ผลของความลึกในการกรอเนื้อฟันต่อความแข็งแรงของสารยึดติดในฟันตัดน้ำนมในสภาวะนอกกาย

Effects of Dentin Depth on Adhesive Bond Strength in Primary Incisors *in vitro*

สิริกานต์ จันทร์ประเสริฐ[1], วริศรา ศิริมหาราช[2], สิทธิชัย วนจันทรรักษ์[3]
[1]หน่วยทันตกรรม โรงพยาบาลกำแพงเพชร อ.เมือง จ.กำแพงเพชร
[2]ภาควิชาทันตกรรมจัดฟันและทันตกรรมสำหรับเด็ก คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
[3]ภาควิชาชีววิทยาช่องปากและวิทยาการวินิจฉัยโรคช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
Sirikan Chanprasert[1], Varisara Sirimaharaj[2], Sitthichai Wanachantararak[3]
[1]Dental Unit, Kamphaeng Phet Hospital, Amphur Muang, Kamphaeng Phet
[2]Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University
[3]Department of Oral Biology and Oral Diagnostic Sciences, Faculty of Dentistry, Chaing Mai University

ชม. ทันตสาร 2557; 35(2) : 83-90
CM Dent J 2014; 35(2) : 83-90

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของความลึกในการกรอเนื้อฟันต่อความแข็งแรงของสารยึดติดในฟันตัดน้ำนมภายใต้การจำลองแรงดันภายในโพรงในตัวฟันในสภาวะนอกกาย นำฟันน้ำนมหน้าล่างจำนวน 36 ซี่ มาทำการทดลองภายหลังการถอนฟันภายใน 24 ชั่วโมง แบ่งฟันโดยวิธีสุ่มออกเป็น 3 กลุ่ม ตามความลึกของการกรอฟันต่ำกว่ารอยต่อเนื้อฟัน–เคลือบฟัน 1.0, 1.5 และ 2.0 มิลลิเมตร กรอตัดฟันจากปลายฟันลงมาตามความลึกของแต่ละกลุ่ม ยึดแท่งเรซินคอมโพสิตปลายเรียบขนาดเส้นผ่านศูนย์กลาง 1 มิลลิเมตร กับผิวฟันที่ถูกตัดด้วยโฟลเอเบิล คอมโพสิตภายใต้การจำลองแรงดันภายในตัวฟัน 15 เซนติเมตรน้ำ เก็บตัวอย่างในน้ำกลั่นที่อุณหภูมิ 37 องศาเซลเซียส 24 ชั่วโมง แล้วนำมาทดสอบความทนแรงดึงเล็กด้วยเครื่องยูนิเวอร์ซัลเทสติ้งแมชชีน หากค่า

Abstract

The objective of this study was to investigate the effects of different dentin depths on the microtensile bond strength of a dentin adhesive in primary incisors under simulated intrapulpal pressure *in vitro*. Thirty-six mandibular primary incisors were collected immediately after extraction and studied within 24 hours. The samples were randomly divided into three groups. The incisal edges were cut to depths of 1.0, 1.5 and 2.0 mm below the DEJ for Groups 1, 2 and 3, respectively. A composite rod with a 1 mm-diameter flat tip was fixed to the cut surface of the tooth, using flowable composite resin under simulated pulpal pressure of 15 cmH_2O. The samples were

Corresponding Author:

สิทธิชัย วนจันทรรักษ์
รองศาสตราจารย์ ดร. ภาควิชาชีววิทยาช่องปากและวิทยาการวินิจฉัยโรคช่องปาก คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ 50200

