# THE INFLUENCE OF A LOCAL ANESTHETIC CONTAINING VASOCONSTRICTOR ON MICROTENSILE BOND STRENGTHS OF TWO ADHESIVE SYSTEMS TO HUMAN DENTIN *IN VIVO*

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TOR ON MICROTENSILE BOND STRENGTHS OF TWO ADHESIVE SYSTEMS TO HUMAN DENTIN *IN VIV*O

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ABSTRACT

Theoretically, performing adhesive techniques *in vivo*, dentinal fluid has detrimental effects on adhesive systems. In this study, the experiment was set up to prove this assumption. A clinical study was performed on upper premolars scheduled for extraction for orthodontic reasons. The experiment was divided into two parts. In part 1, the bond strength test, 47 premolars were prepared on occlusal surfaces in a condition with or without a local anesthetic containing vasoconstrictor prior to cavity preparations, bonded with either Single Bond or Clearfil SE Bond and filled with a resin composite, Filtek Z250. The teeth were then extracted and prepared for the microtensile bond strength test. In part 2, observation of dentin surface, 20 premolars were assigned into 2 groups. In group 1, the teeth were anesthetized with a local anesthetic containing vasoconstrictor before cavity preparation. The prepared dentin was then treated with or without acid etching and impressions were taken with a polyvinyl siloxane impression material. In group 2, as a control group, the teeth were treated in exactly the same procedures as those in group 1 but no local anesthetic was administered. The results in part 1 demonstrated that the conditions with or without a local anesthetic containing vasoconstrictor had no effect on the bond strengths of both adhesive systems ($p>0.05$). In part 2, in the etched group with a local anesthetics containing vasoconstrictor, the results revealed 3 different patterns but the surface of the coalescent dentinal fluid from the tubules was observed in most specimens. On the other hand, fluid covering dentin surface was visible in all replicas in the group using acid etching without a local anesthetic. Granular appearance of smear layer was found in both unetched groups but the granules were slightly larger in the group without a local anesthetic containing vasoconstrictor. This study found that a minute amount of dentinal fluid flow *in vivo* did not have detrimental effects on both adhesive systems.

KEY WORDS: DENTINAL FLUID, MICROTENSILE BOND STRENGTH, ADHESIVE SYSTEMS, VASOCONSTRICTOR

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ผลของยาชาที่มีสารบีบหลอดเลือดต่อค่าแรงยึดแบบไมโครเทนไซล์ของวัสดุยึดฟันสองระบบในมนุษย์ (THE INFLUENCE OF A LOCAL ANESTHETIC CONTAINING VASOCONSTRICTOR ON MICROTENSILE BOND STRENGTHS OF TWO ADHESIVE SYSTEMS TO HUMAN DENTIN *IN VIVO*)

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บทคัดย่อ

ในทางทฤษฎีได้มีการคาดการณ์ไว้ว่าของเหลวในท่อเนื้อฟันจะมีผลในเชิงลบต่อวัสดุยึดฟันเมื่อใช้ทำการบูรณะฟันในมนุษย์ วัตถุประสงค์ของการศึกษานี้เพื่อทดสอบสมมติฐานดังกล่าวโดยทำการทดลองในฟันกรามน้อยบนที่ต้องถอนเพื่อการจัดฟัน การทดลองถูกแบ่งออกเป็น 2 ส่วน คือ ส่วนที่ 1 เป็นการทดลองหาค่าแรงยึดของวัสดุอุดฟันโดยเตรียมโพรงฟันที่ด้านบดเคี้ยวของฟันกรามน้อยบนจำนวน 47 ซี่ อุดฟันด้วยเรซินคอมโพสิต ฟิลเทก ซี 250 ร่วมกับการใช้วัสดุยึดฟันระบบที่กำจัดสเมียร์แลย์โดยการใช้กรด หรือระบบที่ใช้ซิดิคไพร์เมอร์ซึมผ่านสเมียร์แลย์ อย่างใดอย่างหนึ่งโดยเปรียบเทียบในสภาวะที่ฉีดยาชาที่มีส่วนประกอบของสารบีบหลอดเลือดกับสภาวะที่ไม่ฉีดยาชา ผลการศึกษาพบว่าไม่มีความแตกต่างของค่าแรงยึดแบบไมโครเทนไซล์ในสภาวะที่มีการฉีดยาชากับสภาวะที่ไม่ฉีดยาชาในวัสดุยึดฟันทั้ง 2 ระบบ ($p>0.05$) ในการทดลองส่วนที่ 2 เป็นการตรวจสอบลักษณะผิวของเนื้อฟันบริเวณพื้นโพรงฟันด้านบดเคี้ยวของฟันกรามน้อยจำนวน 20 ซี่ ในสภาวะที่มีการใช้กรดกัดและไม่ใช้กรดกัดสเมียร์แลย์ การทดลองนี้จะแบ่งฟันออกเป็น 2 กลุ่มคือกลุ่มการทดลองซึ่งมีการฉีดยาชาที่มีส่วนประกอบของสารบีบหลอดเลือด และกลุ่มควบคุมที่ไม่มีการฉีดยาชา ผลการศึกษาพบว่าในกลุ่มที่มีการฉีดยาชาและใช้กรดกัดสเมียร์แลย์ จะพบผิวของเนื้อฟันได้ 3 ลักษณะ แต่จะพบลักษณะที่มีหยดของเหลวจากท่อในเนื้อฟันมาเชื่อมต่อกันเป็นส่วนใหญ่ ในทางกลับกันจะพบลักษณะของเหลวไหลแผ่ในทุกตัวอย่างของกลุ่มควบคุม ในขณะที่ในสภาวะที่ไม่ใช้กรดกัดสเมียร์แลย์จะพบลักษณะหยดของเหลวที่ถูกปกคลุมด้วยสเมียร์แลย์ในทั้ง 2 กลุ่มแต่จะพบหยดของเหลวที่มีขนาดใหญ่กว่าในกลุ่มที่ไม่ฉีดยาชา การศึกษาในครั้งนี้ได้แสดงให้เห็นว่าของเหลวปริมาณเล็กน้อยในท่อเนื้อฟันไม่มีผลในเชิงลบต่อวัสดุยึดฟันทั้ง 2 ระบบในมนุษย์

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| | |
|---|---|
| mm | millimetre(s) |
| cm | centimetre(s) |
| ml | millilitre(s) |
| nm | nanometre(s) |
| min | minute(s) |
| sec | second(s) |
| HEMA | 2-Hydroxyethyl methacrylate |
| bis-GMA | Bisphenol glycidyl methacrylate |
| PAAC | polyacrylic acid copolymer |
| UEDMA | Urethane dimethacrylate |
| MDP | 10-Methacryloyloxydecyl dihydrogen phosphate |
| SEM | scanning electron microscope |
| SD | standard deviation |
| RDT | remaining dentin thickness |
| MPa | megapascal |

# CHAPTER 1
## INTRODUCTION

From observing the trend of direct restorative techniques, the concepts have been changed. The principle of large preparations and extension for prevention, proposed by Black in 1917, has gradually been replaced by smaller preparations and more conservative techniques combined with using of adhesive restorations.

Adhesive restorations have a number of advantages over traditional, nonadhesive methods. First of all, when more retention and stabilization are required, preparing the cavities for adhesive restorations preserve more tooth structure than traditional cavities. Adhesion also reduces microleakage at the restoration-tooth interface. Prevention of microleakage, or the ingress of oral fluids and bacteria along the cavity wall, reduces clinical problems such as postoperative sensitivity, marginal staining, and recurrent caries, all of which may jeopardize the clinical longevity of restorative efforts (1).

The adhesion between restorative materials and tooth structure has been a goal followed by many researchers and restorative dentists ever since Buonocore established the foundation of adhesive and preventive dentistry (2). Based on the industrial use of phosphoric acid to improve adhesion of paints and resin coatings to metal surfaces, Buonocore proposed that phosphoric acid could be used to transform the surface of enamel to “render it more receptive to adhesion”. Subsequent research indicated that the formation of tag-like resin prolongations into the enamel microporosities was the leading bonding mechanism of resin to phosphoric acid-etched enamel.

Although bonding to enamel provides many preferable results, bonding to dentin still represents an overwhelming task. Dentin is a naturally wet organic tissue

penetrated by a tubular maze containing odontoblastic process and dentinal fluid, which communicates with the pulp (3) This intrinsic moisture may actually degrade the chemistry of adhesive systems partly containing hydrophobic components.

In a total-etching system, an acid has been used to promote the efficacy of adhesive system by removing smear layers, smear plugs and demineralization of underlying sound dentin matrix to expose the collagen fibril network (4-6). Subsequently, hydrophilic monomers penetrate into the collagen fibril network and opened dentinal tubules. The adhesive will be then polymerized to provide micromechanical retention to dentin surface that called hybrid layer and resin tags (4, 6, 7).

Acid etching seems to be the successful method in bonding procedures but some disadvantages still exist. Opening dentinal tubules by removing the smear plugs will provide channels that communicate the pulp and the oral cavity (8). As a result, pulpal interstitial tissue fluid, which transmits into the dentinal tubule compartments (9, 10) and seeps outward to the external dentin surface (11, 12), may adversely affect the proficiency of dentin bonding systems. Previous laboratory experiments performed under simulated clinical condition also reported the detrimental effects of the dentinal fluid to the bond strength and sealing ability of the bonding systems(13-15). However this assumption should be confirmed under the clinical condition.

Theoretically, using a self-etching adhesive system could minimize the detrimental effects of the dentinal fluid seepage. In this system, the primers penetrate the smear layer and simultaneously etch and prime the dentin and enamel without rinsing (16). As the smear layer is not totally removed, there is less dentinal fluid diffusion. From this principle, a self-etching system seems to be better than a total-etching system in case the intrinsic wetness is of concern.

As the dentinal fluid diffusion appears to be a result from the pulpal vasculation, therefore, when the blood supply to the pulp is cut off, the fluid stops immediately (17). Fluid seepage could be decreased when pulpal vascular flow is receded or vessel

is constricted. Many researchers demonstrated that a local anesthetic with vasoconstrictor inhibited or decreased pulpal blood flow significantly (18-21). From this knowledge, it could be assumed that detrimental effects of intrinsic wetness probably are minimized by administration a local anesthetics with vasoconstrictor.

However, very few in vivo or clinical studies were performed. Therefore, it is important to set up the experiments answering the questions. Hence, in this project the efficacy of two dentin bonding systems, a total-etching and a self-etching system, by means of microtensile bond strengths will be tested *in vivo* under two conditions, with or without the influences of local anesthetics containing vasoconstrictor.

# CHAPTER 2

## OBJECTIVE

The purpose of this study was to investigate the effect of bonding systems, a total etching and a self etching system, and the effect of a local anesthetic containing vasoconstrictor, on microtensile bond strengths to human dentin *in vivo*.

**Null hypothesis**

1. There was no difference in microtensile bond strengths to human dentin when using the total-etching or the self-etching system.
2. There was no difference in microtensile bond strengths to human dentin with or without the use of a local anesthetic containing vasoconstrictor.

**Alternative hypothesis**

1. There were differences in microtensile bond strengths to human dentin when using the total-etching or the self-etching system.
2. There were differences in microtensile bond strengths to human dentin with or without the use of a local anesthetic containing vasoconstrictor.

# CHAPTER 3
## REVIEW OF THE LITERATURE

### Pulp-Dentin Complex

Dentin and pulp tissues are specialized connective tissues of mesodermal origin, formed from dental papilla of the tooth bud. Both tissues are considered as a single tissue which thus forms the pulp-dentin complex, with mineralized dentin comprising the mature end product of cell differentiation and maturation. Dentin is formed by cells called odontoblasts. These cells are considered part of both dentin and pulp tissues because their cell bodies are in pulp cavity but their cytoplasmic processes (Tomes fibers) extend into the tubules in the mineralized dentin. Since odontoblastic processes extend into dentinal tubules, dentin is considered as a living tissue with the capability to react to physiologic and pathologic stimuli. Such stimuli can result in changes throughout the life of the tooth, such as secondary dentin, reparative dentin, sclerotic dentin and dead tracts.

Dentin forms the largest portion of the tooth structure, extending almost the full length of the tooth. Externally, dentin is covered by enamel on the anatomic crown and cementum on the anatomic root. Internally, dentin forms the walls of the pulp cavity.

The composition of human dentin by volume is approximately 75% inorganic material, 20% organic material and 5% water and other materials. Dentin is less mineralized than enamel but more mineralized than cementum or bone. The mineral contents of dentin increase with age. This mineral phase is composed primarily of hydroxyapatite crystallites, which are arranged in a less systematic manner than enamel crystallites. These crystallites have a length of 200 to 1000 Å and a width about 30 Å, similar to the sizes seen in bone or cementum (22).

Dentin is harder than bone or cementum but softer than enamel. The hardness of dentin is one fifth that of enamel. At the area near the DEJ, dentin hardness is about 3 times greater than that near the pulp. While dentin is a hard, mineralized tissue, it is somewhat flexible, with a modulus of elasticity of $1.67X10^6$ psi. This flexibility helps support more brittle, nonresilient enamel. Additionally, dentin is not as prone to cleavage as enamel rod structure. The tensile strength of dentin is approximately 40 MPa (6,000 psi), which is less than cortical bone and approximately one half that of enamel. The compressive strength of dentin is much higher that is 266 MPa (40,000 psi) (22).

