Effect of Storage Duration/Solution on Microshear Bond Strength of Composite to Enamel

Gul TOSUN[1], Yagmur SENER[1] and Abdulkadir SENGUN[2]

[1]Department of Pediatric Dentistry, Selcuk University, Faculty of Dentistry Campus, Konya, Turkey
[2]Department of Operative Dentistry, Selcuk University, Faculty of Dentistry Campus, Konya, Turkey
Corresponding author, Gul TOSUN; E-mail: gultosun@hotmail.com

The aim of this study was to determine the effect of three storage solutions and two storage durations on microshear bond strength (μSBS) of a resin composite. Sixty non-carious human permanent molars were stored in three storage solutions (0.1% thymol, 10% formalin, and distilled water). Each tooth was separated mesio-distally into two parts. Specimens of the first part were stored for 24 hours, while specimens of the second part were stored for two months in the solutions. After each storage period, the enamel surface was covered with a composite resin in combination with an etch-rinse adhesive system. Specimens were then serially sectioned into sticks of 1 mm^2 bond area and subjected to μSBS test.

There were no statistically significant differences between the two storage periods for each solution (p>0.05). The thymol solution group showed lower μSBS values than those of distilled water for both storage periods (p<0.05). As for the formalin group, its μSBS values were not statistically different from those of distilled water and thymol groups at each storage period (p>0.05).

In conclusion, the thymol solution caused the μSBS of the resin composite to decrease when compared to both formalin and distilled water after 24 hours and two months. However, the μSBS of the resin composite was not affected by storage duration.

Keywords: Storage solution, Duration, Bond strength

INTRODUCTION

In vitro tests−such as bond strength measurement, microleakage evaluation, and marginal gap measurement−are vital screening tests that serve to predict the clinical behavior of new bonding systems[1]. In a bid to have a more accurate knowledge on the retention capability of bonding sytems in the clinical situation, it usually mandates the evaluation of bond strength to hard dental tissue[2,3]. In these laboratory studies, the most suitable substrate is freshly extracted human teeth. However, freshly extracted teeth are limited in availability. To meet the required number of tooth specimens for an *in vitro* study, freshly extracted teeth are typically stored in different solutions during the collection period to prevent dehydration of collected teeth as well as microorganism growth[4,5].

As mentioned above, a storage solution should ideally prevent dehydration of teeth as well as fungal and bacterial growth[4,5]. To date, several storage solutions have been suggested in published literature[6]. For example, glutaraldehyde, formalin, thymol, and sodium hypochlorite−as disinfectant solutions−are commonly used as storage media[7-10]. However, sodium hypochlorite and glutaraldehyde were found to be less effective than formalin for disinfecting extracted teeth[11,12]. Furthermore, sodium hypochlorite has some serious disadvantages such as discoloration and toxicity[13]. Formalin solution, on the other hand, is not only effective in preventing spore growth, but

that it is also cheap and simple to use−thus making it an apt choice for routine use in preclinical courses, exercises and research purposes[11,12]. Thymol also has antibacterial properties, and it is commonly used as a storage solution in adhesion studies[5,14-16].

Studies on the effects of storage solution and duration on bond strength are generally conducted using dentin as the substrate[3,14,17,18]. As a result, only a few studies on adhesion to enamel are available in published literature. However, enamel adhesion is one of the important factors that determines the success or failure of restorative materials. Apart from the factor of tooth substrate, storage solution and duration may also influence bonding to teeth[3,14].

The objective of this study, therefore, was to determine the effects of these storage solutions (0.1% thymol, 10% formalin, and distilled water) and storage duration (24 hours and two months) on the microshear bond strength (μSBS) of a resin composite to enamel when applied with an etch-and-rinse bonding agent. The null hypothesis tested was that both storage solution and duration would affect the bond strength of the adhesive system.

MATERIALS AND METHODS

Specimen preparation
Sixty non-carious, extracted, fully erupted human third permanent molars were used in this study. Immediately after extraction, the roots of teeth were removed from the crown at approximately 2 mm

below the cementoenamel junction. The teeth were cleaned with wet laboratory pumice and washed with running tap water. Each tooth was separated mesio-distally into two parts by using a low-speed diamond saw under water cooling (Isomet, Buehler Ltd., Lake Bluff, IL, USA). Specimens of the first part were stored for 24 hours, and those of the second part were stored for two months in the storage solutions at room temperature. The samples were stored in one of the storage solutions: 0.1 thymol, 10 formalin, or distilled water.