Sitthichai Wanachantararak
Associate Professor; Dr. Department of Oral Biology and Oral Diagnostic Sciences, Faculty of Dentistry, Chiang Mai Univeristy, Chiang Mai 50200, Thailand.
E-mail: ***sitthichai.w@cmu.ac.th***

p value<0.5 แสดงว่ามีความแตกต่างอย่างมีนัยสำคัญ ความทนแรงดึงเล็ก±ค่าเบี่ยงเบนมาตรฐานของกลุ่ม 1.0, 1.5 และ 2.0 มิลลิเมตรจากรอยต่อเนื้อฟัน–เคลือบฟัน มีค่าเท่ากับ 10.78±1.20, 6.97±0.78 และ 3.57±1.05 เมกะปาสคาล ตามลำดับ ผลการทดลองพบว่าความทนแรงดึงเล็กระหว่างสารยึดติดกับเนื้อฟันลดลงอย่างมีนัยสำคัญในฟันที่มีเนื้อฟันระดับลึกกว่า ที่ระดับ $p<0.001$ จากข้อมูลที่ได้ แนะนำว่าเมื่อมีการจำลองแรงดันภายในโพรงในตัวฟัน ความทนแรงดึงเล็กระหว่างสารยึดติดกับเนื้อฟันระดับตื้นมีค่าสูงกว่าความทนแรงดึงเล็กระหว่างสารยึดติดกับเนื้อฟันระดับลึกกว่า

คำสำคัญ: ความลึกของการกรอตัดเนื้อฟัน ความทนแรงดึงเล็ก แรงดันภายในโพรงในตัวฟัน ฟันน้ำนม

stored in distilled water at 37°C for 24 hours. The microtensile bond strength was tested in a UTM. The *p* value less than 0.05 was considered significant. The mean ± SD microtensile bond strength values of the three groups tested at 1.0 mm, 1.5 mm, and 2.0 mm of dentin depth were 10.78±1.20, 6.97±0.78, and 3.57±1.05 MPa, respectively. The data revealed that the microtensile bond strength between dentin and adhesive decreased significantly in deeper dentin ($p<0.001$). This study found that bonding to superficial dentin obtained higher microtensile bond strength than did bonding to deeper dentin under simulation of intrapulpal pressure.

Keywords: dentin depth, microtensile bond strength, intrapulpal pressure, primary teeth

Introduction

Dentin adhesive is widely used in clinical practice for composite restorations by forming a hybrid layer and resin tags with dentin. The hybrid layer is formed by the infiltration of resin into the demineralized organic matrix of the intertubular and peritubular dentin, which are left over after acid etching, and resin tags are formed by resin flow into the dentinal tubules(1).

It is well documented that the amount of intertubular and peritubular dentin, and the number and diameters of dentinal tubules vary with the depth of the dentin(2). Superficial dentin has lower density with smaller diameters of dentinal tubules and prominent intertubular dentin. In contrast, deep dentin has higher density and has larger diameters of dentinal tubules and less intertubular dentin(2). There is contradictory evidences about the tubular density and tubular diameter in both primary teeth and permanent teeth(3,4). This contradiction raises the possibility that the results of bond strength tested in permanent teeth might not be applicable to primary teeth.

A spontaneous outward flow of fluid in dentinal tubules has been reported after dentin is exposed(5). It increases as dentin thickness decreases because deep dentinal tubules have larger diameters and higher density, corresponding to the findings of Koutsi and coworkers(3), who suggested that the dentin permeability increases in deeper dentin. In addition, pulpal blood flow has a direct effect on the pulpal interstitial fluid pressure, which is related to the fluid flow rate(6). The increased size of fluid droplets emerging from the dentin surface is the result of increased pulpal pressure *in vitro*(7). This moisture can reduce bond strength(8-11).

However, most *in vitro* studies have been conducted to test dentin adhesives in permanent teeth without simulation of pulpal hydrostatic pressure conditions(12-15). These conditions might not represent the physiological condition of the vital tooth or the properties of dentin adhesives in primary teeth. Therefore, the aim of this study was to investigate the effects of dentin depth on the bond strength of an adhesive in primary incisors under simulated pulpal pressure *in vitro*.

