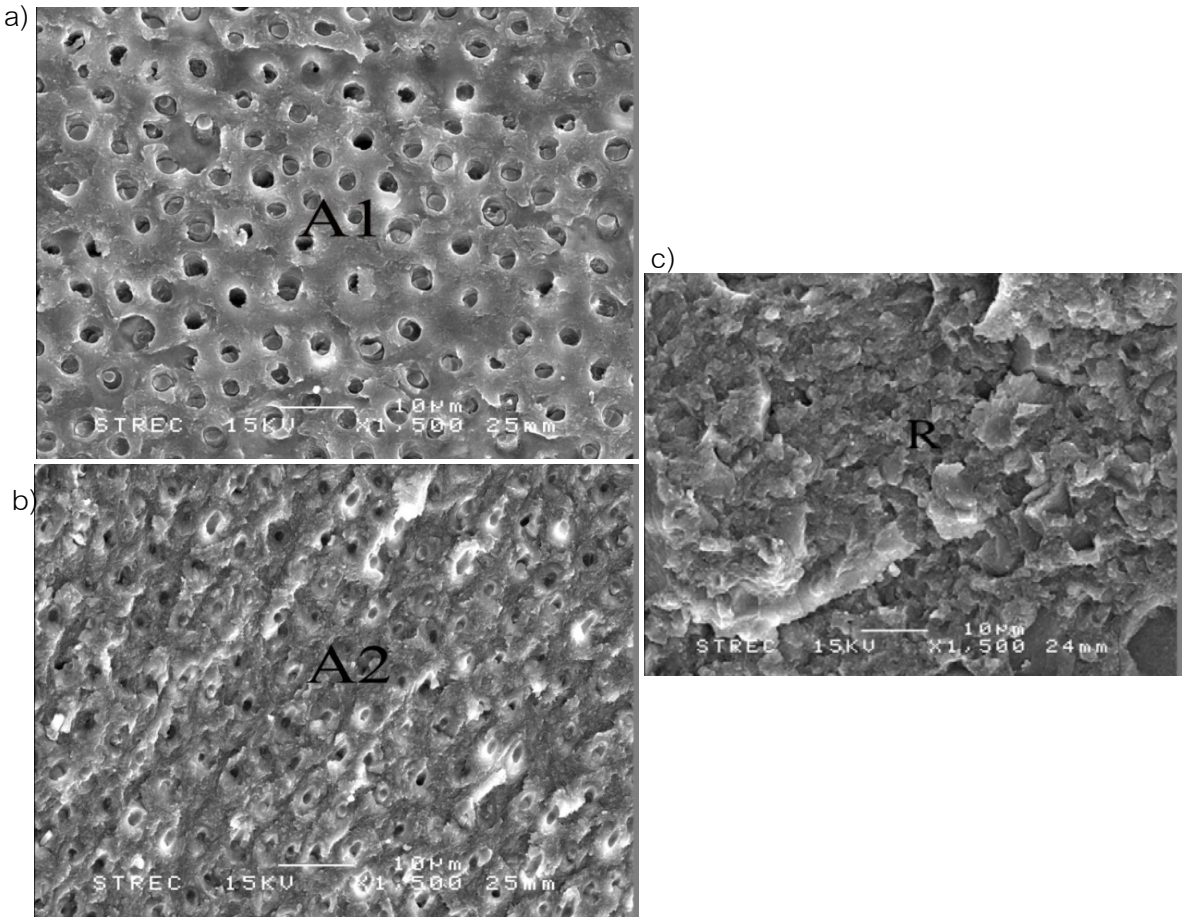


Figure 9. SEM micrograph of dentin side of fractured surface in All-Bond 3 specimen demonstrates mixed failure both in remaining demineralized dentin (A1, A2) and cohesive failure within the cured resin (R) (a, b, c; original magnification x 1500)