After each storage period, 36 phosphoric acid was applied for 15 seconds and rinsed with oil-free water for 15 seconds according to manufacturer's instructions. Bonding resin (Prime&BondNT, Dentsply/De Trey, Konstanz, Germany) was applied to the etched surface and light-cured for 20 seconds by a conventional quartz-tungsten-halogen light unit (Hilux 350, Benlioğlu, Ankara). A hybrid composite (Spectrum TPH, Dentsply/De Trey, Konstanz, Germany) was built up to smooth enamel on the middle part of buccal or lingual surface of the teeth using an incremental technique to a height of 2-3 mm. Each increment was polymerized for 40 seconds using the conventional quartz-tungsten-halogen light unit (Hilux 350, Benlioğlu, Ankara). All specimens were immersed in their respective storage solutions for 24 hours.

The specimens were longitudinally sectioned in both 'x' and 'y' directions to obtain sticks with a cross-sectional area of approximately 1 mm^2 by using a low-speed diamond saw under water cooling (Isomet, Buehler Ltd., Lake Bluff, IL, USA). The cross-sectional area was measured using a digital caliper (Mitutoyo, Tokoya, Japan). Sections cut longitudinally with an 'I' shape were obtained, where the upper half consisted of composite and the lower half consisted of enamel from each tooth. Twenty sticks were randomly selected for each group.

Bond strength test

For μ SBS measurement, samples were mechanically fixed to a testing machine (Harvard Apparatus Co. Inc., Dover, Mass., USA) using a special apparatus[19,20] (Fig. 1). Shear force was applied to the composite-enamel attachment line at a crosshead speed of 1 mm/min until failure occurred. Bond strength values were calculated in MPa.

SEM observation

After the μ SBS test, the fractures surfaces were observed using a scanning electron microscope (SEM). The specimens were dried in an air chamber, and then the fracture surfaces gold sputter-coated and observed using an SEM (JSM-5600, JEOL, Tokyo, Japan).

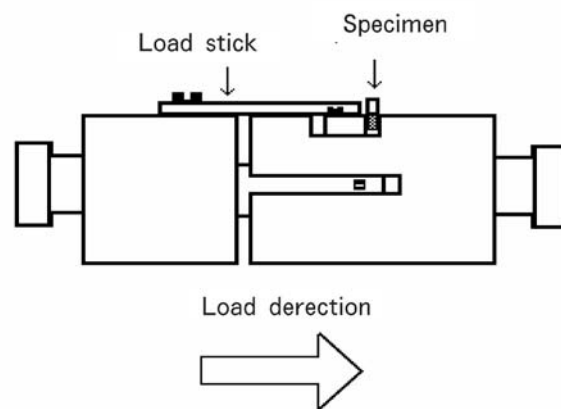Failure modes were classified as adhesive failure

Fig. 1 Microshear bond strength test apparatus.

between enamel and resin, adhesive failure between resin and composite, cohesive enamel failure, or cohesive composite failure[21].

Fracture surface was photographed under × 100 magnification. Specific regions requiring detailed inspection were further examined with × 500 magnification.

Statistical analysis

Data were tested by two-way ANOVA, whereby the parameters were storage solution and time. After obtaining significant differences, the μ SBS values between storage solution and storage time were analyzed by one-way ANOVA and *post hoc* Tukey's HSD test. Failure modes were compared using Kruskall-Wallis and Mann-Whitney U tests. Confidence level of 95 was set for all statistical evaluations.

RESULTS

Table 1 shows the mean μ SBS values (MPa) of 0.1 thymol, 10 formalin, and distilled water groups for two storage periods. There were no statistical differences between the two storage periods for each storage solution (p>0.05).

Specimens stored in thymol solution showed lower μ SBS values than those of distilled water group (p<0.05). The mean μ SBS values of the formalin group for both storage periods were between thymol and distilled water groups with no statistical differences (p>0.05).

Failure modes for each group are shown in Table 2. Statistical analysis indicated that there were significant differences between the failure modes of storage solutions (p<0.05). The majority of failures for formalin and distilled water groups were of adhesive type. In the thymol group, most of the failures appeared to be of cohesive type.