In histological studies, dentin has been described as a biologic composite made up of a collagen matrix (to provide toughness) filled with nanofillers of apatite crystallites (to provide strength) (3). Dentin matrix is penetrated by micrometer-diameter hollow tubules lined by mineral-rich (~95% volume mineral phase) collagen-poor, intratubular dentin or peritubular dentin. These tubules, called dentinal tubules, are very small canals that extend across the entire width of dentin, from the dentinoenamel junction to the pulp. Each tubule contains the cytoplasmic cell process of an odontoblast and filled with dentinal fluid that is saturated in calcium and phosphate ions such as other extracellular fluids (23). The structure of dentin is considered to be both a barrier and a permeable structure, depending on its thickness, age and other variables (24). The surface area of dentin at the dentinoenamel or dentinocemental junction is much larger than the pulpal side. While dentin is formed, odontoblasts are inwardly progressing toward the pulp resulting in closer and larger dentinal tubules. The number of tubules increases from 15,000 to 20,000/$mm^2$ at the DEJ to 45,000 to 65,000/$mm^2$ at the pulp (25). A number of studies have been performed to estimate the sizes of the tubules, the thickness of peritubular region and the amount of intertubular dentin. The calculations of occlusal dentin show the percentage of tubule area and diameter varies from about 22% and 2.5 micrometers near the pulp to 1% and 0.8 micrometer at the DEJ. Intertubular matrix area varies from 12% at predentin to 96% near the DEJ, while peritubular dentin goes from over 60% to 3% at DEJ (26)

**Dentin Permeability**

Dentin is very porous due to its tubular structure that provides the channels for solutes and solvents to permeate through dentin. The density of tubules varies from 15,000 tubules/$mm^2$ at the dentinoenamel junction to 65,000 tubules/$mm^2$ at the pulp boundary (25, 27, 28). As soon as enamel or cementum covering the dentin are lost, these tubules provide a diffusion channel from surface to the pulp. The rate at which diffusional flux of exogenous material crosses dentin to the pulp is highly dependent upon dentin thickness and the hydrolic conductance of dentin (29). Dentin permeability is not uniform throughout the tooth. Coronal dentin is much more permeable than root dentin. Within the coronal dentin (30), dentinal tubules of axial walls are much more permeable than those of pulpal floor.

Normally dentinal tubules are tapered structures measuring approximately 2.5 micrometers in diameter near the pulp and 0.8 micrometer near the DEJ (26). Because the diameter and density of dentinal tubules gradually decline with the distance of dentin from the pulp, it can be concluded that the permeability of dentin is highest at the pulp and lowest at the DEJ. Nevertheless, due to the presence of intratubular material such as collagen fibrils and mineralized constrictions of the tubules (31), the permeability of dentin *in vitro* is far under what would be forecasted from tubule density and diameters (32, 33).

Dentin permeability can be classified into two categories (31): (1) transdentinal movements of substances through the entire thickness of dentin via dentinal tubules (such as fluid shifts in response to hydrodynamic stimuli) and (2) intradentinal movement of exogenous substances into intertubular dentin. The latter occurs during the infiltration of hydrophilic adhesive resins into demineralized dentin surfaces during resin bonding or demineralization of intertubular dentin by bacterially derived acids (34), where the material enters the tubules but does not travel across the tubules. The presence of smear layers, smear plugs and/or intratubular deposits (i.e. sclerotic dentin) is thought to lower intratubular permeability to minimal values (24).

Quantifying hydraulic conductance that measures the ease with which fluid can filter across a unit surface area of dentin in a unit time under a unit pressure gradient (Pashley 1990) is the easiest method of measuring transdentinal permeability. The hydraulic conductance relates inversely with the dentin thickness (33, 35). Some intratubular materials reduce the hydraulic conductance and hence lowers its permeability (31).

Sclerotic dentin, formed in response to stimuli, has the tubules filled with mineral deposits resulting in a low dentinal permeability (36). The advantage of the sclerotic dentin is to impede the penetration of bacteria and its toxin to the pulp. The disadvantage is that it may aid the demineralization by reducing the supply of dentin tissue fluid.

Although the exact composition of dentinal fluid is unknown, it is likely to be similar to serum ultrafiltrate, which is undersaturated with respect to brushite, slightly supersaturated with respect to octacalcium phosphate and moderately supersaturated with respect to hydroxyapatite (37). Thus, a reduction in the supply of dentinal fluid due to tubule occlusion may aid caries progression.

**Dentin bonding and its role on adhesion**

The obtainable of new knowledge about treatment of dental caries and the introduction of new adhesive restorative materials have substantially reduced the need for extensive tooth preparations (38). Nowadays, adhesive restorations have been widely used, for instance, to replace carious dental tissues, to restore fractured teeth, to replace missing enamel or dentin in cervical areas of teeth, or to change the shape and shade of teeth. The capability of clinicians to bond restorative materials to enamel and dentin has tremendous changed the concepts of cavity preparation, orthodontic treatment, caries prevention, and cementation of fixed prostheses, including prefabricated posts.

The idea of applying phosphoric acid on enamel surface was based on the observation that the industrial application of phosphoric acid to metal surfaces resulted in better adhesion of paints and resin coatings (2). Since Buonocore introduced the acid-etch technique, many dental researchers have attempted to achieve methods for reliable and durable adhesion between resins and tooth structure. Acid etching transforms the smooth enamel into a very irregular surface. After rinsing off the etchant with water and drying the enamel surface with air, a fluid resin is applied on the enamel surface. This resin penetrates into the subsurface, drawn by capillary action. Monomers in the fluid resin polymerize and become interlocked with the enamel surface. The formation of resin microtags within the enamel structure is the fundamental mechanism of adhesion of resin to enamel (39, 40).

The success of the enamel etching made the dentin etching a logical next step. Early efforts resulted in very limited micromechanical bonding. Several explanations were obtained: peritubular dentin is preferentially etched, resulting in funnel shaped openings to the tubules which were not conductive to mechanical interlocking; the resins were largely hydrophobic and could not displace dentinal fluid and thus did not penetrate well into the peritubular walls or demineralized intertubular zone; and polymerization shrinkage tended to shrink the tags away from the walls.

As opposed to enamel, which is composed of more than 90% of hydroxyapatite and can be dried easily, dentin is an intrinsically wet organic tissue penetrated by a tubular maze containing the odontoblastic process, which communicates with the pulp. The density of the tubules by unit area is greater close to the pulp than near the dentin-enamel junction (25). The dynamic nature of dentin as a substrate is responsible for inconsistent bond strengths and marginal leakage, which still occur with all resin-based adhesives (41).

Efforts then turned to a wide variety of agents designed to form chemical bonds with the apatite or collagen components of the structure. These approaches have included attempts to bond to the mineral phase via chelation or coordinate bond formation with calcium; covalent bond formation via collagen reaction with glutaraldehyde and HEMA; grafting to collagen; and reaction with mordants following chemical modification. So far these methods have resulted in dentin bond strengths that are not as high or durable as desired.

Although the concept of dentin bonding agent via chemical bond was introduced, an adequate bond to dentin is more difficult to achieve. This is partly due to the presence of dentin smear layer formed immediately after cavity preparation (42). Whenever calcified tissues are cut with hand or rotary dental instruments, smear layers are created. Dentin smear layers consist of tenacious microcrystalline particles of debris, 0.5 to 5 micrometer thick (43, 44), which cannot be removed from dentin surfaces with water. The smear layer is an important factor that can protecting the pulp. A study has been reported that up to 86 percent of fluid movement across dentin can be inhibited by the presence of the smear layer.(45). Moreover, the smear layer can inhibit bacterial colonization in dentinal tubules. Therefore, the desirable properties of the smear layers include limiting the diffusion of substances to the pulp by blocking the orifices of dentinal tubules and providing an impervious barrier to bacteria. On the other hand, the smear layer has been considered to be the weak link in dentin-bonding chain with resin composite because it will prevent intimate contact between adhesive system and substrate, thus nullifying a prerequisite for the

occurrence of an adhesive reaction. Early bonding agents were applied directly on smear layers and they had rather low (1-5 MPa) bond strengths because they did not permeate well in dentin (46). Careful SEM evaluation of both sides of the failed bonds revealed that 5 MPa bond strength was actually a measure of the cohesive forces holding the smear layer particles together since the failure seemed to occur within the layer .

From this knowledge, to achieve higher bond strengths, modification or removal of the smear layer should have been done to allow the bonding resins penetrate through the smear layer into the underlying dentin matrix. Nowadays, technological advancement of dentin adhesives has involved two trends: the total-etching technique, which remove dentin smear layer and open dentinal tubules by acid etching (47) and the self-etching primer techniques, which do not remove the smear layer but make it permeable to the monomers subsequently applied (41).

In the total-etching technique, acids had been used to remove the smear layer and smear plugs and to demineralize the top 3-6 microns of the underlying sound dentin matrix to expose the collagen fibril network (48). By using hydrophilic adhesive monomers, they were able to penetrate into the collagen fibril network which, after polymerization, provided micromechanical retention of the resin to the dentin surface. This resin-infiltrated surface layer is called a hybrid layer. Viewed from this perspective dentin can be regarded as a biologic composite of a collagen matrix which is highly filled with nanometer-sized apatite crystallizes. After solubilizing the crystals and extracting the mineral phase from around the collagen fibrils, they were able to replace them with a resin polymer to form a new therapeutic composite of a resin matrix filled with a fibrous biologic polymer, collagen. This new structure is a hybrid of resin and collagen (49).

Though acid etched dentin tends to facilitate monomer diffusion into dentinal tubules by removing smear layer and increasing the pore size. On the other hand, acid etching will increase outflow of the dentinal fluid from the opening of dentinal tubules

since 86% of the total resistance to fluid flow across dentin was attributed to the presence of smear layer (45). The hydraulic conductance of dentin increased from 2.7 to 32 times when the smear layers was removed with various acidic conditioners (50-53), permitting fluid flow across the dentin tubules to the surface of an acid conditioned dentin which will reduce inward diffusion of the monomer to form the resin tags. The outward flow of fluid through etched dentin will also affect the bonding of dental materials to the dentin surface, because the dentin cannot be dried and the flow of lining or filling materials into the tubules will be restricted.

There are another kinds of bonding systems called self-etching primer systems, which combine etchant and primer together. Self-etching primers are acidic primers capable of penetrating the aqueous channels formed between the smear layer particles, widening these channels and interacting at the top of underlying dentin (54,55). As the same acidic solution remains after conditioning, the smear layer will incorporate itself into the hybrid layer (10) . Thus, self-etching primers offer a simpler clinical application than do the total-etching systems (56) because they are capable of conditioning the tooth surface and simultaneously preparing for adhesion (55, 56). One such system (Clearfil SE Bond: Kuraray Co. Ltd. Osayama Japan) contains phosphate derivatives of hydrophilic monomers, 10-Methacryloyloxydecyl dihydrogen phosphate (MDP). The primer solutions also contain HEMA or other hydrophilic monomers so that they simultaneously etch and prime the dentin. Self-etching primers must have sufficient acidity to overcome the buffering potential of the dentin, and they must also contain sufficient monomer to compete with water when they diffuse to the smear layer. However, the acidity of the primer may be reduced as it penetrates the smear layer, leaving less acid to etch the underlying dentin. As the smear layer might not be totally removed, the partially demineralized smear layer becomes into a hybrid layer. In contrast to the total-etch technique, the outward flow of dentinal fluid is not increased to compete the penetration of bonding resin to form the hybrid layer.

**Vasoconstrictor and its effect on dentinal fluid flow**

Pulpal tissue is an important index of state of the pulpal circulation (9, 57) and pulpal tissue pressure is elevated during pulpal inflammation (58-60). Previous studies measured this tissue pressure by invasive techniques, the direct exposure of pulpal soft tissue using drills (61-64). Because pulpal interstitial tissue pressure is supposed to be transmitted into the dentinal tubule compartment, Moist and Yanoff attempted to measure pulpal tissue pressure through intact dentin by sealing a needle connected to a pressure transducer directly into cavities prepared in dog or human teeth. The experiments were unsuccessful because they did not understand well about the effect of smear layer (65).

Pashley et al. succeeded to measuring pulpal tissue pressure through acid conditioned intact dentin in dog molar and they obtained a mean value of 32.6 $cmH_2O$ (24.1 mmHg) (8). Many years later, Vongsavan and Matthews estimated pulpal tissue pressure in cat teeth by measuring fluid movement across dentin as a function of exogenous pressure. The equilibrium pressure, 15 $cmH_2O$ (11 mmHg) was thought to be equal to pulpal tissue pressure(17).

Ciucchi et al also estimated the pulpal tissue pressure through intact dentin by determining the exogenous pressure required to null dentinal fluid flow but in human teeth(66). The mean normal pulpal pressure of 14.1 $cmH_2O$ were comparable to that of 13.6 $cmH_2O$ reported by Andrews et al. (64). On the basis of capillary experiments and theoretical calculations, it has been suggested that the speed of the fluid flow in the dentinal tubules due to capillary attraction could be about 4mm/sec at a 2-mm distance from the pulp (67) .

Thus the normal outward flow of fluid through exposed dentin appears to result from a process of ultrafiltration from pulpal interstitial fluid. Theoretically when the blood supply to the pulp is cut off, the fluid stops immediately (17). In fact, under these conditions the outward flow is replaced by a very slow inward flow.

The rate of outward flow increases when pulpal afferent nerves are stimulated antidromically (68). This can be attributed to an increase in pulpal tissue fluid pressure accompanied with the vasodilatation. Similarly, stimulation of the sympathetic supply to the pulp causes the flow to reverse into the pulp due to the vasoconstriction (68).

Since the constriction of vessels reduces the fluid flow, there should be some benefits from the use of local anesthetics containing vasoconstrictor. Local anesthetics with vasoconstrictors have been widely used in dentistry since the late 1940s for pain control. Epinephrine is the most commonly used vasoconstrictor. It has been added to local anesthetics in concentration from 1:50,000 to 1:300,000 to produce 2 major beneficial effects: 1) decreasing the plasma concentration of local anesthetic, amount of anesthetic needed and blood loss during surgical procedures and 2) increasing the duration and quality of anesthesia (69). All of these benefits are derived from the vasoconstrictor properties of epinephrine on blood vessels in close proximity to the injection site. The vasoconstrictor helps to contain the anesthetic in a localized area to prolong its effects; this also decreases blood flow and may lead to ischemia of the pulp and other tissues.