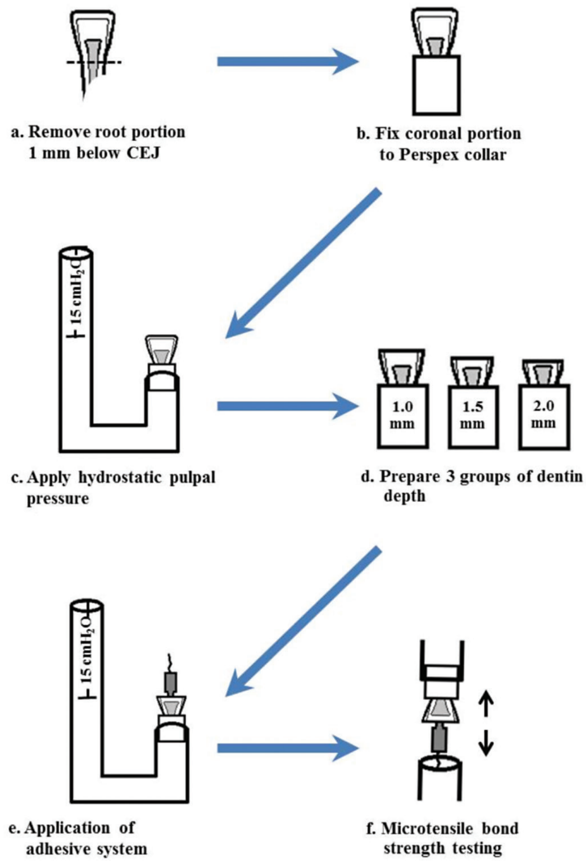
Materials and methods

This study was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand (Ethics reference number 1/2556). Thirty-six non-carious, freshly extracted, primary mandibular incisors (twenty six central incisors and ten lateral incisors) from 5-8-year-old healthy children, which were stored in a solution of 0.01% Thymol at 4°C, were studied within 24 hours of extraction.

Specimen preparation

The tips of the roots were removed at approximately 1 mm apically to the cementoenamel junction (CEJ), using a high-speed cylindrical diamond bur (Intensiv®, Swiss Dental Products, Montagnol, Switzerland) under copious water irrigation. The remaining pulp tissue in the coronal portion was removed with a barbed broach under water to avoid tapping air bubbles. The crown portion was attached to a Perspex collar with self-cure acrylic resin and connected to a water manometer. A hydrostatic pressure of +15 cm H_2O was applied to the pulp cavity during the experiment (Figure 1).

The samples were randomly divided into three groups. The incisal edges were cut to a flat surface at depths of 1.0, 1.5 and 2.0 mm below the dentinoenamel junction (DEJ) for Groups 1, 2 and 3, respectively, with a low-speed diamond disc (Intensiv®, Swiss Dental Products) under plentiful water irrigation.



รูปที่ 1 ขั้นตอนการเตรียมตัวอย่างภายใต้การจำลองแรงดันภายในตัวฟัน และทดสอบความทนแรงดึงเล็กด้วยเครื่องยูนิเวอร์ซัล เทสติ้ง แมชชีน

Figure 1 *Steps in specimen preparation with simulated hydrostatic pulpal pressure and testing microtensile bond strength using a Universal Testing Machine.*

Application of adhesive system

The exposed dentin surface was etched with 35% phosphoric acid gel (ScotchbondTM Etchant #7423, 3M ESPE, Minneapolis, Minnesota, U. S. A) for 15 seconds, then rinsed with distilled water for 10 seconds and excess water was removed by blotting dry with cotton pellets. The dentin adhesive (Adper™ Single Bond 2-step total-etch adhesive, 3M ESPE) was applied, then gently air-blown for five seconds to evaporate the solvents, and light-cured for 10 seconds. A second layer was applied with the same procedure.

A composite rod with a 1 mm-diameter flat tip (a circle area = 0.785 mm^2) was fixed to the cut surface of the tooth, using flowable composite resin (Filtek™ Z350, 3M ESPE) and light-cured for 40 seconds. The tooth surface was etched with 35% phosphoric acid gel (Scotchbond™ Etchant #7423, 3M ESPE) for 15 seconds, then blown dry. The dentin adhesive (Adper™ Single Bond 2-step total-etch adhesive, 3M ESPE) was applied, then gently air-blown for five seconds to evaporate the solvents, and light-cured for 10 seconds. A second layer was applied with the same procedure. The sample was stored in distilled water at 37°C for 24 hours before microtensile bond strength testing.