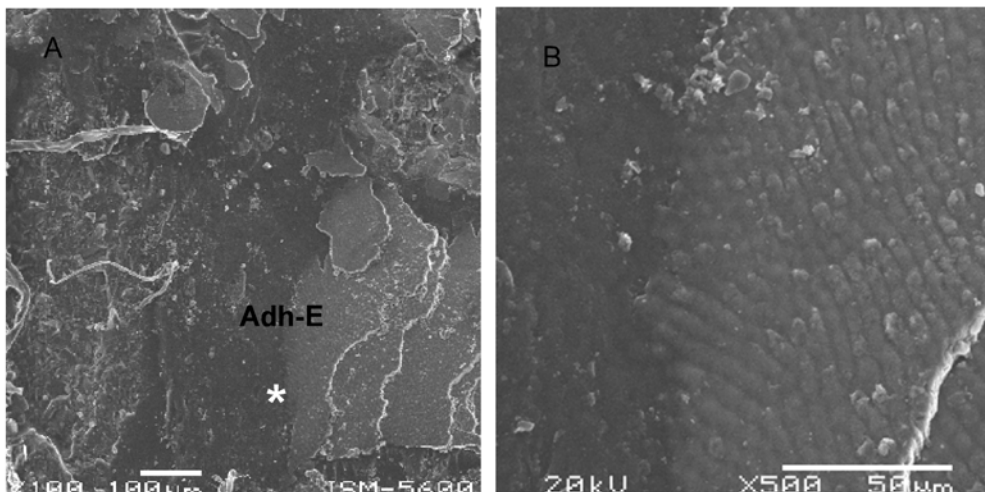Figures 2-4 show the SEM images of the fracture

Fig. 2   A: SEM micrograph of distilled water group with adhesive failure (× 100); B: Fracture surface of enamel and etched enamel pattern after adhesive-enamel failure (× 500); Adh-E: Adhesive-enamel interface.
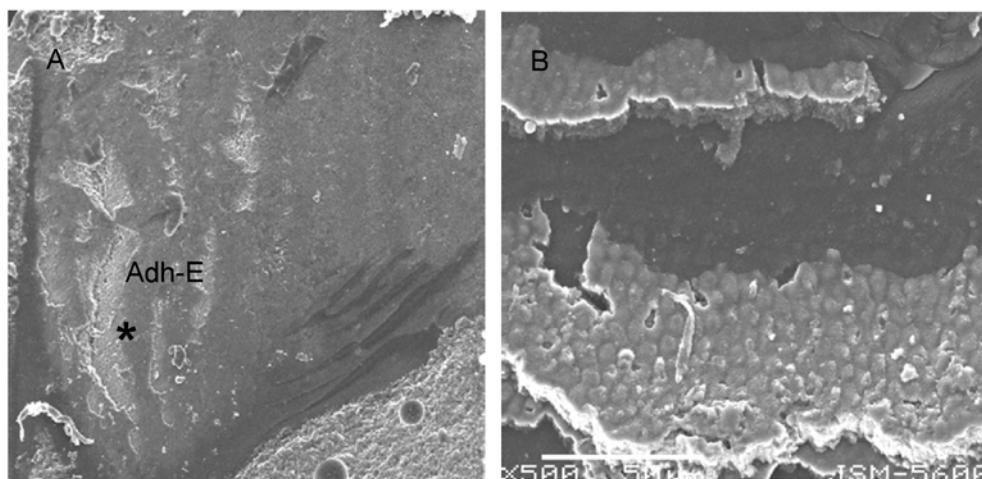

Fig. 3   A: SEM micrograph of formalin group with adhesive failure (× 100); B: Fracture surface of enamel and adhesive-enamel failure (× 500); Adh-E: Adhesive-enamel interface.
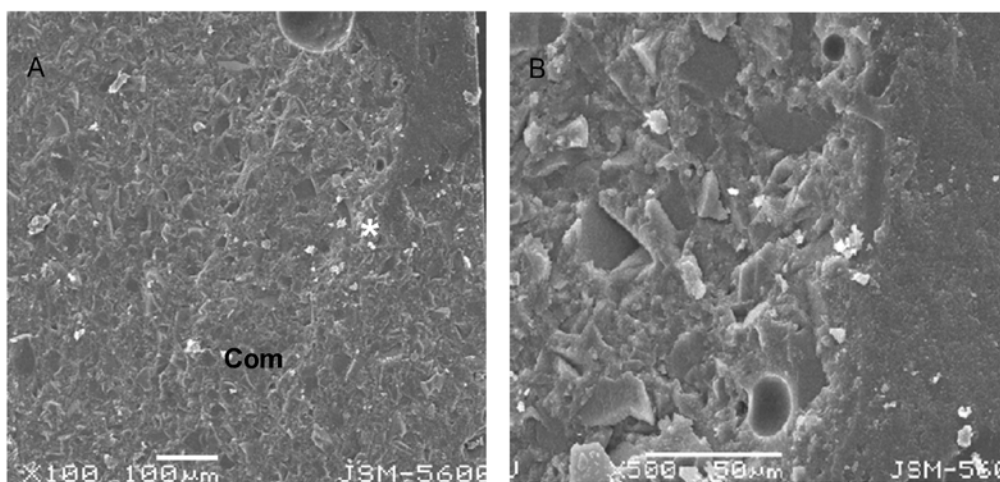

Fig. 4   A: SEM micrograph of thymol group with cohesive composite fracture (× 100); B: Fracture surface of composite material (× 500); Com: composite.