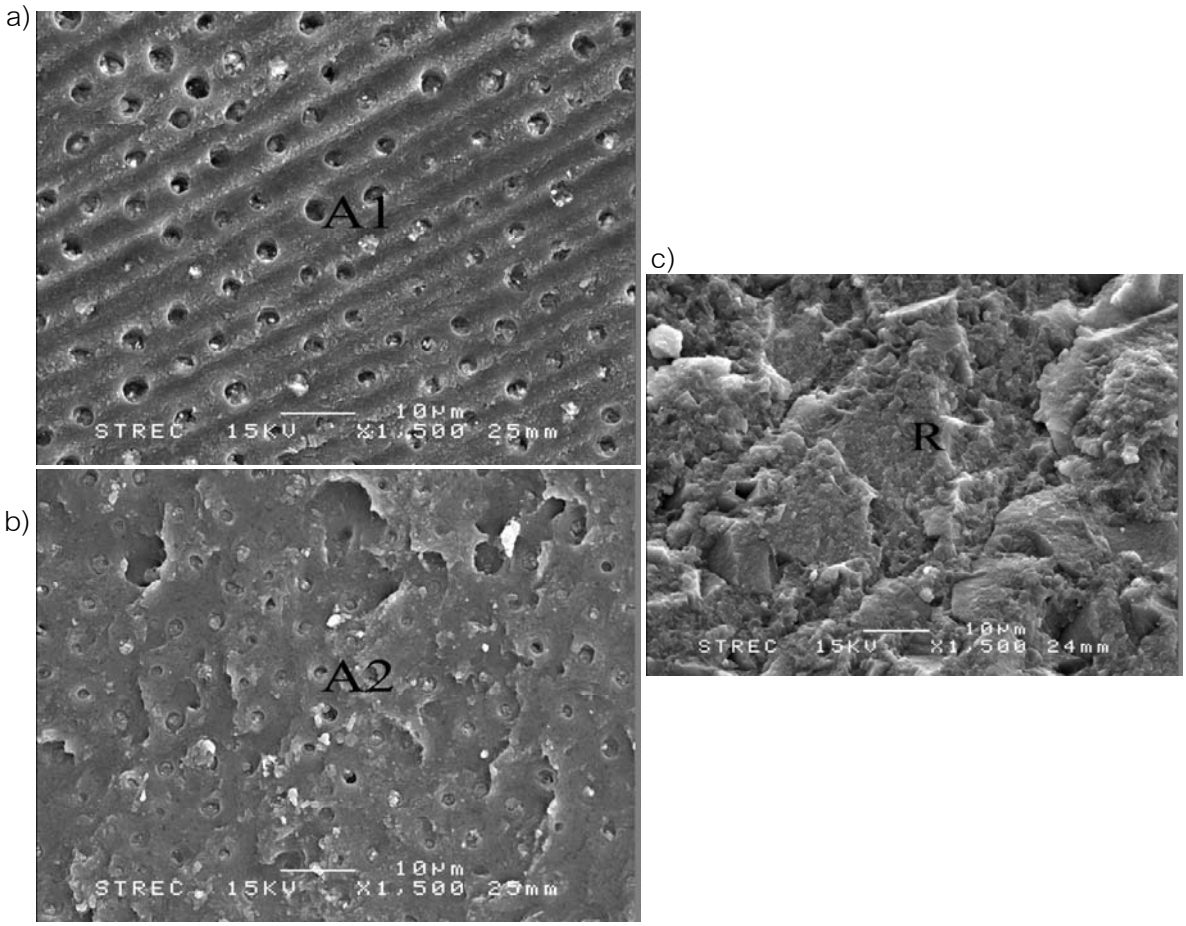


Figure 10. SEM micrograph of dentin side of fractured surface in Opibond FL specimen demonstrates mixed failure of adhesive failure (A1), cohesive failure in remaining demineralized dentin (A2) and cohesive failure within the cured resin (R) (a, b, c; original magnification x 1500)

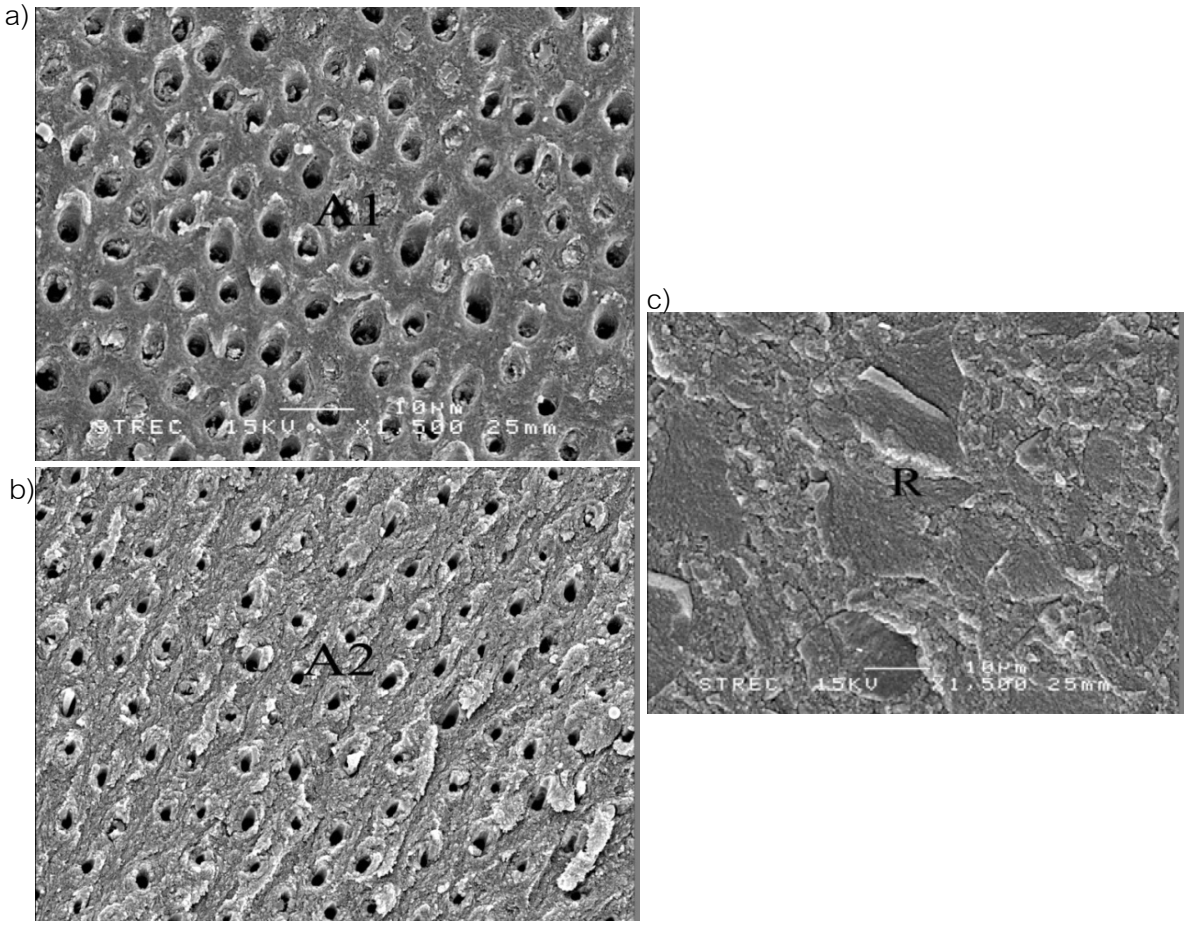


Figure 11. SEM micrograph of dentin side of fractured surface in Scotchbond MP specimen demonstrates mixed failure of adhesive failure (A1), cohesive failure in remaining demineralized dentin (A2) and cohesive failure within the cured resin (R) (a, b, c; original magnification x 1500)

Table3. Thicknesses of the dentin-resin interface of all dentin adhesives bonded specimens before and after chemical modification