Microtensile bond strength (µTBS) testing

Each sample was attached to the microtensile bond testing apparatus of a Universal Testing Machine (UTM) (Instron®, Instron (Thailand) Limited, Bangkok, Thailand) and pulled at a cross-head speed of 0.5 mm/min until fracture (Figure 1f).

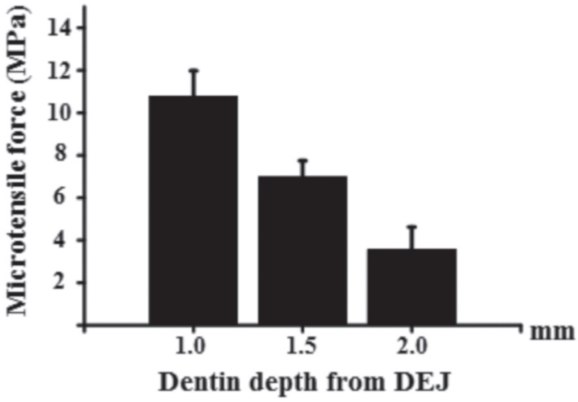
All specimens were sectioned longitudinally (bucco-lingual) using a chisel and hammer. The fractured surface was observed under a Scanning Electron Microscope (SEM) (JEOL® JSM-6610LV; JEOL Ltd., Tokyo, Japan) at x1000 magnification and was classified according to one of four types: type 1, adhesive failure between bonding resin and dentin; type 2, partial adhesive failure between the bonding resin and dentin, and partial cohesive failure in the bonding resin; type 3, partial cohesive failure in dentin; and type 4, cohesive failure in the bonding resin[16].

Statistical analysis

The data were analyzed by one-way ANOVA and Tukey tests. The *p* value less than 0.05 was considered significant.

Results

The mean ± SD microtensile bond strength values of the three groups tested at 1.0 mm, 1.5 mm, and 2.0 mm of dentin depth were 10.78±1.20, 6.97±0.78, and 3.57±1.05 MPa, respectively (Figure 2). The data passed the test of normality and had homogenous variances. One-way ANOVA statistical analysis showed that there were statistically significant differences among those groups (P<0.001). In addition, significant differences between each group were detected with the Tukey Multiple Comparison test (P<0.001). These results suggested that the microtensile bond strength significantly decreases in deeper dentin.



รูปที่ 2 *แสดงค่าความทนแรงดึงระดับไมโคร±ค่าเบี่ยงเบนมาตรฐาน ที่ระดับความลึกของเนื้อฟันที่แตกต่างกัน*

Figure 2 *Mean microtensile bond strength ± SD values at different depths of dentin*

Distribution of failure modes is described in Table 1. Most samples exhibited adhesive failure between the bonding resin and dentin (Figure 3). Few samples showed cohesive failure in the bonding resin. No samples exhibited partial adhesive failure between the bonding resin and dentin, and partial cohesive failure in the bonding resin, or partial cohesive failure in dentin. Nevertheless, there were no statistically significant differences among the groups of dentin depth.

ตารางที่ 1 แสดงการกระจายของลักษณะของการแตกหักที่ระดับความลึกของเนื้อฟันที่แตกต่างกัน