Table 1   μ SBSs (MPa) of composite resin to enamel of teeth stored in the three media for 24 hours and
2 months (n=20). Data are presented in Mean ±　SD.

Solution	Storage Period		*p
	24 hours	2 months	
Thymol	7.35 ± 2.28	8.08 ± 1.47	a
Formalin	8.48 ± 2.38	9.25 ± 1.75	a,b
Distilled water	9.24 ± 2.56	9.70 ± 2.32	b

*p   Means having same letters were not statistically different (p>0.05).

Table 2   Distribution (in percentage) of failure modes of each group.

	24 hours			2 months		
	Adhesive (%)	Cohesive (%)	*p	Adhesive (%)	Cohesive (%)	*p
Thymol	8 (40)	12 (60)	a	7 (35)	13 (65)	a
Formalin	15 (75)	5 (25)	b	14 (70)	6 (30)	b
Distilled water	16 (80)	4 (20)	b	14 (70)	6 (30)	b

*p   Means having same letters were not statistically different (p>0.05).

surface of all groups. Figure 2A shows a specimen of the distilled water group. Figure 2B is a higher-magnification view of the asterisk area in Fig. 2A, and which shows adhesive failure.

Figure 3A is a view of the fracture surface of formalin group. Figure 3B is a higher-magnification view of the asterisk area in Fig. 3A, and which shows adhesive failure.

Figure 4A shows the fracture surface of thymol group after the μ SBS test. Figure 4B is a higher-magnification view of the asterisk area in Fig. 4A, and which shows cohesive failure in the composite.

DISCUSSION

Bond strength measurement in the laboratory is one of the proven, effective methods in characterizing commercial restorative materials[22-25]. However, an essential prerequisite in achieving consistent results for bond strength studies is that the tooth specimens used must be stored after extraction. At this juncture, it should be highlighted that detractors of conventional shear bond strength tests claimed that for contemporary adhesives with improved bonding efficacy, the eccentric stress distribution often resulted in cohesive failure within the tooth substrates[26]. To address this concern, microtensile bond test was designed and developed to permit evaluation of bond strength between an adhesive material and a small region of dental tissue[27]. As specimens used in the microtensile test are prepared with a minimal surface area (ca. 1 mm^2), it will theoretically produce a more uniform distribution of stress to the adhesion substrate   which means that there should be fewer cohesive failures than found with conventional testing[24,27].

Microshear test is a modification of the microtensile test. This testing method also uses small surface areas like in a microtensile test, and which generally shows adhesive failures at the bonded interface[20]. In the present study which used the microshear bond strength test method, cross-sectional surface area of specimens was prepared at about 1 mm^2 and most of the specimens showed adhesive failure, except for the thymol group (Figs. 2-4).

Although cohesive failure occurred predominantly in the thymol group (Table 2), the μ SBS values of thymol group were lower than those of distilled water and formalin groups for both storage periods (Table 1). While some reports suggested that cohesive resin fracture was indicative of higher bond strength, other reports indicated that there was no correlation between cohesive resin fracture and bond strength[28,29]. For example, Almuammar et al.[30] suggested that there was no direct relationship between fracture mode and shear bond strength value. Further, Hosoya et al.[28] found that cohesive resin fractures were easily caused by the lower physical properties of the composite resin such as lower compressive and bending strengths. As for our results, they could be explained by the findings of Fujisawa and Kadomo[31], whereby phenolic compounds such as thymol were found to inhibit the polymerization of methyl methacrylate by reacting with free radicals. Due to the inhibited polymerization of methyl methacrylate, it caused the thymol group to exhibit

lower bond strength as well as for the composite resin to fracture easily (Fig. 4). Indeed, the SEM image confirmed that the failure type of thymol group was cohesive composite failure.