The circulatory effects of 1:80,000 epinephrine injected supraperiosteally in the area of the canine teeth of cats have been reported by Olgart and Gazelius. Pulpal blood flow was monitored by employing the tracer disappearance method which measure the washout rate of radioactive iodide ($^{131}I$) placed in a deep cavity prepared at the tooth crown. They reported almost complete cessation of pulpal blood flow within a few minutes following the anesthetic procedure. During the following 2 hours, a complete standstill of the tracer washout was observed (18).

Kim *et al* revealed that the pulpal blood flow in mongrel dogs determined using the 15 microns radioisotope-labeled microsphere injection method decreased significantly when 2% lidocaine with 1:100,000 epinephrine administered by the various local anesthetic techniques, infiltration, mandibular block and intraseptal injection. However, the most drastic reduction occurred in the molar teeth with intraseptal injection. But when 2% lidocaine without epinephrine was used in the

intraseptal injection, pulpal blood flow increased significantly (19). This study was similar to Beveridge and Brown's experiment in 1965, that infiltration of lidocaine without vasoconstrictor provided sufficient local anesthesia to prepare a cavity without lowering pulpal blood flow but produced a transient increase in pulpal pressure (70). The study of the effects of dental local anesthesia solutions to pulpal blood flow assessed by a laser Doppler flowmeter were also done by Pitt Ford et al (1993). The results showed that following injection of 1 ml of 2% lignocaine with epinephrine 1:80,000 caused a significant reduction (31%) of pulpal blood flow and the duration was 68.5 minutes (20). These results are confirmed in humans by Ahn's experiment (1998) that 2% lidocaine with 1:100,000 epinephrine was able to decrease the blood flow in the dental pulp up to 73% within 5 minutes (21).

Recently, Ittagarun and Tay succeeded in recording dentinal fluid flow in human by impression and replica technique and examining with scanning electron microscope. The experiment was set to compare surface features of deep acid conditioned dentin from vital human molars that were anesthetized with a local anesthetic with or without a vasoconstrictor. Following the use of a local anesthetic containing a vasoconstrictor, patent tubular orifices without fluid were observed in all specimens. In contrast to the group using a local anesthetic without a vasoconstrictor, fluid was visible leaving tubular orifices in all replica (71).

Removal of the smear layer by acid etching increases the permeability of the dentin substrate, transdentinal permeation (11). In addition to this permeation through the dentinal tubules, an intradentinal permeation has been described, which is essential for resin infiltration into the perifibrillar spaces around collagen fibrils (5, 6) The extent of resin infiltration depends on the amount of apatite removed by conditioning and the moisture of the dentin. The intrinsic moisture, i.e. the outward flow of dentinal fluid, may interfere with monomer infiltration into the dentin, depending on the monomer composition of the dentin bonding systems (7). It has therefore been suggested to perform *in vitro.* testing of dentin bonding systems under hydrostatic pulpal pressure in order to simulate physiological bonding conditions (24, 72). Interestingly, Moll et

al. demonstrated that simulated pulpal pressure, 30 $cmH_2O$, adversely affects the efficacy of dentin bonding system (73).

From this knowledge, it could be assumed that the total-etching system probably performs well in some clinical cases when a local anesthetic with vasoconstrictor is used to minimize the intrinsic wetness.

An alternative strategy is to use a self-etching primer system. Although self-etching primers can modify the smear layer, the weaker acidity of the primer solutions results in minimal dissolution of the smear plugs and limits the opening of the dentin tubules, which, in turn, will reduce the permeability of dentin. With the use of self-etching primers, the need of having a moist dentin surface, which allows the collagen fibrils to remain plasticized and flexible for optimal resin infiltration, is less when compared to adhesive systems that utilize strong acid conditioners.

# CHAPTER 4
# MATERIALS AND METHODS

This project was approved by the committee on human rights related to human experimentation, Mahidol University. All the patients participated in this study were informed about the operative procedures and the consequential symptoms that might occur. The patients agreed to participate the experiments and the informed consent were signed before the operation.

**Materials**

1. The adhesive systems used in this study are listed below

| Adhesive system | Manufacturer | compositions | Batch No |
|---|---|---|---|
| Single Bond | 3M ESPE St.Paul, Minnesota, USA | Etchant: 35% phosphoric acid Adhesive: ethanol, HEMA, Bis-GMA, PAAC, water, dimethacrylates (UEDMA, GDMA) | 20011211 |
| Clearfil SE Bond | Kuraray Medical Inc. Okayama, Japan | Primer: MDP,HEMA, hydrophilic dimethacrylate, dicamphoquinone, N,N-diethanol-p-toluidine, water Bonding: MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, dicamphoquinone, N,N-diethanol-p-toluidine, silanated colloidal silica | 51142 |

2. Resin composite shade A2 ( Filtek™ Z250 , 3M ESPE, St. Paul, Minnesota, USA)
3. Topical anesthetic agent ( Benzo-Jel, Melville,NY,USA)
4. Local anesthesia (Scandonest 2% special with 1:100,000 epineprine, Septodont, Kent, UK)
5. Silicone impression material (Xantopren L, Hareus Kulzer, Germany)
6. Cyanoacrylate cement (Zapit ,MDS Products Co., Corona, CA, USA)
7. Epoxy resin

**Instruments**

1. Fissure diamond bur (No.214 , Intensive, Viganello-Lugano, Switzerland)
2. Ultrafine diamond bur (No.FG 4205 L , Intensive, Viganello-Lugano, Switzerland)
3. Pumice and brush
4. Dental needle gauge 27
5. Rubber dam sheet
6. Clamp #2
7. Elevator
8. Extracting forceps #150
9. Light curing unit (Optilux 501, Kerr/Demetron)
10. Micrometer model ICD-S 15M (Mitutoyo corporation, Japan)
11. Universal testing machine (Instron 5566H 1612, England)
12. Measuring microscope model NM 11 (Nikon, Japan)
13. Microcutting instrument (Accutom-50 Streuers, Denmark)
14. Scanning electron microscope (JSM-5410LV, JEOL Ltd., Tokyo, Japan)
15. Bencore multi-T device (Danville engineering, Danville, California, USA)

## Methods

### Clinical Procedures

**Part I: The microtensile bond test**

The study was performed on upper premolars scheduled for extraction for orthodontic reasons. All teeth were intact, non-carious and restoration-free. The patients were randomly assigned into 2 groups that were subjected to the following treatments

Group 1: Experiment group: using a local anesthetic with vasoconstrictor
Group 2: control group, no anesthesia

In each group the patients were randomly assigned into 2 subgroups that were subjected to the materials

Subgroup 1: Single Bond group
Subgroup 2: Clearfil SE Bond group

**In group 1 (Experiment group):** Patients were asked to abstain from hot, cold or alcoholic foods or beverages for at least 2 hours before attending. The mucous membrane in the vestibule above the tooth was placed with a cotton pellet soaked in a surface anesthetic agent for 1 minute. Then the tooth was anesthetized with approximately 1.5 ml 2% Scandonest special with 1:100,000 epinephrine. The teeth were isolated from the oral environment with rubber dam to exclude contamination of the sampling area from oral fluids.The rubber dam was disinfected with tincture iodine and 70% ethyl alcohol. A class I cavity about 4x8 mm (MDxBL) and 1.5 mm under DEJ depth was prepared. Preparations were cut with a cylindrical diamond bur operated at a high speed under copious water irrigation.

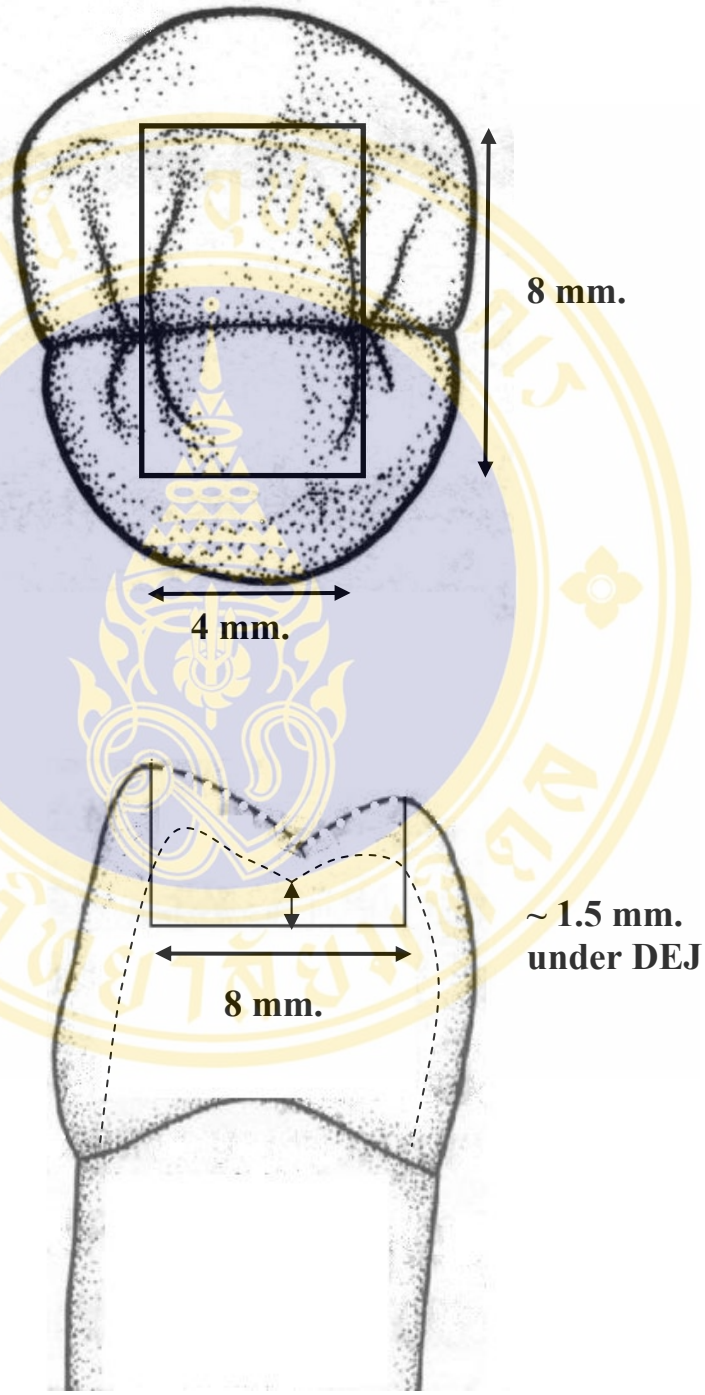


**Figure 4.1 Diagram of the outline of the cavity preparation on occlusal surface of the maxillary premolar**

**In subgroup 1 (Single Bond)** After the cavities had been prepared, the teeth were polished with pumice and rinsed with water. Then the cavity surfaces were etched with 35% phosphoric acid gel for 15 seconds followed by 20 seconds water rinse. The adhesive was applied according to a manufacturer's instruction and light cured for 10 seconds with a light curing unit. Then a hybrid resin composite was placed into the cavities by an incremental technique until the bulk of resin composite about 5 mm. in height over the occlusal surface was created. The teeth were then carefully extracted with either forceps or elevator to avoid direct pressure on the filling or its nearest surrounding area. After extraction, the teeth were cleaned and then stored in a humidity chamber at room temperature for one day before laboratory procedures.

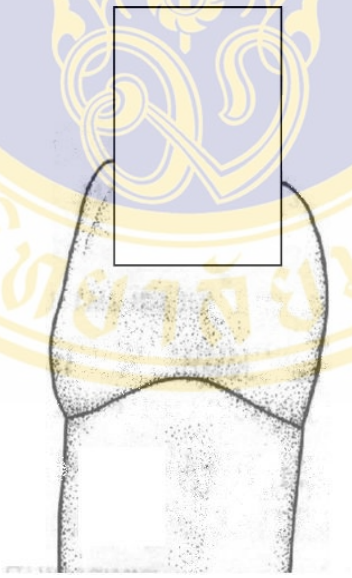


**Figure 4.2 Diagram of a bulk of resin composite after restoration**

**In subgroup 2 (Clearfil SE Bond)** Once the cavities had been prepared, the teeth were cleaned with pumice, rinsed with water and dried with oil-free air. The primer

was applied to the entire cavity wall with a sponge or a disposable brush tip and left for 20 seconds. Then the volatile ingredients were evaporated with a mild oil-free air stream. The bonding was then applied to the cavity surfaces. The bonding film were made as uniform as possible by a gentle oil-free air stream and was light-activated for 10 seconds with a visible light curing unit. The following procedures of resin composite filling, extraction of the teeth and storing process were the same as those in subgroup 1.

**In group 2 (control group):** The teeth were divided into 2 subgroups and treated in exactly the same procedure as previously mentioned except that the teeth were not anesthetized with either topical anesthesia or local anesthetic solutions to avoid the consequence of pulpal blood flow effects.

**Part II: The observation of the dentin surface**

The condition of tested teeth and patients were the same as those in the microtensile bond test studies. The patients were randomly assigned into 2 groups that were subjected to the following treatment.

Group 1: administration of a local anesthetic with vasoconstrictor

Group 2: control group: no anesthesia

**In group 1 (administration of a local anesthetic with vasoconstrictor)** Patients were asked to abstain from hot, cold or alcoholic foods or beverages for at least 2 hours before attending. The mucous membrane in the vestibule above the tooth was placed with a cotton pellet soaked in a topical anaesthetic agent for 1 minute. The teeth were anaesthetized with approximately 1.5 ml 2% Scandonest with 1:100,000 epinephrine. The teeth were isolated and the cavities were prepared in exactly the same way as in microtesile bond tests. The cavities were cleaned with pumice, rinsed with water and dried with cotton pellets and then recorded with a silicone impression material. After fully polymerization, the set impressions were removed from cavities, rinsed and kept for further laboratory procedures. The same cavities were again

cleaned with slurry pumice and water. Then the specimens were etched with 35% phosphoric acid gel for 15 seconds followed by 20 seconds water rinse. The impressions were taken, removed and kept with the prior technique.