Adhesives	Thickness of dentin-resin interface (μm)	
	Original dentin	Modified dentin
All-Bond 3	2-4	0-2
OptiBond FL	2-4	0-2
Scotchbond MP	2-4	0-2

Cross-sectional SEM examination revealed similar interfacial morphology for all tested dentin adhesives as they presented a 2-4 μm dentin-resin interdiffusion zone with penetration of resin monomers into both the dentinal tubules and the small lateral tubule branches, regardless of differences in the chemical compositions of their primer and resin bonding and the bonding technique. However the thickness of the dentin-resin interface of the bonded specimens for all groups were obviously thin, discontinuous and detachable after chemical modification in 6 mol/L HCL 30 seconds and followed by 60 minute immersion in 1% NaOCL (Figure 12-14). The decreasing in thicknesses of the dentin-resin interfaces after dual challenges are listed in Table 3. The dentin-resin interfaces which were not identifiable (disappeared) in some area of all specimens were shown in Figure 12d and 14d. Higher magnification of the dentin-resin interface of all bonded specimens clearly revealed degradation of non-resin impregnated exposed collagen fiber (Figure 15).

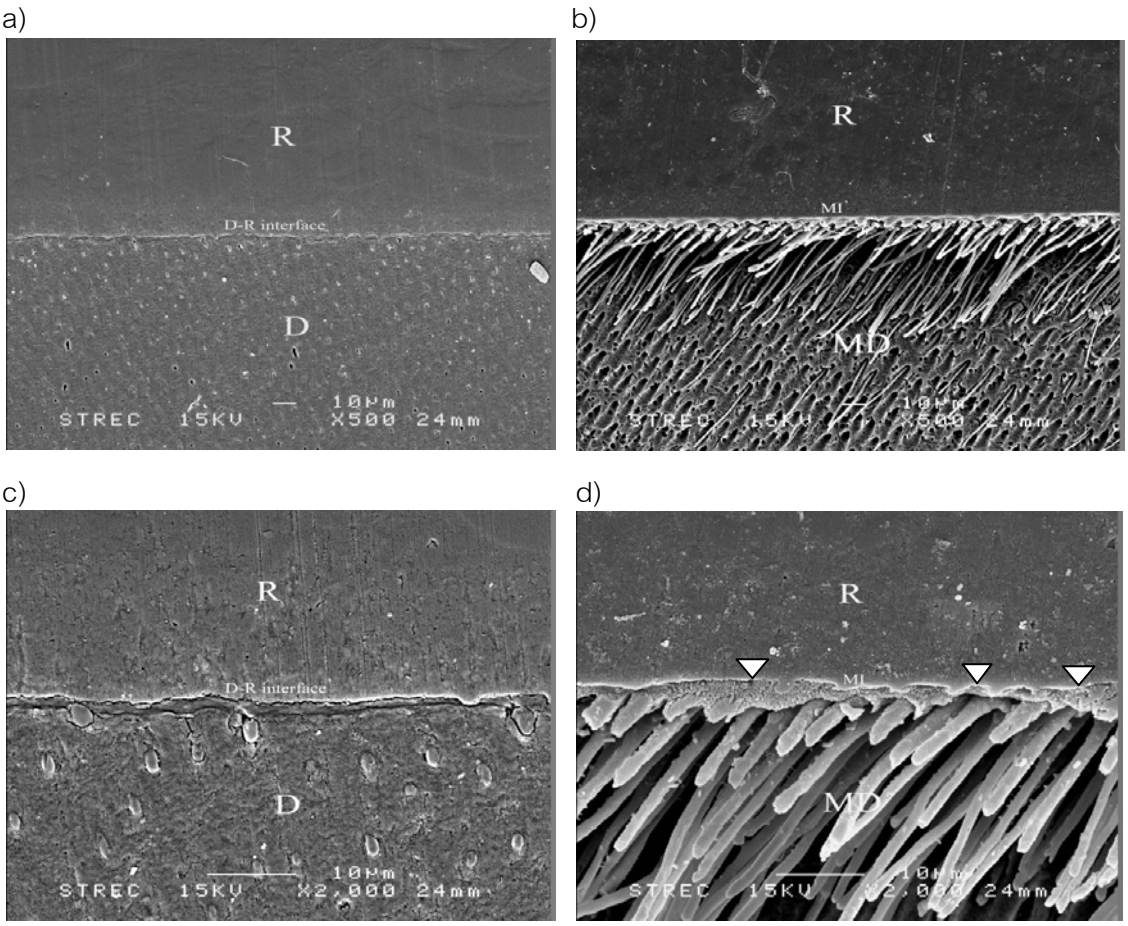


Figure 12. Cross-sectional SEM micrographs at mid dentin of All-Bond 3 restored specimen after HCL and NaOCL immersion (b, d) demonstrate that the thickness of dentin-resin interface is detached (arrow) and degraded compared to the original thickness (a, c). The thin dentin-resin interface is hardly identified in some area of interfacial layer (d). [(a, b; original magnification x 500), (c, d; original magnification x 2000)]; R=resin, MI= modified interface, MD=modified dentin treated with HCL and NaOCL.