Table 1 Distribution of failure modes at different depths of dentin

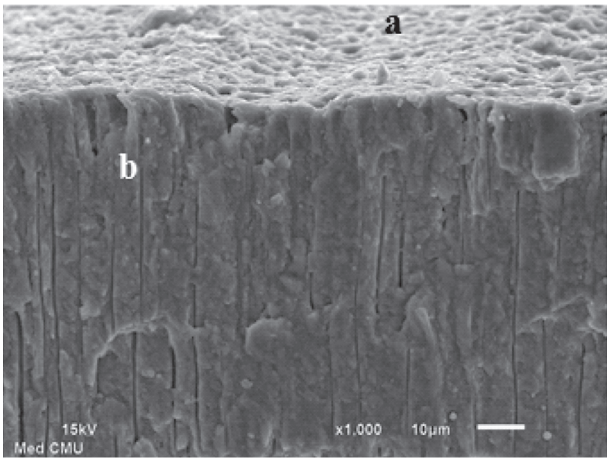
Modes of failure (Type)	Depths from the DEJ (mm)		
	1.0 (N)	1.5 (N)	2.0 (N)
1	9	9	10
2	0	0	0
3	0	0	0
4	3	3	2

Type 1: adhesive failure between the bonding resin and the dentin

Type 2: partial adhesive failure between the bonding resin and the dentin, and partial cohesive failure in the bonding resin

Type 3: partial cohesive failure in the dentin

Type 4: cohesive failure in the bonding resin



รูปที่ 3 *ภาพจากกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดแสดงลักษณะการแตกหักระหว่างบอนด์ดิ้ง เรซินกับเนื้อฟันในกลุ่มทดสอบที่ความลึก 1 มิลลิเมตร ที่กำลังขยาย 1000 เท่า a) พบสารเมทริกซ์อินทรีย์และรูเปิดของท่อเนื้อฟันซึ่งแสดงการแตกหักที่บริเวณระหว่างบอนด์ดิ้ง เรซินกับเนื้อฟัน b) ไม่พบการสร้างเรซิน แทก หรือชั้นไฮบริด*

Figure 3 *SEM image of a sample in the 1 mm dentin depth group after labio-lingual fracture (magnification x1000).*
a) the sponge of organic matrix and the opening of dentinal tubules indicated adhesive failure between the bonding resin and dentin
b) no resin tag or hybrid layer formation was observed.

The mean ± SD values of remaining dentin thickness (RDTs) from the dental pulp were approximately 1.47±0.19 mm, 0.93±0.16 mm, and 0.46±0.10 mm in the groups with 1 mm, 1.5 mm, and 2.0 mm of dentin reduction from DEJ, respectively. From these results, therefore, the dentin thickness of primary mandibular incisors is 2.45±0.16 mm.

Discussion

This study revealed that the microtensile bond strength is significantly different in each group. The bond strength was significantly decreased in deeper dentin, similar to the findings of other studies(12-15). Pegado and coworkers(12) showed similar results to ours when they compared two levels of dentin depth. Pashley and coworkers(14) found that shear bond strength in superficial dentin (0.5-10 mm from the DEJ) was greater than in middle (0.5-1.0 mm deeper than superficial dentin) or deep dentin (0.5 ± 0.4 mm from the pulp chamber). Besides, the bond strength in intermediate dentin was greater than that in deep dentin. Other studies(13,15) also found that shear bond strength in superficial is significant greater than in deeper dentin.

Pashley and colleagues(17) suggested that the higher bond strength in superficial dentin could be due to anatomical variation in dentin; in larger areas of intertubular dentin, more hard tissue is available for hybrid layer formation(17,18). However, this explanation might contradict another(19), which proposed that a greater amount of resin tag formation is provided and the resin is well hybridized to the lateral walls of the demineralized dentinal tubules in deeper dentin. The latter explanation might be true in the absence of intrapulpal hydrostatic pressure.

Our study used simulated pulpal pressure at 15 cmH_2O (equivalent to 11 mmHg) to simulate the physiologic pulpal pressure, as suggested in the experiment in cat canine teeth by Vongsavan and Matthews[20]. This value of pressure is close to normal pulpal pressure of human teeth (14.1 cm H_2O), as studied by Ciucchi and colleagues[21]. After dentin is exposed, a spontaneous outward flow of dentinal fluid can be detected on exposed dentinal tubules in the presence of positive pressure inside the pulp[5]. A number of studies in permanent teeth reported a deterioration of bond strength when intrapulpal pressure was applied[8-10,22]. Moll and Haller[9] found that continuous intrinsic moisture from hydrostatic pulpal pressure adversely affects the efficacy of dentin bonding systems. Özok and colleagues[10] supported the idea that dentin permeability to dentinal fluid can decrease the sealing ability of a total-etch adhesive to dentin. Hydrostatic pressure affects bond strength not only in permanent teeth, but also in primary teeth[11].