**In group 2 (Control group)** The premolars were treated in exactly the same procedures as those in group 1 except that the teeth were not anesthetized with either topical anaesthesia or local anesthetic solutions to avoid the consequence of pulpal blood flow effects.

**Laboratory procedures**

**Part I: The Microtensile bond test**

After the storage time in 100% humidity chamber at room temperature for 24 hours, the specimens were sectioned with a diamond saw under copious water lubrication to separate them serially perpendicular to the bonded surfaces, creating 4 slabs per tooth of approximately 0.7 mm. thick. The remaining dentin thickness (RDT) from the resin dentin interface to the nearest portion of the pulp chamber was measured in each slab and the specimens were trimmed into an hour-glass shape with the narrowest portion of approximately 1.4 mm. at the adhesive-dentin interface by mean of an ultrafine diamond point attached in a high speed handpiece under copious air-water spray.

The trimmed specimens were mounted on a testing apparatus (Bencor Multi T Device) with a cyanoacrylate adhesive, subjected to tensile forces at a crosshead speed of 1 mm/min in a universal testing machine until until the bond fractured.

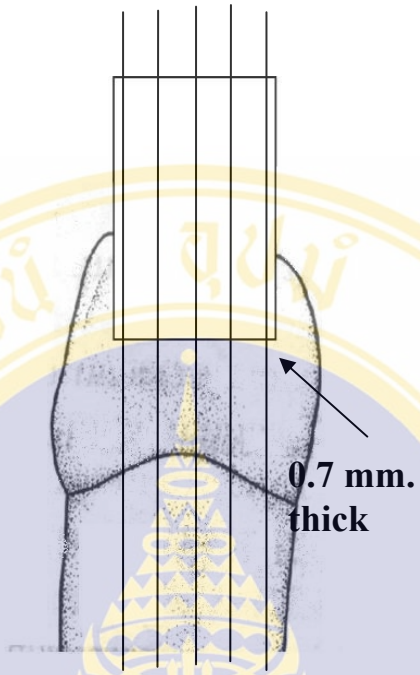



**Figure 4.3 Diagram of the outline of sectioned slabs for the microtensile bond tests**

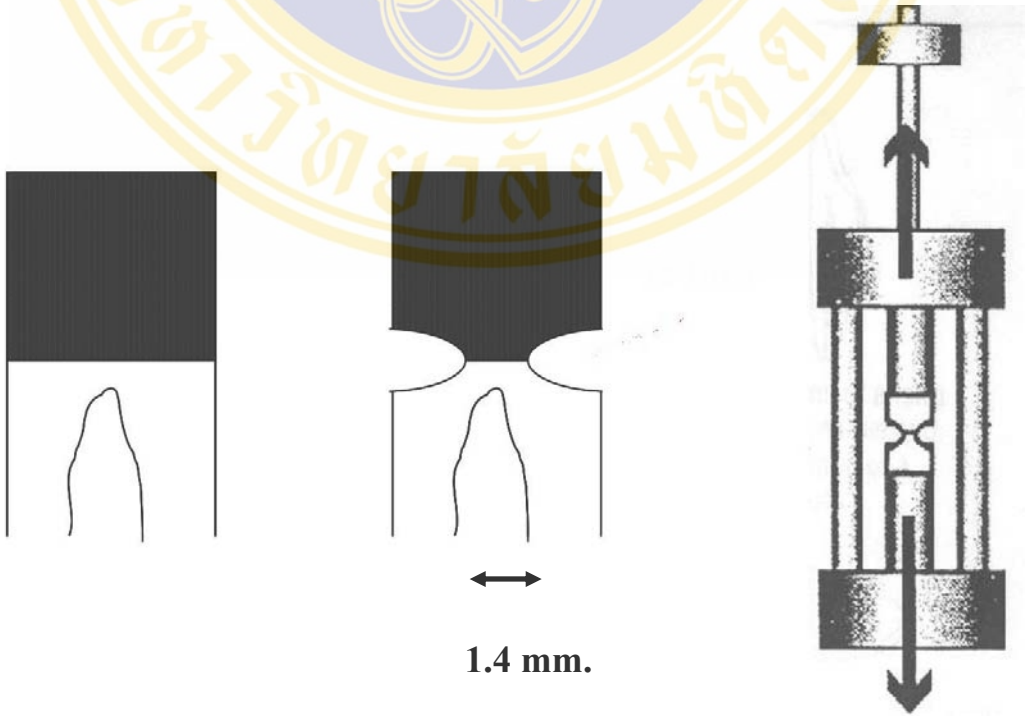



**Figure 4.4 The diagram of the microtensile bond test**

**Part II: The observation the dentin surface.**

After the impressions of the cavities had been taken, each impression was rinsed with distilled water, dried with oil-and dust-free air and left for 1 hour. Then the replica was prepared by pouring a freshly mixed epoxy resin into the impression which was allowed to polymerize at room temperature for 8 hours. Specimens were then ultrasonically cleaned in distilled water for 3 minutes, air-dried and sputter-coated with gold. Observations were then performed under the SEM.

# CHAPTER 5
# RESULTS

## The microtensile bond strength test

The results obtained with microtensile bond strength test of two adhesive systems are illustrated in Table 5.1. No specimens were lost during preparation for the bond test.

These results varied from 24.39 ± 5.58 MPa for Single Bond with a local anesthetic containing vasoconstrictor to 29.69 ± 6.09 MPa for Clearfil SE Bond without a local anesthetic containing vasoconstrictor. The t-test for independent revealed that injection of a local anesthetic containing vasoconstrictor had no statistically significant influence on dentin microtensile bond strengths ($p > 0.05$).

For the total-etching system, the average values obtained from the normal tooth (25.03 ± 7.42) on one hand, and from the tooth using a local anesthetic containing vasoconstrictor (24.39 ± 5.58) on the other hand were not statistically different ($p > 0.05$). However, the microtensile bond strength to the dentin on a normal tooth, a group without a local anesthetic containing vasoconstrictor was slightly higher. The results in the self-etching system was similar to those in the total-etching system, no statistical difference was also noted between the normal group and the group using a local anesthetic containing vasoconstrictor ($p > 0.05$), in which the microtensile bond strength to normal tooth, a group without a local anesthetic containing vasoconstrictor (29.69 ± 6.09) was also slightly higher than a group with a local anesthetic containing vasoconstrictor (28.65 ± 7.89).

Between the total-etching and the self-etching system, the microtensile bond strengths expressed as a mean and standard deviation for each product and test

condition were shown in table 5.1. Statistically significant differences existed at the 0.05 level between the mean microtensile bond strengths recorded by Clearfil SE Bond (the adhesive with a self-etching primer) and Single Bond (the total-etching system), with the values being higher for either with or without a local anesthetic containing vasoconstrictor condition in Clearfil SE Bond group.

**TABLE 5.1: Mean microtensile bond strengths (MPa) to normal dentin and dentin on the tooth using a local anesthetic with vasoconstrictor**

| | Mean±SD(MPa) (n) | |
|---|---|---|
| Adhesive | normal dentin | dentin on the tooth using a local anesthetic with vasoconstrictor |
| Single Bond | 25.03 ± 7.42 [a] (48) | 24.39 ± 5.58 [a] (45) |
| Clearfil SE Bond | 29.69 ± 6.09 [b] (49) | 28.65 ± 7.89 [b] (53) |

The values with the same superscript are not significant different.

**Remaining dentin thickness evaluation**

The remaining dentin thickness (RDT) of the specimens for microtensile bond strengths test was recorded. Table 5.2 summarized the RDT of debonded specimens. The mean RDT varied from 2.17 ± 0.51 mm. for Clearfil SE Bond with a local anesthetic containing vasoconstrictor to 2.29 ± 0.57 mm. for Single Bond with a local anesthetic containing vasoconstrictor. While the dentin thickness for Single Bond without a local anesthetic with vasoconstrictor and Clearfil SE Bond without a local anesthetic containing vasoconstrictor are 2.28 ± 0.53 mm. and 2.23 ± 0.69 mm. After statistical analysis using the one-way ANOVA, the result revealed that the RDT in all groups had no statistically significant difference ($p < 0.05$).

**TABLE 5.2: The mean and standard deviation of remaining dentin thickness of debonded specimens for microtensile bond strength test (mm.)**

| | RDT |
|---|---|
| | mm. ± SD |
| | (n) |
| Single Bond without a local anesthetic containing vasoconstrictor | 2.28 ± 0.53 [a] (42) |
| Single Bond with a local anesthetic containing vasoconstrictor | 2.29 ± 0.57 [a] (41) |
| Clearfil SE Bond without a local anesthetic containing vasoconstrictor | 2.23 ± 0.69 [a] (30) |
| Clearfil SE Bond with a local anesthetic containing vasoconstrictor | 2.17 ± 0.51 [a] (39) |

The values with the same superscript are not significant different.

**The evaluation of the dentin surface**

Resin replicas of the acid-etched dentin from the tooth that were anesthetized with 2% Lidocaine with 1:100,000 epinephrine showed 3 different patterns. First, the smear layer was absent and the patent tubular orifice without dentinal fluid was observed (Figure 5.1). Second, the dentin surface with the patent dentinal tubules with emerging fluid droplets (Figure 5.2). Third, the surface with the coalesce dentinal fluid from the tubules (Figure 5.3).

In contrast, the exudation of dentinal fluid was detected in all specimens from the unanesthetized group. From the replicas observations, the entire dentin surface was covered with a smooth amorphous film of dentinal fluid (Figure 5.4).

In unetched groups, resin replicas from dentin surface showed the presence of smear layer with a granular appearance in both anesthetized and non-anesthetized groups. The SEM observation of replicas identified that the granules from the group that was anesthetized with 2% Lidocaine with 1:100,000 epinephrine (Figure 5.5) were slightly smaller than those of non-anesthetized group (Figure 5.6).

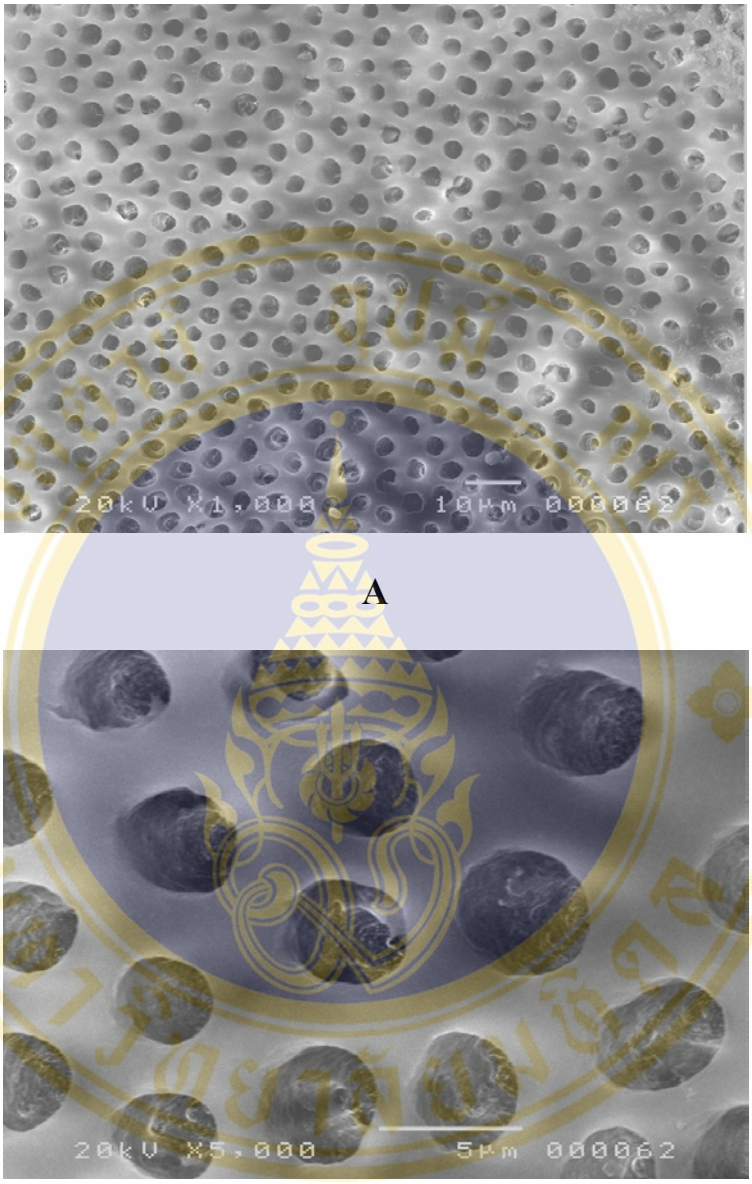
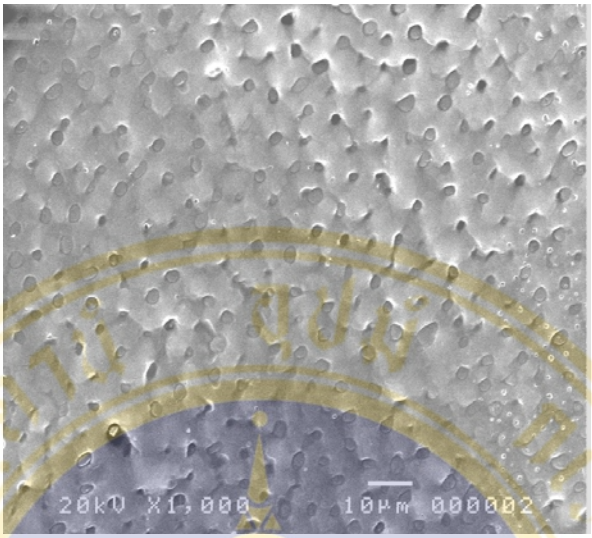
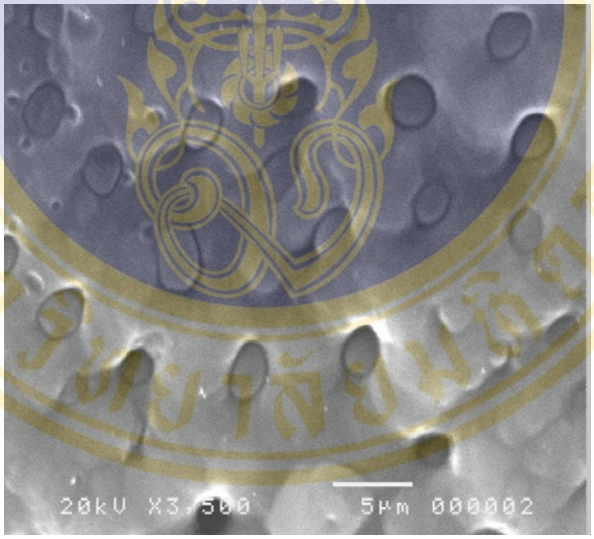