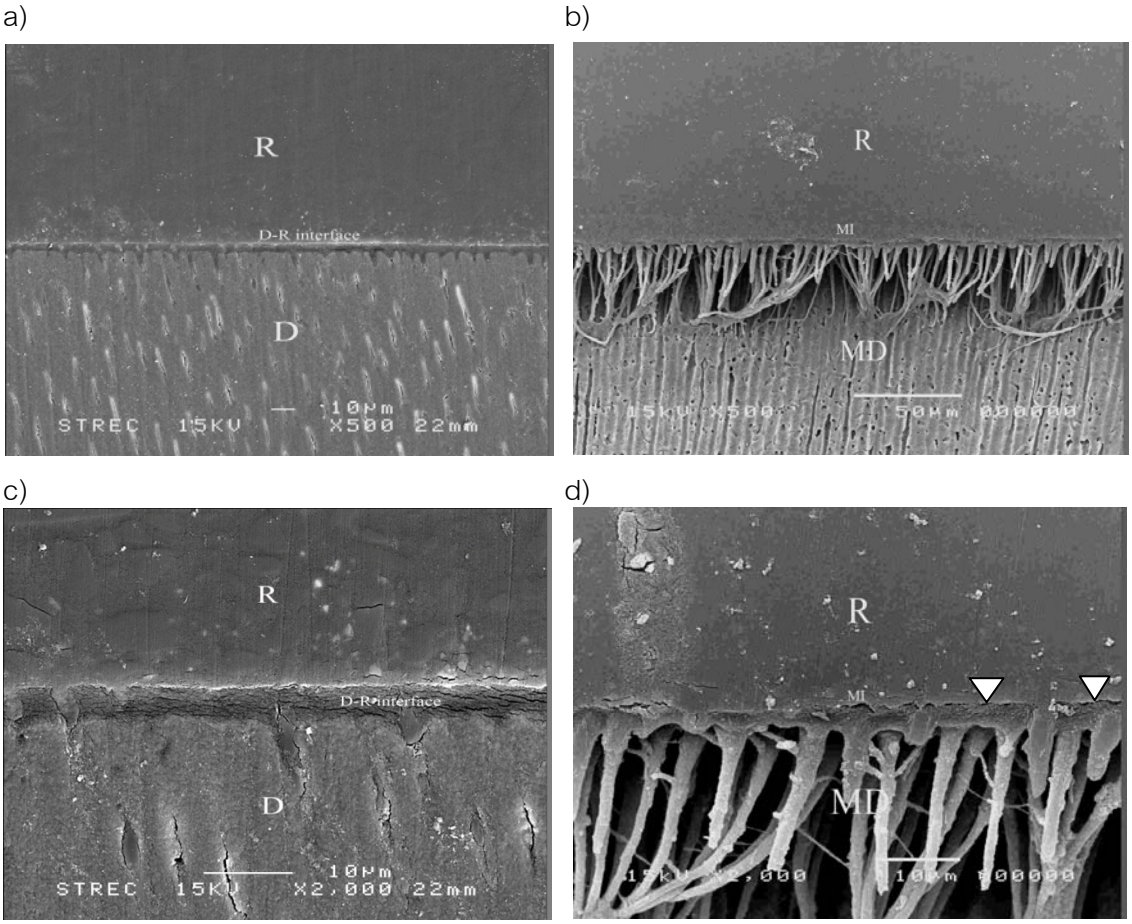


Figure 13. Cross-sectional SEM micrographs at mid dentin of OptiBond FL restored specimen after HCL and NaOCL immersion (b, d) demonstrate that the thickness of dentin-resin interface is detached (arrow) and degraded compared to the original thickness (a, c). [(a, b; original magnification x 500), (c, d; original magnification x 2000)]; R=resin, MI= modified interface, MD=modified dentin treated with HCL and NaOCL.