The decrease of bond strength in primary incisors as pulpal pressure increases was reported by Saelim and her colleagues[11]. They investigated the effects of various steps of pulpal hydrostatic pressures on bond strength at 1 mm of dentin depth from the DEJ. Dry teeth and various pulpal pressures of -30 cmH_2O, 0 cm H_2O, and +30 cmH_2O yielded bond strength values of 12.09 MPa, 11.29 MPa, 10.14 MPa, and 6.36 MPa, respectively. The mean microtensile bond strength value in the +15 cmH_2O group at the same depth in our study was 10.78±1.20 MPa, which was close to the values identified by Saelim and her colleagues[11]. However, our value is slightly higher than those they obtained at 0 and +30 cmH_2O. Although the same sample groups and bonding system were used in both studies, the inconsistent results may result from the differences in the techniques. In our study, additional equipment was used to stabilize the composite rod and the tooth during the bonding process; therefore, our technique may be more stable and may have produced the higher values.

Itthagarun and Tay[22] suggested that the intrinsic wetness of dentin, which results from the perfusion of dentinal fluid, affects the bonding process in deep dentin. Hebling and coworkers[8] supported the idea that intrapulpal pressure caused a significant reduction of bond strength in deeper layers of dentin, whereas in the absence of intrapulpal pressure, no significant difference could be detected among different depths of dentin. We also found that under simulated pulpal pressure, a significant reduction of bond strength occurs in deeper layers of dentin, where Koutsi and colleagues[3] showed that dentinal tubule diameter and density are larger, leading to increased dentin permeability. The evidence of the fluid droplets was also observed by Rangcharoen and her coworkers[7] on unetched dentin surfaces of primary incisor using the replica technique under simulation of pulpal pressure. Their results suggested that the dentin surface is wet by outward fluid flow from the pulp through dentinal tubules present on the dentin surface. Therefore, when bonding to dentin *in vitro*, simulation of intrapulpal pressure should also be considered.

In all three groups, adhesive failure between the bonding resin and the dentin was mainly observed. This result was correlated to that of the study of Pegado and coworkers[12], who found that most failures in both superficial and deep dentin were adhesive fractures, with few cohesive fractures. Also, Pereira and coworkers[23], who experimented on specimens under positive pulpal pressure, found interface/ adhesive fractures in most of the specimens. Burrow and colleagues[24] reported the majority of failures were adhesive fractures at the interface in both permanent and primary dentin. The possible mechanism of an adhesive failure between the bonding resin and the dentin could be explained by the outward flow of dentinal fluid disturbing the polymerization of the resin to form a hybrid layer to demineralized dentin.

Conclusions

The results in this study suggest that the bond strength of dentin adhesive decreases as dentin thickness decreases in primary incisors under simulation of pulpal pressure.

Acknowledgements

This research was supported by the Faculty of Dentistry and The Graduate School, Chiang Mai University. We are grateful for Dr. Thanapat Sastraruji for his assistance in statistical analysis. We wish to thank Professor Dr. M. Kevin O Carroll, Professor Emeritus of the University of Mississippi School of Dentistry, USA and Faculty Consultant at Faculty of Dentistry, Chiang Mai University, Thailand, for his assistance in the preparation of the manuscript.