**Figure 5.1: Scanning electron micrographs of replicas recorded from etched dentin surface on a tooth using a local anesthetic containing vasoconstrictor. (A) The patent lumina without the dentinal fluid were observed. (B) A higher magnification of dried dentinal tubules (5000X)**

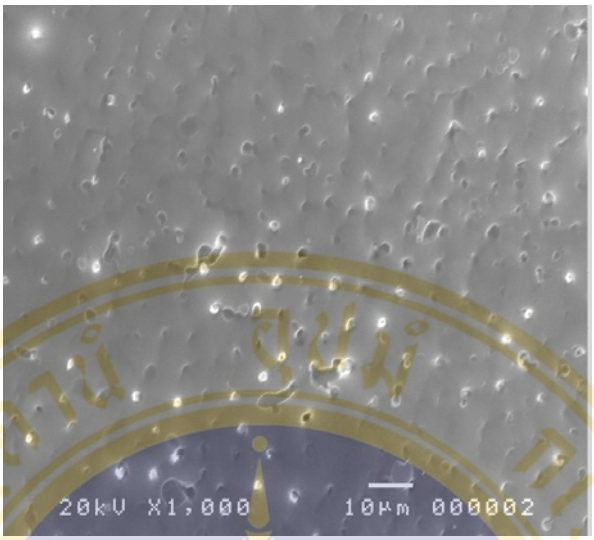



**(A)**

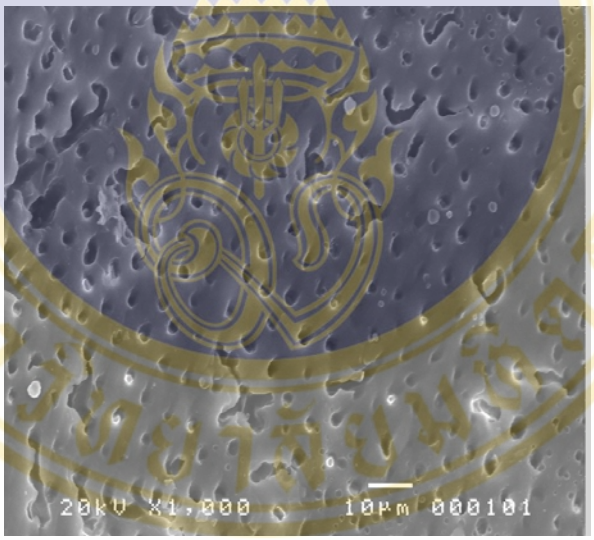



**(B)**

**Figure 5.2: Scanning electron micrographs of replicas recorded from etched dentin surface on a tooth using a local anesthetic containing vasoconstrictor. (A) The patent lumina with the emerging fluid droplets were detected. (B) A higher magnification of (A) (3500X)**

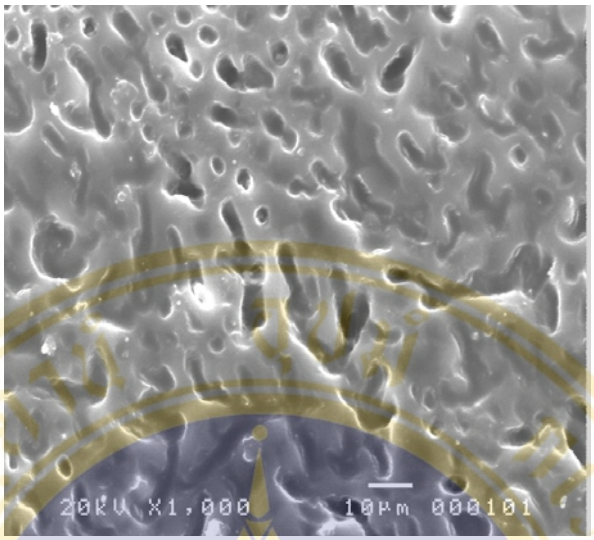



**(A)**

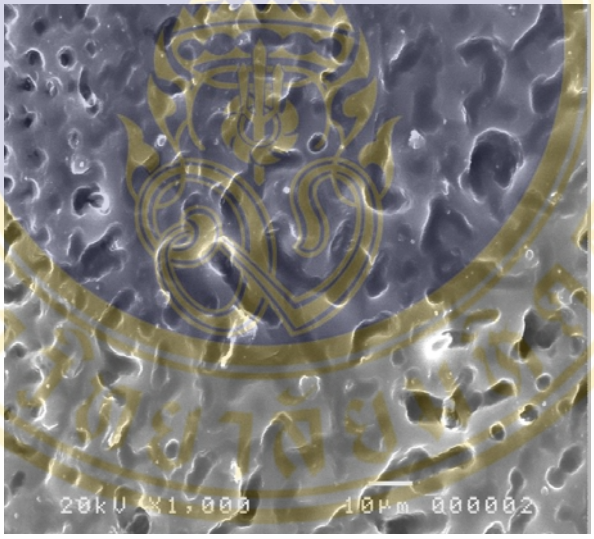



**(B)**

**Figure 5.3: Scanning electron micrographs of replicas recorded from etched dentin surface on a tooth using a local anesthetic containing vasoconstrictor. (A) and (B) the surface with the coalesce dentinal fluid from the tubules**

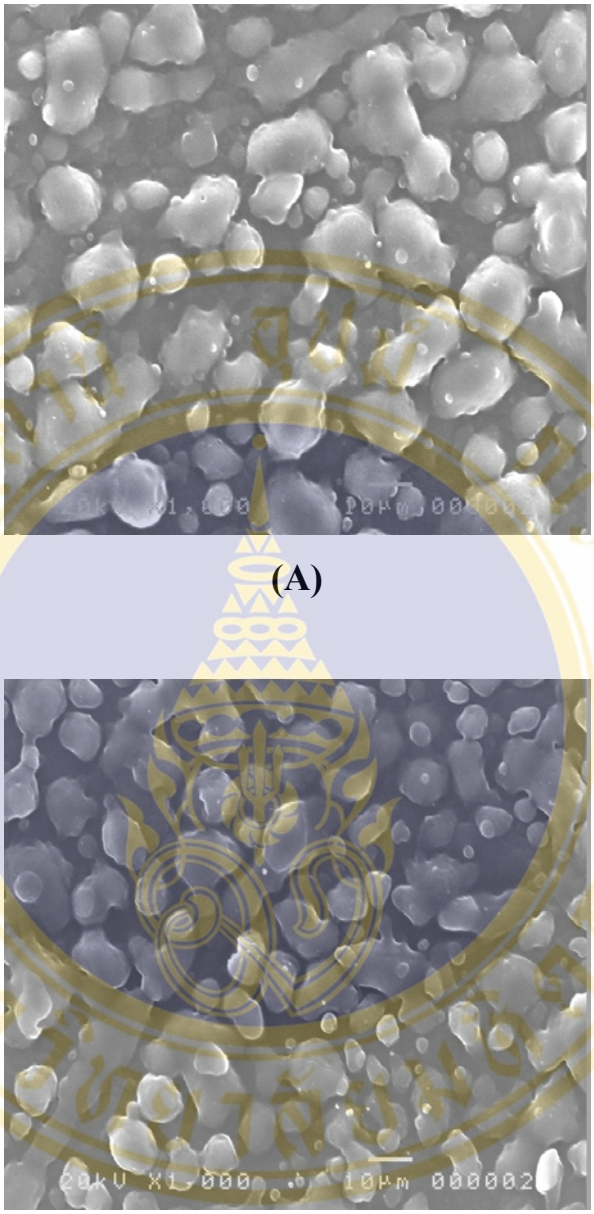



**(A)**




**(B)**

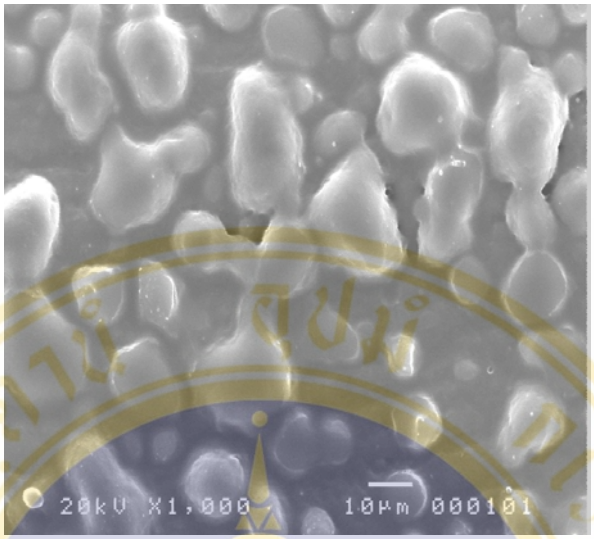
**Figure 5.4: Scanning electron micrographs of a replica recorded from etched dentin on the unanesthetized tooth. (A) and (B) The lumina were covered with a smooth amorphous film of dentinal fluid**



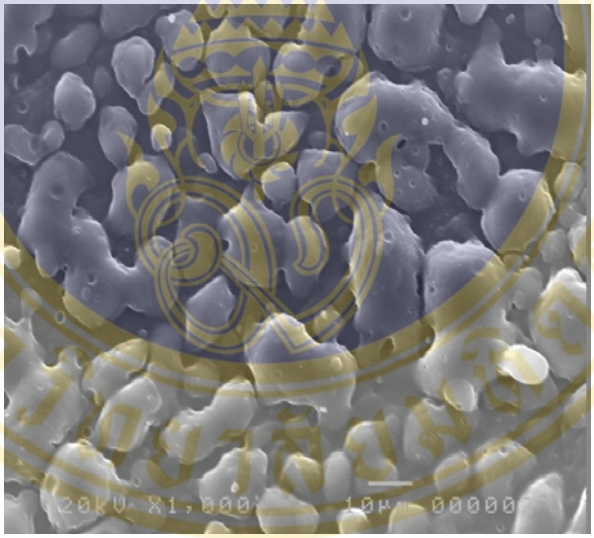

**(A)**

**(B)**

**Figure 5.5: Scanning electron micrographs of a replica recorded from unetched dentin surface on the tooth using a local anesthetic with vasoconstrictor. (A) and (B) The small-sized emerging fluid droplets covered by the smear layer were detected**




**(A)**




**(B)**

**Figure 5.6: Scanning electron micrographs of a replica recorded from dentin surface on the unanesthesize tooth. (A) and (B) The size emerging fluid droplets covered by the smear layer were larger than the droplets in the anesthetized group (Figure 5.5).**

# CHAPTER 6
# DISCUSSION

Most studies of the bonding capabilities of adhesive systems have concentrated on the use of *in vitro* testing conditions. Prior experiments investigated bond strengths to dentin on extracted teeth that were stored in water. A gradient of water content throughout the dentin may lead to higher or lower dentin bonds, depending upon the bonding systems. Although recently developed adhesive systems are much less sensitive to the degree of wetness of dentin than earlier bonding agents (74)-(75), moisture effects have been repeatedly demonstrated (73). Pashley and others (1984) revealed that fluid-filtration occurred from pulpal surface to the prepared surface in freshly cut dentin of vital dog teeth. This fluid was presumably pulpal fluid and could act as a source of moisture contamination during polymerization of dental adhesive systems. From this experiment we can assume that dentinal fluid is one of the factors affecting bonding capabilities of the dentin hence the *in vitro* and *in vivo* tests simulating the clinical condition of the dentin bonding systems under hydrostatic pulpal pressure (24, 72) should be performed. According to the suggestion, the pressurized dentin was used to study the effects on bond strengths and sealing properties of adhesive systems (13, 74, 76-82). The perfusing solutions used in these studies were sterile phosphate-buffered saline (13, 74, 76-77, 79) sterile physiologic saline (81), horse serum (82) and water (78, 80).

None of previous *in vitro* models have been able to mimic *in vivo* conditions flawlessly. Those models had provided only preliminary information to substantiate clinical phenomina. One important factor affecting the efficacy of adhesive systems is the permeability characteristics of dentin. However, the dentin permeability of teeth after extraction was shown to increase over time (45, 83). After tooth extraction, dentin permeability almost doubles in a week (45) and it continues to increase even a

week after (83). The most likely explanation of the increase in dentin permeability *in vitro* was the discharge of the loosely bound organic contents of tubules that had already started to degrade after extraction (84). This means that the bond test results from in vitro conditions may be not reliable.

To achieve the accurate results, this study was performed *in vivo*. on upper premolars scheduled for extraction for orthodontic reasons. Because all teeth were intact, non-carious and restoration free, it could be assumed that the experimental dentin in this study was intact. Even though the intact dentin may not represent the dentin in ordinary clinical situation which mostly is caries-affected dentin, we decided to use this dentin in the study. The caries-affected dentin has some disadvantages from its unpredictable structures that affect the microtensile bond strength. First, the dentinal tubules of excavated carious dentin are filled with a various degree of mineral deposits, therefore, this dentin is much less permeable than normal dentin (24, 85). The whitlockite crystals in the lumina of dentinal tubule may impede the penetration of the resin monomer especially that from the self-etching system that may interfere the forming of the resin tag. Second, the smear layer created on caries-affected dentin includes acid-resistant crystals that formed during cycles of demineralization. This smear layers are more resistant to the self-etching primer that is the weak acid (86). Third, caries-affected dentin has lost its mineral phase in various degree (85). This revealed by a Knoop hardness of caries-affected dentin that is only half that of normal dentin (87-88). The loss of mineral from intertubular dentin may have the effect to the bonding procedures particulary those in a total-etching system. To avoid the variabilities from the structure of caries-affected dentin, using the intact dentin in this experiment was suitable.