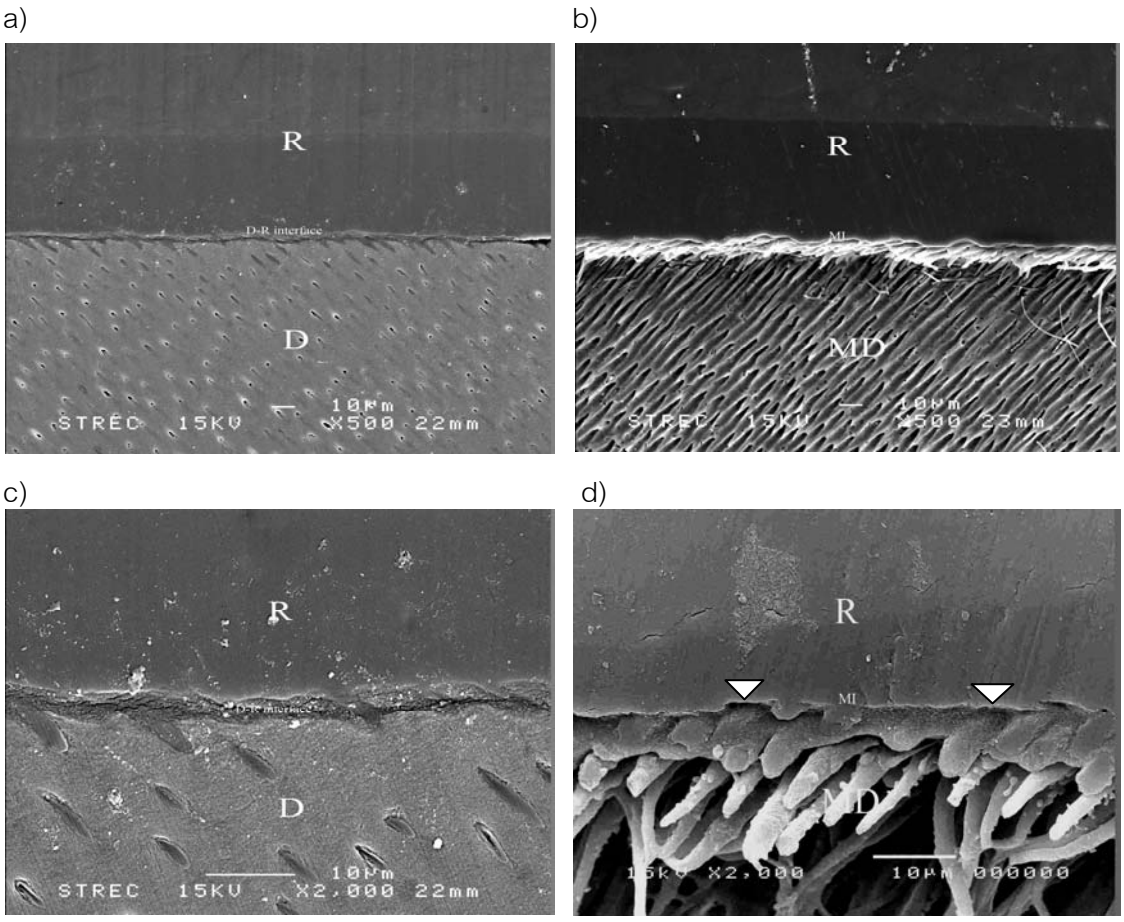


Figure 14 Cross-sectional SEM micrographs at mid dentin of Scotchbond MP restored specimen after HCL and NaOCL immersion (b, d) demonstrate that the thickness of dentin-resin interface is detached (arrow) and degraded compared to the original thickness (a, c). The thin dentin-resin interface is hardly identified in some area of interfacial layer (d). [(a, b; original magnification x 500), (c, d; original magnification x 2000)]; R=resin, MI= modified interface, MD=modified dentin treated with HCL and NaOCL.

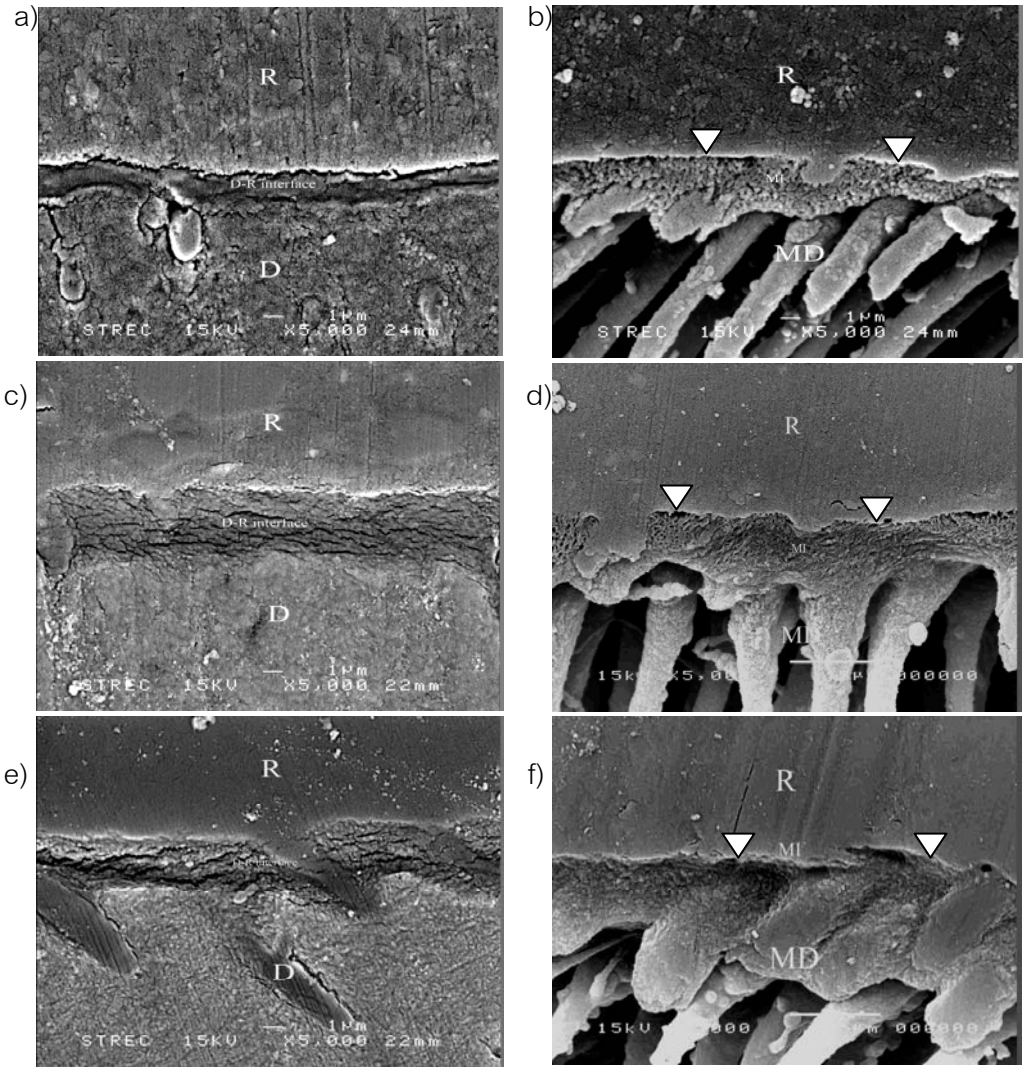


Figure 15. Higher magnification of cross-sectional SEM micrographs at mid dentin of all groups after HCL and NaOCL immersion demonstrate the similar detached (arrow) and degraded interfacial layers (b, d, f) compared to the original thickness (a, c, e). (original magnification x 5000); a, b) All-Bond3, c, d) OptiBond FL, e, f) Scotchbond MP; R=resin, MI= modified interface, MD=modified dentin treated with HCL and NaOCL.

CHAPTER V

DISCUSSION AND CONCLUSION

DISCUSSION

Wet bonding technique with All-Bond 3, Optibond FL and Scotchbond MP in which 32-37.5% phosphoric acid was used as dentin conditioner to create diffusion pathways for monomers infiltration resulted no significant difference in tensile bond strength of 11-12 MPa after tensile loading to failure of mini-dumbbell shaped specimen. The dual immersion in HCL and NaOCL solutions demineralize any mineral within the hybrid layer that was not protected by resin infiltration and solubilize all non-protected demineralized dentin matrix beneath the hybrid layer, respectively (Nakabayashi et al., 1992b). The present study also found that all dentin-resin interfaces of bonded specimens were not resistant to this dual chemical challenge in all depths of dentin, although 2-4μm dentin-resin interface had been revealed before this challenge under SEM. This can be assuredly confirmed the result of this tensile test and indicated that there is some non-resin infiltrated demineralized dentin situated in all depths of these dentin-resin interfaces. The amount of remaining non-protected demineralized dentin in these bonded specimens suggests that interfibrillar channels between phosphoric acid etched collagen fibers were not preserved for adhesive monomer diffusion and impregnation of these substrates. Consequently some demineralized dentin which was not entangled with resin copolymer chain, an exposed collagenous dentin, remained in the dentin-resin interface. The mechanical strength of this remaining demineralized dentin is the weakest in the bonded specimens (Nakabayashi et al., 1998b), comparing to dentin and bonding resin.