References

1. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. *J Biomed Mater Res* 1982 May;16(3):265-73.
2. Pashley DH. Dentin: a dynamic substrate–a review. *Scanning Microsc* 1989 Mar;3(1):161-74; discussion 174-6.
3. Koutsi V, Noonan RG, Horner JA, Simpson MD, Matthews WG, Pashley DH. The effect of dentin depth on the permeability and ultrastructure of primary molars. *Pediatr Dent* 1994 Jan-Feb;16(1):29-35.
4. Sumikawa DA, Marshall GW, Gee L, Marshall SJ. Microstructure of primary tooth dentin. *Pediatr Dent* 1999 Nov-Dec;21(7):439-44.
5. Vongsavan N, Matthews B. The permeability of cat dentine in vivo and in vitro. *Arch Oral Biol* 1991;36(9):641-6.
6. Matthews B, Vongsavan N. Interactions between neural and hydrodynamic mechanisms in dentine and pulp. *Arch Oral Biol* 1994;39 Suppl:87S-95S.
7. Rangcharoen M. The use of replica technique to study dentin permeabilty in primary teeth. M.S. Thesis, Chiang Mai University. 2011.
8. Hebling J, Castro FL, Costa CA. Adhesive performance of dentin bonding agents applied in vivo and in vitro. Effect of intrapulpal pressure and dentin depth. *J Biomed Mater Res B Appl Biomater* 2007 Nov;83(2):295-303.
9. Moll K, Haller B. Effect of intrinsic and extrinsic moisture on bond strength to dentine. *J Oral Rehabil* 2000 Feb;27(2):150-65.
10. Ozok AR, Wu MK, De Gee AJ, Wesselink PR. Effect of dentin perfusion on the sealing ability and microtensile bond strengths of a total-etch versus an all-in-one adhesive. *Dent Mater* 2004 Jun;20(5):479-86.
11. Saelim T. Effects of pulpal pressure on adhesives bond strength in primary incisors. M.S. Thesis, Chiang Mai University. 2012.
12. Pegado RE, do Amaral FL, Florio FM, Basting RT. Effect of different bonding strategies on adhesion to deep and superficial permanent dentin. *Eur J Dent* 2010 Apr;4(2):110-7.
13. Villela-Rosa AC, Goncalves M, Orsi IA, Miani PK. Shear bond strength of self-etch and total-etch bonding systems at different dentin depths. *Braz Oral Res* 2011 Mar-Apr;25(2):109-15.
14. Pashley EL, Tao L, Matthews WG, Pashley DH. Bond strengths to superficial, intermediate and deep dentin in vivo with four dentin bonding systems. *Dent Mater* 1993 Jan;9(1):19-22.

15. Lopes GC, Perdigao J, Lopes Mde F, Vieira LC, Baratieri LN, Monteiro S, Jr. Dentin bond strengths of simplified adhesives: effect of dentin depth. *Compend Contin Educ Dent* 2006 Jun;27(6):340-5.
16. Phrukkanon S, Burrow MF, Tyas MJ. The influence of cross-sectional shape and surface area on the microtensile bond test. *Dent Mater* 1998 Jun;14(3):212-21.
17. Pashley DH, Ciucchi B, Sano H, Carvalho RM, Russell CM. Bond strength versus dentine structure: a modelling approach. *Arch Oral Biol* 1995 Dec;40(12):1109-18.
18. Gwinnett AJ. Quantitative contribution of resin infiltration/hybridization to dentin bonding. *Am J Dent* 1993 Feb;6(1):7-9.
19. Marshall GW, Jr., Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. *J Dent* 1997 Nov;25(6):441-58.
20. Vongsavan N, Matthews B. Fluid flow through cat dentine in vivo. *Arch Oral Biol* 1992 Mar;37(3):175-85.
21. Ciucchi B, Bouillaguet S, Holz J, Pashley D. Dentinal fluid dynamics in human teeth, in vivo. *J Endod*. 1995 Apr;21(4):191-4.
22. Itthagarun A, Tay FR. Self-contamination of deep dentin by dentin fluid. *Am J Dent* 2000 Aug;13(4):195-200.
23. Pereira PN, Okuda M, Sano H, Yoshikawa T, Burrow MF, Tagami J. Effect of intrinsic wetness and regional difference on dentin bond strength. *Dent Mater* 1999 Jan;15(1):46-53.
24. Burrow MF, Nopnakeepong U, Phrukkanon S. A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin. *Dent Mater* 2002 May;18(3):239-45.