The bond strengths of the adhesive systems tested in this study were measured through a microtensile test, which allows a better distribution of stress on the adhesive interface, when compared to the conventional tensile or shear tests. The conventional tests served well when resin-dentin bond strengths were relatively low (10 to 15 MPa). However, as bonding techniques and materials improved, the bond strengths became high enough to cause cohesive failures in dentin, leaving the resin-dentin interface

intact (72, 89). To avoid cohesive failures of dentin during bond testing, the microtensile bond test was used in the study. Additionally, it was difficult to obtain intact vital premolars for the experiment. The microtensile testing method permitted multiple specimens to be prepared from each tooth. Thus, there was a trade-off between the extra labor involved in using this method, and the extra data that could be obtained per tooth.

The adhesive systems in previous studies showed dramatic decrease in bond strengths under simulated pulpal pressure (13-15, 81). The response of adhesive systems to intrinsic moisture depends on several factors. First is the ability of adhesive resin to polymerize and cross-link in the presence of water. Although some studies revealed that no detrimental effect of hydrostatic pulpal pressure on shear bond strength has been observed with autocuring 4-META/MMA-TBB systems (79, 90), however, water seems to have an inhibitory effect on the polymerization of light-curing bonding resins. The addition of 0.2 mL water/mL bonding resin resulted in the reduction of the conversion rate of the bonding resin by approximately 25% (91). Second, the ability of resin monomers to penetrate into the demineralized dentin and form a hybrid layer on intertubular and peritubular dentin against the outward flow of dentinal fluid. The degree to which perifibrillar water in the exposed collagen is replaced by resin monomers depends on the water-displacing effect of the solvents in bonding resins and their ability to promote the infiltration of monomers into the microporosities of the exposed collagen network. Acetone is known to have excellent water-chasing properties, ethanol is less effective, while water is the least (91-93).

The animal study, measuring the shear bond strengths of dentin bonding systems demonstrated that remaining dentin thickness (RDT) affected the shear bond values (6). In addition, most bonding systems showed higher bond strengths to superficial dentin and progressively lower bond strengths to deep dentin (6, 94-96). In our study, the means RDT in all groups did not show statistically significant difference ($p > 0.05$), therefore, the effect of the RDT to the bond strengths could be excluded.

The results of this study showed that the bond strength to dentin was material dependent. Both bonding approach (self-etching and total-etching) and the fillers in the composition of the adhesives gave some contributions on the performance of the systems**.** The higher bond strength obtained by Clearfil SE Bond may be attributed to the presence of fillers. This adhesive formed thick intermediate layers between the hybrid layers and the resin composite restoration. An intermediate filled adhesive layer provides an elastic buffer zone 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 (97). Moreover, in a microtensile bond test, the alignment of the specimen with respect to the tensile load is critical to avoid stress concentration at the interface during testing. The specimen on the long axis of the testing device may not always be exactly parallel to the long axis of the testing device. The thicker adhesive layer may permit self-alignment of specimen that corrects for minor deviation in specimen placement, thereby, improving stress distribution during testing, yielding higher bond strength (98). Contrast, the unfilled Single Bond showed lower microtensile bond strength compared with the highly-filled Clearfil SE Bond. This corresponded to the study that filler content was necessary to increase the bond strength and improve the mechanical properties of bonding agents (97).

Another possible explanation of the lowered bond strength obtained from the Single Bond was the differences in adhesive ingredient. Clearfil SE Bond contains 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) monomer, which has two hydroxyl groups that may chelate to calcium ions of dentin, thereby improving bonding (99). On the other hand, Single Bond contains polyalkenoic acid as the copolymer. This high molecular weight resin component (from 14,000 to 20,000) is difficult to penetrate the narrow (20 to 30 nm.) intertubular spaces of demineralized dentin matrix (5, 100). It seems likely that the copolymer may be restricted to the superficial portion of the hybrid layer and form a continuous layer along the dentin surface through chelation with remnant calcium ions that are present in the demineralized dentin (100). The inclusion of polyalkenoic acid copolymer may cause incomplete resin infiltration which affects the bond strengths.

When a vital tooth is anesthetized with a local anesthetic containing vasoconstrictor, pulpal tissue pressure is lowered. It has been shown that epinephrine acted on α-adrenergic receptors to constrict blood vessels. When the time passes, the vasoconstrictor effect is decreased, the fluid flow returns to normal. The outward dentinal fluid movement is corresponded to blood flow. From the principle of dental adhesives, the reduction of dentinal fluid seepage will probably enhance the adhesion between the dentin bonding system and the tooth surface, particularly the total-etching systems. These were proved by many *in vitro* and *in vivo* studies. Conversely, there was a study found that the presence of pulpal pressure did not significantly affect bonding to vital baboon teeth prepared *in vivo* (101). Similarly, in this study, the use of 2% Scandonest with 1:100,000 epinephrine did not promote the microtensile bond strengths to dentin of both total-etching and self-etching systems. From the finding of the dentinal fluid dynamic in human dentin, the mean value of spontaneous fluid flow approximately 0.35 microlitre/$cm^2$/min was recorded (66). Another research revealed that 20% water added bonding resin had a result in the reduction of the conversion rate of the bonding resin by approximately 25% (91). However, in this study, the dentinal fluid changes due to a local anesthetic containing vasoconstrictor had very little effect on the microtensile bond strengths of both adhesive systems. The possible explanation may be that the different amount of the dentinal fluid between anesthetized and unanesthetized group detected on dentin surfaces before bonding procedures might be very little compared with the amount of adhesive resin.

Nowadays, the experiments of pulpal blood flow and other related factors usually perform with the laser Dopler flowmetry. This technique affords new opportunities for the clinical researchers by providing continuous, noninvasive method (20-21, 102-104). In this study, we did not use this technique since the experiments consisted of many steps and the process of a laser Dopler flowmetry is complicated. Instead of detecting blood flow which is the source of dentinal fluid with a laser apparatus, we observed the dentinal fluid droplets directly by the impression technique as earlier studies (71, 105).

With the impression technique, Kerdvongbundit and Itthagarun demonstrated the acid-conditioned vital deep dentin was dried on the anesthetized tooth. The SEM images of the resin replica showed that the patent tubular structure without dentinal fluid was observed. Conversely, the observation of the resin replica in our study revealed that the etched dentin surface of a 2% Scandonest with 1:100,000 epinephrine infiltrated tooth showed 3 different patterns. These are 1) the dentin surface with patent dentinal tubules without fluid exudation, 2) the dentin surface with the patent dentinal tubules with emerging fluid droplets and 3) the surface with the coalesce dentinal fluid from the tubules. The dentin surface obtained in this study seemed to be wetter than those from others. These differences were probably related to some factors. First, the dentin structure, the dentin tested in Itthagarun's work was the caries-affected dentin obtained from deep occlusal carious molars that planed for indirect resin-based composite inlay. This caries-affected dentin had the whitlockite crystals occluding lumina therefore, its permeability is lower than that of normal dentin. This probably affected the fluid permeation. Second, the anesthetized technique, the intraligamental technique performed in Kerdvongbundit's experiment reduced more pulpal circulation than those from the infiltration in our studies.

When the replicas of unetched dentin surface were observed, the emerging of fluid droplets covered by smear layer in was found as earlier studies (71, 106). However, our SEM images were different from the previous study. In this experiment, after injection of lidocaine with vasoconstrictor, the granular appearance was still detected on the dentin surface but the size was smaller than those of the surface on unanesthetized tooth. Contrast to Kerdvongbundit's work, with intraligamental technique, the specimen was dried at 5 minutes after injection with lidocaine with vasoconstrictor and the droplets were increased in size after the local anesthetic effect was removed. The different SEM results probably depended on the local anesthetic administration technique. The intraligamental technique may be better than the infiltration technique in term of pulpal circulation

As mentioned above, there were many techniques to administrate a local anesthetic in previous studies(18-21, 105). The intraseptal and the intraligamental

technique was shown to be the optimum technique to lower the dentinal fluid movement (19, 105). In this study, to simulate clinical practice, 1.5 ml 2% Scandonest with 1:100,000 epinephrine was infiltrated to reduce the dentinal fluid seepage. However, the action of a local anesthetic and vasoconstrictor might not be uniform due to the variation of the distance between the anesthetized area and the root tip. Moreover, the variable density of the maxilla might affect the penetration of a local anesthetic and vasoconstrictor into the root tip of the tooth. This uncontrolled condition might lead to the variable dentinal fluid flow that affects the mean microtensile bond strength. The high value of standard deviation of the mean bond strength in this experiment obtained might be a result from these variations. When regard to fluid flow, the infiltration technique provided unsatisfied result. However, due to its simplicity, this technique is usually performed when dealing with upper maxillary premolar in ordinary clinical situation. Lastly, the infiltration of lidocaine with vasoconstrictor provided a slight difference of dentinal fluid on the dentin surface compared to unanesthetized tooth, the microtensile bond strength of adhesive resin to these dentin was not different. From this study it could be assumed that when a local anesthetic with vasoconstrictor is infiltrated, the reduction of intrinsic wetness is not enough to promote the bond strength of the adhesive systems.

# CHAPTER 7
# CONCLUSION

The results of this study showed that:-

1. For the total-etching system, microtensile bond strengths obtained in the anesthesized dentin, with a local anesthetic containing vasoconstrictor, and in unanesthetized dentin were not statistically different ($p > 0.05$).
2. For the self-etching system, microtensile bond strengths obtained in the anesthesized dentin, with a local anesthetic containing vasoconstrictor, and in unanesthetized dentin were not statistically different ($p > 0.05$).
3. The administration of a local anesthetic containing vasoconstrictor influenced the dentinal fluid flow. The reduction of fluid flow was observed when a local anesthetic agent with vasoconstrictor was applied.

This study suggests that a minute amount of dentinal fluid flow *in vivo*. does not have detrimental effects to both total-etching and self-etching adhesive systems used in this study.

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# APPENDIX

**The original data used in this experiment**

1. The original data obtained from the group using Single Bond without a local anesthetic.

| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| #25 | SB | N | 1 | 0.68 | 1.55 | 1.054 | - | 29.06 |
| | | | 2 | 0.63 | 1.48 | 0.9324 | 1.7 | 31.04 |
| | | | 3 | 0.64 | 1.52 | 0.9728 | 2.15 | 18.69 |
| | | | 4 | 0.64 | 1.54 | 0.9856 | 2.24 | 29.23 |
| #24 | SB | N | 1 | 0.74 | 1.41 | 1.0434 | - | 32.84 |
| | | | 2 | 0.73 | 1.42 | 1.0366 | 2.27 | 29.57 |
| | | | 3 | 0.71 | 1.44 | 1.0224 | 2.36 | 26.48 |
| | | | 4 | 0.7 | 1.14 | 0.798 | 3.12 | 32.42 |
| #14 | SB | N | 1 | 0.69 | 1.4 | 0.966 | 3.46 | 34.47 |
| | | | 2 | 0.72 | 1.42 | 1.0224 | 2.44 | 18.95 |
| | | | 3 | 0.72 | 1.42 | 1.0224 | 2.33 | 15.33 |
| | | | 4 | 0.68 | 1.36 | 0.9248 | 3.01 | 24.87 |
| #15 | SB | N | 1 | 0.69 | 1.58 | 1.0902 | - | 33.79 |
| | | | 2 | 0.67 | 1.42 | 0.9514 | 1.79 | 19.25 |
| | | | 3 | 0.66 | 1.49 | 0.9834 | 1.84 | 14.41 |
| | | | 4 | 0.64 | 1.62 | 1.0368 | 2.96 | 24.87 |
| #25 | SB | N | 1 | 0.72 | 1.38 | 0.9936 | 3.22 | 24.18 |
| | | | 2 | 0.72 | 1.36 | 0.9792 | 2.57 | 24.95 |
| | | | 3 | 0.7 | 1.33 | 0.931 | 2.31 | 21.53 |
| | | | 4 | 0.71 | 1.44 | 1.0224 | 3.04 | 29.24 |
| #14 | SB | N | 1 | 0.59 | 1.6 | 0.944 | - | 35.47 |
| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |
| | | | 2 | 0.67 | 1.39 | 0.9313 | 1.63 | 19.89 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3 | 0.67 | 1.48 | 0.9916 | 1.57 | 13.14 |
| | | | 4 | 0.61 | 1.64 | 1.0004 | 2.72 | 22.78 |
| #24 | SB | N | 1 | 0.64 | 1.54 | 0.9856 | 2.48 | 29.76 |
| | | | 2 | 0.68 | 1.49 | 1.0132 | 1.94 | 30.06 |
| | | | 3 | 0.63 | 1.55 | 0.9765 | 1.66 | 17.59 |
| | | | 4 | 0.65 | 1.53 | 0.9945 | - | 30.24 |
| #14 | SB | N | 1 | 0.68 | 1.48 | 1.0064 | 2.39 | 18.51 |
| | | | 2 | 0.71 | 1.41 | 1.0011 | 1.82 | 8.68 |
| | | | 3 | 0.7 | 1.43 | 1.001 | 1.66 | 11.45 |
| | | | 4 | 0.63 | 1.52 | 0.9576 | 1.88 | 14.77 |
| #34 | SB | N | 1 | 0.74 | 1.43 | 1.0582 | 2.72 | 38.88 |
| | | | 2 | 0.71 | 1.42 | 1.0082 | 1.81 | 37.7 |
| | | | 3 | 0.71 | 1.38 | 0.9798 | 1.54 | 26 |
| | | | 4 | 0.69 | 1.45 | 1.0005 | 2.61 | 27.62 |
| #24 | SB | N | 1 | 0.68 | 1.36 | 0.9248 | - | 33.93 |
| | | | 2 | 0.72 | 1.36 | 0.9792 | 2.41 | 30.56 |
| | | | 3 | 0.71 | 1.43 | 1.0153 | 2.31 | 27.48 |
| | | | 4 | 0.67 | 1.44 | 0.9648 | 2.74 | 31.24 |
| #14 | SB | N | 1 | 0.75 | 1.35 | 1.0125 | 2.88 | 21.08 |
| | | | 2 | 0.73 | 1.33 | 0.9709 | 1.69 | 21.85 |
| | | | 3 | 0.73 | 1.39 | 1.0147 | 1.82 | 26.42 |
| | | | 4 | 0.72 | 1.41 | 1.0152 | 2.33 | 19.68 |
| #24 | SB | N | 1 | 0.68 | 1.4 | 0.952 | 2.73 | 35.47 |
| | | | 2 | 0.71 | 1.41 | 1.0011 | 1.65 | 19.89 |
| | | | 3 | 0.72 | 1.36 | 0.9792 | 1.46 | 13.13 |
| | | | 4 | 0.69 | 1.44 | 0.9936 | 2.55 | 22.78 |