The similarity of both low tensile bond strength values and the degradation in all depths of the dentin-resin interfaces of all tested specimens after dual challenges also suggests that phosphoric etched dentin substrate was difficult for monomer's impregnation to form a complete resin infiltrated collagen rich fibrous network, unrelated to various chemical compositions in their primer and bonding resin and different techniques to keep wet or moist on etched dentin. 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.

There are two possible explanations why phosphoric etched dentin adhesives produced the imperfect dentin-resin interfaces in restored specimens for the current study. Firstly, when prepared dentin was decalcified by acid conditioners particularly phosphoric acid during smear layer removal, the acidity of the conditioner not only removed mineral phase from the dentinal matrix, but also it may denature collagen peptides, leading to increase their solubility. The unsupported collagen mesh together with the fused or clumped collagen fibers shrank so that the interfibrillar spaces between exposed collagen fibrils were eliminated, thereby decreasing monomer permeability of etched dentin substrates (Asmussen et al., 1993, Pashley et al., 1993). Very large and rapid shrinkages of the exposed collagen, relative impermeable state occurred when demineralized dentin was air dried with air syringe (Inokoshi et al., 1990, Marshall et al., 1995, Pashley et al., 1993). Such incident might be occurred in case of gentle air drying for 2-3 or 5 seconds after rinse out their conditioners of OptiBond FL and Scotchbond MP, respectively.

Ability of water resulting from its high dielectric constant to break the hydrogen bonds between the collagen fibers has been proposed to re-expand the collapsed and shrunken collagen network (Maciel et al., 1996). However the results of current study showed that wet bonding technique in which the tooth surface is left moist (Kanca, 1992, Gwinnett, 1992c) by adsorbing the wet etched dentin surface with pellet foam in All-Bond 3 cannot help to preserve channels between the phosphoric-denatured collagen fibers for adhesive monomer penetration and impregnation. HEMA 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 (Nakabayashi et al., 1992b) but also it is good for supplying water due to its hydrophilicity. Thirty percent aqueous HEMA primer was therefore used to reversal the collapsed demineralized dentin. It required 60 minute for complete recovery of this collapsed matrix (Nakabayashi et al., 1992b, Nakabayashi et al., 2004b), while in this study took 15-30 seconds for complete priming step followed manufactures' instructions of all adhesives. It might be possible that some collapsed phosphoric acid demineralized dentin was still remained and consequently affected the diffusion and impregnation of the applied adhesive monomers. Hence a non-resin infiltrated demineralized dentin was situated between dentin-resin interface and mineralized dentin. This would probably be the explanation for inferior results of Optibond FL and Scotchbond MP restored specimens even though water supplied by aqueous HEMA primer was used to re-expand their collapsed collagen network.

An alternative possibility is the presence of excess water that occupied the interfibrillar spaces of the intertubular dentin. Dramatic effect of water to interfere with polymerization process of dentin bonding system has been reported (Jacobsen et al., 1995). Therefore when the benefit of using wet bonding to keep the interfibrillar spaces

within the demineralized collagen network from collapsing during primer infiltration is fulfilled, water occupied these spaces has to be completely displaced and replaced by non-volatile HEMA which contribute to increase the concentration of the resin components in the hybridized dentin. Unfortunately, due to the high boiling temperature, low vapor pressure of the water and the negative effect of HEMA to lower the vapor pressure of water (Pashley et al., 1998), the removal of last amounts of water is absolutely impossible. The volatile polar solvent in the primer such as ethanol in case of All-Bond 3 and water-ethanol mixtures in case of OptiBond FL and Scotchbond MP has been employed for wet or moist bonding technique to be better media for evaporation of water (Maciel et al., 1996). However the imperfect hybridization of dentin-resin interfaces of all groups indicates that their primers did not completely replace water within the pores between the collagen fibrils, then some water remained within these interfibrillar spaces of the demineralized dentin. This remaining water competes with a hydrophilic resin monomer (HEMA) for space, resulting in decreasing its density within the collagen mesh and then decreasing in the degree of monomer conversion (Jacobsen et al., 1995).

Non-resin impregnated collagen network of etched dentin (residual demineralized dentin) and the entrapped resin-water blister or primer globules described as overwet phenomenon (Tay et al., 1995, Tay et al., 1996b, Tay et al., 1996c) or water treeing (Tay et al., 2003) are the weakest in terms of mechanical strength in the boned specimen (Kato et al., 1996, Nakabayashi et al., 1998b, Piemjai et al., 2010). Hence these phosphoric etched dentin adhesives bonded specimens not only failed in dentin-resin interface at quite low tensile bond strength but also they provided the low resin content dentin-resin interfaces that were insufficient to resist the chemical challenge in all depths of dentin. Since the dual chemical challenges that be

akin to the chemical reaction of dental caries (Nakabayashi et al., 1992b), it can be indicated that these bonding systems could not provide impermeable and stable dentin-resin interface that can protect weak exposed dentin against acid demineralization and proteolytic degradation.