2. The original data obtained by the group using Single Bond and a local anesthetic with vasoconstrictor.

| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| #24 | SB | Y | 1 | 0.66 | 1.45 | 0.957 | - | 30.32 |
| | | | 2 | 0.7 | 1.44 | 1.008 | 1.45 | 15.59 |
| | | | 3 | 0.67 | 1.38 | 0.9246 | 1.68 | 21.13 |
| | | | 4 | 0.67 | 1.32 | 0.8844 | 2.76 | 16.44 |
| #14 | SB | Y | 1 | 0.74 | 1.34 | 0.9916 | 2.83 | 29.8 |
| | | | 2 | 0.71 | 1.29 | 0.9159 | 1.84 | 30.06 |
| | | | 3 | 0.73 | 1.38 | 1.0074 | 1.63 | 17.58 |
| | | | 4 | 0.68 | 1.4 | 0.952 | 2.58 | 30.25 |
| #44 | SB | Y | 1 | 0.62 | 1.48 | 0.9176 | 2.72 | 30.32 |
| | | | 2 | 0.6 | 1.67 | 1.002 | 1.71 | 17.59 |
| | | | 3 | 0.66 | 1.52 | 1.0032 | 2.53 | 21.13 |
| | | | 4 | 0.59 | 1.69 | 0.9971 | 3.06 | 16.44 |
| #44 | SB | Y | 1 | 0.72 | 1.3 | 0.936 | 2.62 | 19.41 |
| | | | 2 | 0.72 | 1.31 | 0.9432 | 2.46 | 26.99 |
| | | | 3 | 0.71 | 1.37 | 0.9727 | 1.88 | 21.97 |
| | | | 4 | 0.7 | 1.4 | 0.98 | 2.77 | 21.04 |
| #44 | SB | Y | 1 | 0.72 | 1.41 | 1.0152 | - | 22.5 |
| | | | 2 | 0.72 | 1.31 | 0.9432 | 1.66 | 24.18 |
| | | | 3 | 0.66 | 1.47 | 0.9702 | 1.42 | 21.85 |
| | | | 4 | 0.7 | 1.37 | 0.959 | 2.75 | 24.246 |
| #44 | SB | Y | 1 | 0.68 | 1.42 | 0.9656 | 2.38 | 25.97 |
| | | | 2 | 0.71 | 1.31 | 0.9301 | 1.5 | 15.59 |
| | | | 3 | 0.68 | 1.34 | 0.9112 | 2.39 | 33.56 |
| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 4 | 0.7 | 1.43 | 1.001 | 2.5 | 30.01 |
| #14 | SB | Y | 1 | 0.72 | 1.4 | 1.008 | - | 31.16 |
| | | | 2 | 0.71 | 1.41 | 1.0011 | 1.41 | 18.45 |
| | | | 3 | 0.69 | 1.44 | 0.9936 | 2.04 | 22.43 |
| | | | 4 | 0.73 | 0.68 | 0.4964 | 2.36 | 27.54 |
| #14 | SB | Y | 1 | 0.68 | 1.42 | 0.9656 | 3.32 | 27.16 |
| | | | 2 | 0.74 | 1.33 | 0.9842 | 1.55 | 19.15 |
| | | | 3 | 0.72 | 1.32 | 0.9504 | 1.73 | 23.65 |
| | | | 4 | 0.67 | 1.4 | 0.938 | 2.67 | 29.66 |
| #25 | SB | Y | 1 | 0.74 | 1.35 | 0.999 | 3.28 | 33.48 |
| | | | 2 | 0.73 | 1.3 | 0.949 | 2.56 | 24.26 |
| | | | 3 | 0.76 | 1.28 | 0.9728 | 2.21 | 21.51 |
| | | | 4 | 0.72 | 1.4 | 1.008 | 2.92 | 30.21 |
| #15 | SB | Y | 1 | 0.68 | 1.44 | 0.9792 | 2.84 | 27.55 |
| | | | 2 | 0.7 | 1.36 | 0.952 | 1.64 | 16.41 |
| | | | 3 | 0.71 | 1.32 | 0.9372 | 1.73 | 14.52 |
| | | | 4 | 0.69 | 1.34 | 0.9246 | 2.82 | 24.32 |
| #24 | SB | Y | 1 | 0.74 | 1.35 | 0.999 | - | 35.14 |
| | | | 2 | 0.71 | 1.35 | 0.9585 | 2.83 | 29.67 |
| | | | 3 | 0.74 | 1.26 | 0.9324 | 1.69 | 22.66 |
| | | | 4 | 0.73 | 1.3 | 0.949 | 2.01 | 24.79 |
| | | | 5 | 0.73 | 1.34 | 0.9782 | 3.03 | 30.04 |

3. The original data obtained from the group using Clearfil SE Bond without a local anesthetic.

| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| 14 | SE | N | 1 | 0.66 | 1.34 | 0.8844 | 1.72 | 34.42 |
| | | | 2 | 0.71 | 1.35 | 0.9585 | 2.51 | 29.05 |
| | | | 3 | 0.67 | 1.47 | 0.9849 | 2.2 | 33.76 |
| | | | 4 | 0.67 | 1.37 | 0.9179 | 1.21 | 29.42 |
| 14 | SE | N | 1 | 0.75 | 1.32 | 0.99 | - | 27.6 |
| | | | 2 | 0.66 | 1.22 | 0.8052 | 1.21 | 29.05 |
| 14 | SE | N | 1 | 0.7 | 1.4 | 0.98 | - | 19.28 |
| | | | 2 | 0.71 | 1.42 | 1.0082 | 1.55 | 21.14 |
| | | | 3 | 0.71 | 1.25 | 0.8875 | 3.26 | 28.51 |
| | | | 4 | 0.75 | 1.3 | 0.975 | 2.5 | 24.51 |
| 14 | SE | N | 1 | 0.71 | 1.43 | 1.0153 | - | 46.11 |
| | | | 2 | 0.64 | 1.37 | 0.8768 | 1.76 | 35.12 |
| | | | 3 | 0.72 | 1.33 | 0.9576 | 2.48 | 27.04 |
| | | | 4 | 0.69 | 1.44 | 0.9936 | 2.25 | 32.46 |
| 14 | SE | N | 1 | 0.71 | 1.4 | 0.994 | - | 32.17 |
| | | | 2 | 0.71 | 1.39 | 0.9869 | 2.5 | 29.26 |
| | | | 3 | 0.55 | 1.35 | 0.7425 | 3.07 | 31.22 |
| | | | 4 | 0.7 | 1.4 | 0.98 | - | 41.36 |
| 24 | SE | N | 1 | 0.72 | 1.36 | 0.9792 | - | 20.16 |
| | | | 2 | 0.69 | 1.44 | 0.9936 | 1.6 | 21.14 |
| | | | 3 | 0.74 | 1.23 | 0.9102 | 3.11 | 28.46 |
| | | | 4 | 0.73 | 1.34 | 0.9782 | 2.56 | 26.31 |
| 15 | SE | N | 1 | 0.76 | 1.3 | 0.988 | - | 28.4 |
| | | | 2 | 0.73 | 1.26 | 0.9198 | 2.29 | 16.48 |
| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3 | 0.68 | 1.26 | 0.8568 | 1.24 | 30.15 |
| 25 | SE | N | 1 | 0.71 | 1.41 | 1.0011 | - | 21.38 |
| | | | 2 | 0.72 | 1.42 | 1.0224 | 1.48 | 22.16 |
| | | | 3 | 0.69 | 1.45 | 1.0005 | 3.24 | 29.15 |
| | | | 4 | 0.73 | 1.36 | 0.9928 | 2.6 | 26.46 |
| 25 | SE | N | 1 | 0.7 | 1.43 | 1.001 | - | 32.46 |
| | | | 2 | 0.69 | 1.42 | 0.9798 | 2.52 | 30.36 |
| | | | 3 | 0.58 | 1.34 | 0.7772 | 3.1 | 32.14 |
| | | | 4 | 0.7 | 1.38 | 0.966 | - | 43.63 |
| 25 | SE | N | 1 | 0.7 | 1.4 | 0.98 | 1.73 | 32.23 |
| | | | 2 | 0.68 | 1.37 | 0.9316 | 2.61 | 30.06 |
| | | | 3 | 0.66 | 1.45 | 0.957 | 2.13 | 35.14 |
| | | | 4 | 0.67 | 1.39 | 0.9313 | 1.26 | 30.28 |
| 25 | SE | N | 1 | 0.73 | 1.31 | 0.9563 | - | 28.6 |
| | | | 2 | 0.68 | 1.26 | 0.8568 | 1.24 | 27.05 |
| 25 | SE | N | 1 | 0.72 | 1.39 | 1.0008 | - | 33.71 |
| | | | 2 | 0.72 | 1.41 | 1.0152 | 2.61 | 28.62 |
| | | | 3 | 0.65 | 1.45 | 0.9425 | 3.36 | 32.21 |
| | | | 4 | 0.71 | 1.4 | 0.994 | - | 38.66 |

4. The original data obtained by the group using Clearfil SE Bond and a local anesthetic with vasoconstrictor.

| Tooth No | Adhesive | Anesthesia | Specimen no | Thickness | Width | Area | RDT | MTBS |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| 25 | SE | Y | 1 | 0.7 | 1.4 | 0.98 | - | 29.96 |
| | | | 2 | 0.72 | 1.42 | 1.0224 | 1.98 | 17.91 |
| | | | 3 | 0.72 | 1.4 | 1.008 | 2.74 | 28.62 |
| 24 | SE | Y | 1 | 0.72 | 1.36 | 0.9792 | - | 34.11 |
| | | | 2 | 0.72 | 1.4 | 1.008 | 1.41 | 32.92 |
| | | | 3 | 0.73 | 1.44 | 1.0512 | 1.92 | 29.54 |
| | | | 4 | 0.67 | 1.47 | 0.9849 | 3.02 | 29.89 |
| 25 | SE | Y | 1 | 0.78 | 1.23 | 0.9594 | 2.36 | 25.79 |
| | | | 2 | 0.75 | 1.23 | 0.9225 | 1.48 | 29.84 |
| | | | 3 | 0.73 | 1.32 | 0.9636 | 2.39 | 15.53 |
| 24 | SE | Y | 1 | 0.72 | 1.36 | 0.9792 | - | 28.16 |
| | | | 2 | 0.7 | 1.41 | 0.987 | 1.83 | 16.92 |
| | | | 3 | 0.71 | 1.28 | 0.9088 | 1.61 | 15.82 |
| | | | 4 | 0.7 | 1.36 | 0.952 | 2.72 | 28.14 |
| 25 | SE | Y | 1 | 0.74 | 1.28 | 0.9472 | 1.71 | 38.72 |
| | | | 2 | 0.67 | 1.5 | 1.005 | 2.45 | 32.79 |
| | | | 3 | 0.73 | 1.42 | 1.0366 | 2.2 | 35.39 |
| | | | 4 | 0.71 | 1.4 | 0.994 | 2.61 | 44.11 |
| 25 | SE | Y | 1 | 0.73 | 1.28 | 0.9344 | 1.72 | 39.18 |
| | | | 2 | 0.69 | 1.5 | 1.035 | 2.44 | 34.69 |
| | | | 3 | 0.72 | 1.41 | 1.0152 | 2.3 | 32.19 |
| | | | 4 | 0.7 | 1.42 | 0.994 | 2.51 | 42.21 |
| 25 | SE | Y | 1 | 0.8 | 1.27 | 1.016 | - | 15.73 |
| | | | 2 | 0.77 | 1.24 | 0.9548 | 2.39 | 26.06 |

| Tooth No | Adhesive | Anesthesia | Specimen No | Thickness | Width | Area | RDT | MTBS |
|---|---|---|---|---|---|---|---|---|
| | | | 3 | 0.74 | 1.25 | 0.925 | 1.5 | 27.81 |
| | | | 4 | 0.74 | 1.3 | 0.962 | 2.38 | 15.53 |
| 24 | SE | Y | 1 | 0.71 | 1.37 | 0.9727 | - | 35.16 |
| | | | 2 | 0.71 | 1.42 | 1.0082 | 1.46 | 32.93 |
| | | | 3 | 0.74 | 1.43 | 1.0582 | 2.01 | 28.45 |
| | | | 4 | 0.66 | 1.46 | 0.9636 | 2.44 | 28.89 |
| 24 | SE | Y | 1 | 0.72 | 1.29 | 0.9288 | 1.69 | 37.27 |
| | | | 2 | 0.67 | 1.5 | 1.005 | 2.43 | 32.87 |
| | | | 3 | 0.73 | 1.42 | 1.0366 | 2.33 | 33.79 |
| | | | 4 | 0.71 | 1.4 | 0.994 | 3.16 | 44.19 |
| 25 | SE | Y | 1 | 0.7 | 1.4 | 0.98 | - | 28.86 |
| | | | 2 | 0.7 | 1.41 | 0.987 | 2.4 | 19.71 |
| | | | 3 | 0.72 | 1.32 | 0.9504 | 3.2 | 16.82 |
| | | | 4 | 0.73 | 1.42 | 1.0366 | 2.71 | 27.16 |
| 24 | SE | Y | 1 | 0.81 | 1.26 | 1.0206 | - | 16.67 |
| | | | 2 | 0.76 | 1.24 | 0.9424 | 2.41 | 26.49 |
| | | | 3 | 0.75 | 1.24 | 0.93 | 1.42 | 28.92 |
| | | | 4 | 0.73 | 1.33 | 0.9709 | 1.38 | 17.53 |
| | | | 5 | 0.73 | 1.36 | 0.9928 | 2.59 | 16.41 |
| 24 | SE | Y | 1 | 0.71 | 1.4 | 0.994 | - | 34.41 |
| | | | 2 | 0.71 | 1.4 | 0.994 | 1.48 | 32.9 |
| | | | 3 | 0.73 | 1.43 | 1.0439 | 2.02 | 29.53 |
| | | | 4 | 0.69 | 1.46 | 1.0074 | 1.89 | 29.87 |

# Table of statistical analysis

**Table 1: Case Processing Summary[a]**

| | Cases | | | | | |
|---|---|---|---|---|---|---|
| | Included | | Excluded | | Total | |
| | N | Percent | N | Percent | N | Percent |
| MTBS * BONDING * LA | 183 | 100.0% | 0 | .0% | 183 | 100.0% |

a Limited to first 500 cases.

**Table 2: Case Summaries**

| | | | | MTBS |
|---|---|---|---|---|
| BONDING Single Bond | LA | control | 1 | 29.060 |
| | | | 2 | 31.040 |
| | | | 3 | 18.690 |
| | | | 4 | 29.230 |
| | | | 5 | 32.840 |
| | | | 6 | 29.570 |
| | | | 7 | 26.480 |
| | | | 8 | 32.420 |
| | | | 9 | 34.470 |
| | | | 10 | 18.950 |
| | | | 11 | 15.330 |

| | | |
|---|---|---|
| | 12 | 24.870 |
| | 13 | 33.790 |
| | 14 | 19.250 |
| | 15 | 14.410 |
| | 16 | 24.870 |
| | 17 | 24.180 |
| | 18 | 24.950 |
| | 19 | 21.530 |
| | 20 | 29.240 |
| | 21 | 35.470 |
| | 22 | 19.890 |
| | 23 | 13.140 |
| | 24 | 22.780 |
| | 25 | 29.760 |
| | 26 | 30.060 |
| | 27 | 17.590 |
| | 28 | 30.240 |
| | 29 | 18.510 |
| | 30 | 8.680 |
| | 31 | 11.450 |
| | 32 | 14.770 |
| | 33 | 38.880 |
| | 34 | 37.700 |

| | | | |
|---|---|---|---|
| | | 35 | 26.000 |
| | | 36 | 27.620 |
| | | 37 | 33.930 |
| | | 38 | 30.560 |
| | | 39 | 27.480 |
| | | 40 | 31.240 |
| | | 41 | 21.080 |
| | | 42 | 21.850 |
| | | 43 | 26.420 |
| | | 44 | 19.680 |
| | | 45 | 35.470 |
| | | 46 | 19.890 |
| | | 47 | 13.130 |
| | | 48 | 22.780 |
| | | Total | N 48 |
| | | | Mean 25.02542 |
| | | | Std. Deviation 7.418525 |
| | | | Minimum 8.680 |
| | | | Maximum 38.880 |
| | LA | 1 | 30.320 |
| | | 2 | 15.590 |
| | | 3 | 21.130 |
| | | 4 | 16.440 |

| | | |
|---|---|---|
| | 5 | 29.800 |
| | 6 | 30.060 |
| | 7 | 17.580 |
| | 8 | 30.250 |
| | 9 | 30.320 |
| | 10 | 17.590 |
| | 11 | 21.130 |
| | 12 | 16.440 |
| | 13 | 19.410 |
| | 14 | 26.990 |
| | 15 | 21.970 |
| | 16 | 21.040 |
| | 17 | 22.500 |
| | 18 | 24.180 |
| | 19 | 21.850 |
| | 20 | 24.246 |
| | 21 | 25.970 |
| | 22 | 15.590 |
| | 23 | 33.560 |
| | 24 | 30.010 |
| | 25 | 31.160 |
| | 26 | 18.450 |
| | 27 | 22.430 |

| | | | |
|---|---|---|---|
| | 28 | | 27.540 |
| | 29 | | 27.160 |
| | 30 | | 19.150 |
| | 31 | | 23.650 |
| | 32 | | 29.660 |
| | 33 | | 33.480 |
| | 34 | | 24.260 |
| | 35 | | 21.510 |
| | 36 | | 30.210 |
| | 37 | | 27.550 |
| | 38 | | 16.410 |
| | 39 | | 14.520 |
| | 40 | | 24.320 |
| | 41 | | 35.140 |
| | 42 | | 29.670 |
| | 43 | | 22.660 |
| | 44 | | 24.790 |
| | 45 | | 30.040 |
| | Total | N | 45 |
| | | Mean | 24.39391 |
| | | Std. Deviation | 5.581803 |
| | | Minimum | 14.520 |
| | | Maximum | 35.140 |

| | | | | |
|---|---|---|---|---|
| | | Total | N | 93 |
| | | | Mean | 24.71985 |
| | | | Std. Deviation | 6.566360 |
| | | | Minimum | 8.680 |
| | | | Maximum | 38.880 |
| SE Bond | LA | control | 1 | 34.420 |
| | | | 2 | 29.050 |
| | | | 3 | 33.760 |
| | | | 4 | 29.420 |
| | | | 5 | 27.600 |
| | | | 6 | 29.050 |
| | | | 7 | 19.280 |
| | | | 8 | 21.140 |
| | | | 9 | 28.510 |
| | | | 10 | 24.510 |
| | | | 11 | 46.110 |
| | | | 12 | 35.120 |
| | | | 13 | 27.040 |
| | | | 14 | 32.460 |
| | | | 15 | 32.170 |
| | | | 16 | 29.260 |
| | | | 17 | 31.220 |
| | | | 18 | 41.360 |

| | | |
|---|---|---|
| | 19 | 20.160 |
| | 20 | 21.140 |
| | 21 | 28.460 |
| | 22 | 26.310 |
| | 23 | 28.400 |
| | 24 | 16.480 |
| | 25 | 30.150 |
| | 26 | 21.380 |
| | 27 | 22.160 |
| | 28 | 29.150 |
| | 29 | 26.460 |
| | 30 | 32.460 |
| | 31 | 30.360 |
| | 32 | 32.140 |
| | 33 | 43.630 |
| | 34 | 32.230 |
| | 35 | 30.060 |
| | 36 | 35.140 |
| | 37 | 30.280 |
| | 38 | 28.600 |
| | 39 | 27.050 |
| | 40 | 33.710 |
| | 41 | 28.620 |

| | | | |
|---|---|---|---|
| | 42 | | 32.210 |
| | 43 | | 38.660 |
| | Total | N | 43 |
| | | Mean | 29.69488 |
| | | Std. Deviation | 6.093786 |
| | | Minimum | 16.480 |
| | | Maximum | 46.110 |
| LA | 1 | | 29.960 |
| | 2 | | 17.910 |
| | 3 | | 28.620 |
| | 4 | | 34.110 |
| | 5 | | 32.920 |
| | 6 | | 29.540 |
| | 7 | | 29.890 |
| | 8 | | 25.790 |
| | 9 | | 29.840 |
| | 10 | | 15.530 |
| | 11 | | 28.160 |
| | 12 | | 16.920 |
| | 13 | | 15.820 |
| | 14 | | 28.140 |
| | 15 | | 38.720 |
| | 16 | | 32.790 |

| | | |
|---|---|---|
| | 17 | 35.390 |
| | 18 | 44.110 |
| | 19 | 39.180 |
| | 20 | 34.690 |
| | 21 | 32.190 |
| | 22 | 42.210 |
| | 23 | 15.730 |
| | 24 | 26.060 |
| | 25 | 27.810 |
| | 26 | 15.530 |
| | 27 | 35.160 |
| | 28 | 32.930 |
| | 29 | 28.450 |
| | 30 | 28.890 |
| | 31 | 37.270 |
| | 32 | 32.870 |
| | 33 | 33.790 |
| | 34 | 44.190 |
| | 35 | 28.860 |
| | 36 | 19.710 |
| | 37 | 16.820 |
| | 38 | 27.160 |
| | 39 | 16.670 |

| | | | | |
|---|---|---|---|---|
| | | 40 | | 26.490 |
| | | 41 | | 28.920 |
| | | 42 | | 17.530 |
| | | 43 | | 16.410 |
| | | 44 | | 34.410 |
| | | 45 | | 32.900 |
| | | 46 | | 29.530 |
| | | 47 | | 29.870 |
| | | Total | N | 47 |
| | | | Mean | 28.64660 |
| | | | Std. Deviation | 7.889573 |
| | | | Minimum | 15.530 |
| | | | Maximum | 44.190 |
| | Total | N | | 90 |
| | | Mean | | 29.14744 |
| | | Std. Deviation | | 7.069161 |
| | | Minimum | | 15.530 |
| | | Maximum | | 46.110 |
| Total | N | | | 183 |
| | Mean | | | 26.89736 |
| | Std. Deviation | | | 7.152574 |
| | Minimum | | | 8.680 |
| | Maximum | | | 46.110 |

**Univariate analysis of variance**

**Table 3: Between-Subjects Factors**

| | | Value Label | N |
|---|---|---|---|
| BONDING | 1 | Single Bond | 93 |
| | 2 | SE Bond | 90 |
| LA | 1 | Control | 91 |
| | 2 | LA | 92 |

**Table 4: Levene's Test of Equality of Error Variances[a]**
Dependent Variable: MTBS

| F | df1 | df2 | Sig. |
|---|---|---|---|
| 2.284 | 3 | 179 | .081 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+BONDING+LA+BONDING * LA

**Table 5: Tests of Between-Subjects Effects**
Dependent Variable: MTBS

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Noncent. Parameter | Observed Power |
|---|---|---|---|---|---|---|---|
| Corrected Model | 930.563 | 3 | 310.188 | 6.625 | .000 | 19.876 | .971 |
| Intercept | 132579.762 | 1 | 132579.762 | 2831.808 | .000 | 2831.808 | 1.000 |
| BONDING | 908.855 | 1 | 908.855 | 19.412 | .000 | 19.412 | .992 |
| LA | 32.216 | 1 | 32.216 | .688 | .408 | .688 | .131 |
| BONDING * LA | 1.983 | 1 | 1.983 | .042 | .837 | .042 | .055 |
| Error | 8380.433 | 179 | 46.818 | | | | |
| Total | 141705.588 | 183 | | | | | |
| Corrected Total | 9310.996 | 182 | | | | | |

a Computed using alpha = .05
b R Squared = .100 (Adjusted R Squared = .085)

**T-test for equality of mean MTBS between the control group, Single Bond or Clearfil SE Bond without a local anesthetic containing vasoconstrictor**

**Table 6: Group Statistics**

| | BONDING | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| MTBS | Single Bond | 48 | 25.02542 | 7.418525 | 1.070772 |
| | SE Bond | 43 | 29.69488 | 6.093786 | .929294 |

**Table 7: Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| MTBS | Equal variances assumed | 4.480 | .037 | -3.258 | 89 | .002 | -4.66947 | 1.433175 | -7.517154 | -1.821780 |
| | Equal variances not assumed | | | -3.293 | 88.366 | .001 | -4.66947 | 1.417794 | -7.486872 | -1.852063 |

**T-test for equality of mean MTBS between the control group, Single Bond or Clearfil SE Bond with a local anesthetic containing vasoconstrictor**

**Table 8: Group Statistics**

| | BONDING | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| MTBS | Single Bond | 45 | 24.39391 | 5.581803 | .832086 |
| | SE Bond | 47 | 28.64660 | 7.889573 | 1.150813 |

**Table 9: Independent Samples Test**

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| MTBS | Equal variances assumed | 2.377 | .127 | -2.973 | 90 | .004 | -4.25268 | 1.430546 | -7.094714 | -1.410655 |
| | Equal variances not assumed | | | -2.995 | 82.964 | .004 | -4.25268 | 1.420119 | -7.077262 | -1.428108 |

**Statistical analysis using the one-way ANOVA to determine the RDT in all groups.**

**Table 10: Case Processing Summary**

| | Cases | | | | | |
|---|---|---|---|---|---|---|
| | Included | | Excluded | | Total | |
| | N | Percent | N | Percent | N | Percent |
| RDT * BONDING * LA | 152 | 83.1% | 31 | 16.9% | 183 | 100.0% |

**Table 11: Levene's Test of Equality of Error Variances**
Dependent Variable: RDT

| F | df1 | df2 | Sig. |
|---|---|---|---|
| 1.948 | 3 | 148 | .124 |

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+BONDING+LA+BONDING * LA

**Table 12: Tests of Between-Subjects Effects**
Dependent Variable: RDT

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. | Noncent. Parameter | Observed Power |
|---|---|---|---|---|---|---|---|
| Corrected Model | .344 | 3 | .115 | .354 | .786 | 1.063 | .118 |
| Intercept | 750.675 | 1 | 750.675 | 2319.393 | .000 | 2319.393 | 1.000 |
| BONDING | .259 | 1 | .259 | .799 | .373 | .799 | .144 |
| LA | 2.603E-02 | 1 | 2.603E-02 | .080 | .777 | .080 | .059 |
| BONDING * LA | 3.834E-02 | 1 | 3.834E-02 | .118 | .731 | .118 | .063 |
| Error | 47.900 | 148 | .324 | | | | |
| Total | 813.969 | 152 | | | | | |
| Corrected Total | 48.244 | 151 | | | | | |

a Computed using alpha = .05
b R Squared = .007 (Adjusted R Squared = -.013)

# BIOGRAPHY

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