High filler content in the adhesive resin of OptiBond FL and All-Bond 3 which has been proposed to potentially absorb a part of the tensile stress imposed by polymerization shrinkage on the dentin-resin interface (Cardoso et al., 2001, Labella et al., 1999, Nunes et al., 2001) did not help to promote complete resin-impregnated deminerlized dentin. The tensile bond strength values and the characteristic of dentin-resin interface among them were therefore not different. As the initial fracture occurred in the demineralized dentin the weakest part remaining in the restored specimens.

The results of this study are confirmed the research hypothesis and agreed with the previous studies (Kato et al., 1996, Piemjai et al., 2004, Piemjai et al., 2010, Piemjai et al., 2011a) that incomplete resin-impregnated demineralized dentin serves as a defect in the adhesive interface of bonded specimens. The remaining demineralized dentin in the restored tooth is the pathway of microleakage which is one of the contributed factors of restorative failure (Kidd, 1976). Unprotected demineralized dentin at the dentin adhesive interface has been also shown to be short-stable due to hydrolytic degradation (Kiyomura et al., 1987). Marginal discoloration, post-operative hypersensitivity, recurrent caries, short-term detachment of restoration from the prepared teeth and finally pulpal inflammation can be the consequences of the microleakage (Garcia-Godoy et al., 2006, Kato et al., 1998, Tay et al., 1995, Tay et al., 2003).

The bond strength values of restored phosphoric etched dentin specimens obtained using mini-dumbbell shaped specimen tensile test in the current study are significantly lower (11-12 MPa) than those obtained with previous micro-tensile tests using both stick-type specimen (40-60 MPa) (Reis et al., 2010, Sadek et al., 2010, Sarr et al., 2010) and an hour-glass specimen (20-40 MPa) (Bouillaquet et al., 2001,Inoue et al., 2001, Phrukkanon et al., 1998a). Using mini-dumbbell shaped specimen, stress is directed to its cross-section area so that failure is initiated at and propagates from this weakest point of the bonded assembly and consequently fracture in non-resin impregnated phosphoric etched dentin with low tensile bond strength values (Nakabayashi et al., 2004a). In contrast to the mini-dumbbell shaped specimen tensile testing, the high tensile bond strength values with high incidence of cohesive failure within dentin or resin obtained with both a stick-type and an hour-glass shaped specimen micro-tensile bond strength testing attributed from the small surface area that reduced number of internal defects was reported (Phrukkanon et al., 1998a, Sano et al., 1994b). Due to the fact that the stress from tensile loading was concentrated at the narrowest part of an hour-glass shaped specimen, this uneven stress distribution often occurred and progressed through the resin or the dentin where the narrowest part of an hour-glass was placed rather than through the interface. Hence cohesive failure within either dentin or resin occurred more often easily during bond strength testing, leading to misinterpretation of the test result even defect in bonded specimen existing (Nakabayashi et al., 2004a). In the same way the tensile stress is not always concentrated at the interface when the rectangular stick-type specimens were tested. The “true” interfacial bond strength between the adhesive and the dentin was not being measured with these techniques. As the size of these micro-specimens is too small to trim test specimens for the micro-tensile test, premature debonded failures are often occurred. These premature debonded specimens should be recorded as zero bond

strength values and then considered for statistical analysis. However weak or zero bond strength values were only recorded but they were not included in the mean value for statistical purposes. Consequently higher tensile bond strength values with lesser their standard deviations, resulting from this selection bias were reported (Inoue et al., 2001, Reis et al., 2010, Sadek et al., 2010).

Both tensile bond strength values and SEM micrograph of tensile fractured surface in the present study confirm the superiority of using mini-dumbbell specimens for tensile loading test to clearly reveal defects with in bonded specimen due to more uniform stress distribution during loading across the interface compare to other techniques (Nakabayashi et al., 1998b, Nakabayashi et al., 2004a).This technique is also the worldwide standard method in materials science and engineering (Nakabayashi et al., 2004a). It can be concluded that tensile test using mini-dumbbell specimens is a standardized method for measuring tensile bond strength and analyzing existence defects in restored tooth helping to predict its clinical performance.

The quality of hybrid layer, the amount of resin content in the substructure of substrate, rather than the thickness of the hybrid layer is a critical factor for longevity of restored tooth with dentin adhesives (Kato et al., 1996, Kato et al., 1998, Nakabayashi et al., 1998a). It is thus essential to prevent the collapse of demineralized dentin after pretreatment an smear layer via acid etching (Nakabayashi et al., 1982, Nakabayashi et al., 1998a) in order to obtain good dentin hybridization by choosing proper conditioners. Inclusion of 1-5% ferric chloride in a citric acid or phosphoric acid conditioners has been used as a chemical stabilized of exposed collagen fibrils by means of cationic ion interfered the collapse of deminerlized dentin (Piemjai et al., 2003, Iwasaki et al., 2004). Cations such as Fe^{3+}, Ca^{2+}dissolved in pure acid are easily attracted by polyelectrolytes (PG, GAG, PP) distributed along the collagen fibrils of demineralized dentin and make

them insoluble in the demineralized dentin. This results 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 (Piemjai et al., 2003). Complete resin-impregnated conditioned dentin without defect or existing demineralized dentin leads to better hybrid layer with higher tensile bond strength and leakage free interface (Piemjai et al., 2011b) 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 10-3 is available, dry bonding is beneficial in minimizing the water contamination compared to wet bonding technique (Nakabayashi et al., 2000, Piemjai et al., 2011a).

All adhesives used in this study could not provide complete hybridization of resin into phosphoric acid etched dentin, resulting in adhesive failure with low tensile bond strength and low quality of dentin-resin interface. It can be assumed that demineralized dentin with phosphoric acid to prepared hybridized dentin is not yet accomplished, thus this layer is not a good barrier to prevent the ingression of bacteria and theirs by-product. To prepare the perfect hybridization of resin into phosphoric acid conditioned dentin, future research is needed to emphasize the improvement in the permeability of conditioned tissue.

CONCLUSION

Tensile bond strength of wet bonding using 3-step phosphoric etched dentin adhesives was not significant different even there are differences in chemical compositions in primer and bonding resin and techniques to keep moist. Adhesive failure and cohesive failure in remaining demineralized dentin after tensile loading and the dentin-resin interfaces that were not resistant to the strong acid and NaOCL solution were occurred in all different type of adhesives used in this study. This suggests that wet bonding using phosphoric acid as dentin conditioner caused low permeability of the demineralized dentin for monomers diffusion and impregnation around the exposed collagen fibrils to form a perfect dentin-resin interface. All types of primer used in this study did not improve the permeability of moist demineralized dentin that had been etched with phosphoric acid.

Comparison of generated TBS, SEM micrographs of tensile-fractured surfaces and cross-sectional SEM micrographs of adhesive interfaces of bonded specimens after chemical modification yields much useful information concerning the influence of tissue permeability. Careful examination for the presence of remaining demineralized dentin that is not adequately reinforced by impregnating resin is essential to understanding adhesion mechanisms and their potential for restoration failure.

A further study is required in order to understand how to maintain the structural integrity of the interfibrillar spaces within the demineralized collagen network after removal of the mineral phase by acid etching which were essential to the preparation of good hybridized dentin to protect the dentin and pulp from infection for long-term durability of restored tooth.

Clinical implication: Dentin demineralized with phosphoric acid and bonded with adhesives used in this study has not been protected from oral acid or external stimuli. The remaining demineralized dentin is not stable against hydrolysis and chemical challenge.

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Appendix

Adhesive	Number of specimens	TBS(MPa)	Mode of failure
All-Bond 3	1	8.1	A,R
	2	9.4	A,R
	3	9.9	A,R
	4	11.6	A,R
	5	11.9	A
	6	12.0	A,R
	7	12.1	A,R
	8	12.3	A,R
	9	12.6	A,R
	10	12.6	A,R
	11	12.9	A,R
	12	13.5	A,R

OptiBond FL	1	8.8	A,R
	2	9.3	A,R
	3	10.3	A,R
	4	11.0	A,R
	5	12.0	A,R
	6	12.1	A,R
	7	12.9	A,R
	8	13.4	A,R
	9	13.9	A,R
	10	14.4	A,R
	11	14.7	A,R
	12	15.0	A,R

Scotchbond MP	1	7.6	A,R
	2	8.8	A,R
	3	9.3	A,R
	4	9.5	A,R
	5	10.3	A,R
	6	10.4	A,R
	7	11.2	A,R
	8	11.8	A,R
	9	12.5	A,R
	10	12.7	A,R
	11	14.0	A,R
	12	14.8	A,R

Statistical analysis for tensile bond strength test for each dental adhesive

One-Sample Kolmogorov-Smirnov Test

Group			TBS
All-Bond3	N		12
	Normal Parameters[a,,b]	Mean	11.564167
		Std. Deviation	1.6145613
	Most Extreme Differences	Absolute	.261
		Positive	.121
		Negative	-.261
	Kolmogorov-Smirnov Z		.904
	Asymp. Sig. (2-tailed)		.387
OptiBond FL	N		12
	Normal Parameters[a,,b]	Mean	12.339167
		Std. Deviation	2.1175005
	Most Extreme Differences	Absolute	.115
		Positive	.102
		Negative	-.115
	Kolmogorov-Smirnov Z		.399
	Asymp. Sig. (2-tailed)		.997
Scotchbond MP	N		12
	Normal Parameters[a,,b]	Mean	11.530833
		Std. Deviation	1.9395991
	Most Extreme Differences	Absolute	.144
		Positive	.144
		Negative	-.115
	Kolmogorov-Smirnov Z		.498
	Asymp. Sig. (2-tailed)		.965

a. Test distribution is Normal.

b. Calculated from data.

Oneway

Descriptives

TBS

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
All-Bond3	12	11.564167	1.6145613	.4660837	10.538323	12.590010	8.0600	13.4500
OptiBond FL	12	12.339167	2.1175005	.6112697	10.993771	13.684562	8.8400	15.0300
Scotchbond MP	12	11.530833	1.9395991	.5599140	10.298471	12.763196	8.7600	14.7700
Total	36	11.811389	1.8852801	.3142133	11.173502	12.449276	8.0600	15.0300

Test of Homogeneity of Variances

TBS

Levene Statistic	df1	df2	Sig.
.880	2	33	.424

ANOVA

TBS

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.021	2	2.510	.694	.507
Within Groups	119.379	33	3.618		
Total	124.400	35			

Post Hoc Tests

Multiple Comparisons

TBS

Tukey HSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
All-Bond3	OptiBond FL	-.7750000	.7764829	.583	-2.680329	1.130329
	Scotchbond MP	.0333333	.7764829	.999	-1.871996	1.938663
OptiBond FL	All-Bond3	.7750000	.7764829	.583	-1.130329	2.680329
	Scotchbond MP	.8083333	.7764829	.557	-1.096996	2.713663
Scotchbond MP	All-Bond3	-.0333333	.7764829	.999	-1.938663	1.871996
	OptiBond FL	-.8083333	.7764829	.557	-2.713663	1.096996

Homogeneous Subsets

TBS

TukeyHSD[a]

group	N	Subset for alpha = 0.05
		1
Scotchbond MP	12	11.530833
All-Bond3	12	11.564167
OptiBond FL	12	12.339167
Sig.		.557

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 12.000.

Biography

Miss SudaratNubdee was born on June 7,1975 in Bangkok, Thailand. She received degree of Doctor of Dental Surgery (D.D.S.) from Chulalongkorn University in 2000,degree of Graduate Diploma of Clinical Sciences in Prosthodontics in 2002 and Diploma Thai Board of Prosthodontics in 2008. She worked as a general dentist at Sankraburi Hospital, Ministry of Public Health in 2000-2002 and a lecturer in Department Prosthodontics, Faculty of Dentistry at Chulalongkorn University from 2007 to the present day.