Dentin has a relatively high organic content as compared with enamel. In this connection, questions have been raised concerning how the changes within dentin after extraction may influence adhesion in *in vitro* dentin bonding studies[9]. Titley *et al.*[14] stated that post-mortem changes could occur in dentin, which in turn could affect the outcome of shear bond strength tests. They therefore suggested that fresh teeth are required for achieving the highest possible shear bond strength of resin to dentin. However, Retief *et al.*[3] showed that although there was a tendency for shear bond strength to increase with prolonged storage, the bond strengths obtained with teeth stored in physiological saline, chloramine, formalin, 0.05 thymol, and 70 ethanol for two days and six months were not significantly different.

For enamel bonding, it was reported that no histological changes were observed in ground tooth sections of enamel that had been stored in distilled water for up to six months[32]. In the present study, the tooth specimens were stored for 24 hours and two months after extraction and it was found that storage duration did not affect bond strength to enamel. These results agreed with those of Williams and Svare[33]. Thus, the null hypothesis of this study concerning the effect of storage duration was rejected. Conversely, the effect of storage solution was accepted. The results showed clearly that storage solutions had a significant influence on composite-enamel bond strength.

Thymol has antibacterial properties, and it can also prevent dehydration because of its aqueous structure[4,13]. However, it was found that the μ SBS values of thymol group were lower than distilled water and formalin groups in our study. As mentioned previously, this result could be explained by thymol inhibiting the polymerization of methyl methacrylate.

The distilled water group yielded the highest μ SBS values of resin to enamel, although there were no statistically significant differences between the bond strengths of distilled water and formalin groups in this study. Distilled water does not have antimicrobial properties, and it will not prevent disease transmission to the investigators with the presence of microorganisms in human extracted teeth. On the other hand, formalin has been found to be effective for infection control purpose[11,12]. However, some investigators debate on the use of formalin as a storage medium for dentin adhesion studies, due to differences in dentin bond strength arising from its use. It is known that dentin is composed of 70 inorganic materials, 20 organic materials, and 10

water; on the other hand, enamel is composed of 96 inorganic matter, 3 water, and less than 1 organic matter[34]. Owing to the vast differences in structural composition, the effect of a storage solution on bond strength to dentin would most probably differ from that to enamel. With due understanding of how storage solutions would affect the tooth structure, 10 formalin solution which is routinely used for inorganic substrates[35] may therefore be an appropriate storage solution for adhesion studies to enamel, since it is the most highly mineralized tissue in the human body.

CONCLUSIONS

The present study sought to investigate the effect of storage solution and duration on bond strength of composite to enamel. To meet this objective, a new bond strength test method was used to determine the shear bond strength values.

It is suggested that freshly extracted teeth are the most suitable substrate for *in vitro* evaluation of adhesive systems. However, to acquire the sufficient number of tooth specimens, freshly extracted teeth must be collected over time which means that they must be stored after extraction. Storage solutions are thus used to prevent dehydration of teeth as well as cross-contamination between extracted teeth. Based on the results of this study, we recommended that care should be taken when selecting storage solutions for *in vitro* studies. This is because the chemical nature of the storing agent may affect the tooth structure and material properties at the tested interface.

REFERENCES

1) Van Meerbeek B, Peumans M, Verschueren M, Gladys S, Braem M, Lambrechts P, Vanherle G. Clinical status of ten dentin adhesive systems. J Dent Res 1994; 73: 690-702.

2) Qilo G. Bond strength testing what does it mean?. Int Dent J 1993; 43: 492-498.

3) Retief DH, Wendt SL, Bradley EL, Denys FR. The effect of storage media and duration of storage of extracted teeth on the shear bond strength of Scotchbond 2/Silux to dentin. Am J Dent 1989; 2: 269-273.

4) Dewald JP. The use of extracted teeth for *in vitro* bonding studies: a review of infection control considerations. Dent Mater 1997; 13: 74-81.

5) Ziskind D, Gleitman J, Rotstein I, Friedman M. Evaluation of cetylpyridinium chloride for infection control in storage solution. J Oral Rehabil 2003; 30: 477-481.

6) Rueggeberg FA. Substrate for adhesion testing to tooth structure review of the literature. Dent Mater 1991; 7: 2-10.

7) Aquilino SA, Williams VD, Svare CW. The effect of storage solutions and mounting media on the bond

strengths of a dentinal adhesive to dentin. Dent Mater 1987; 3: 131-135.

8) McGuckin RS, Pashley DH. Effect of disinfection / sterilization treatments on Gluma-mediated dentin shear bond strengths. Am J Dent 1990; 3: 278-282.

9) Mitchem JC, Gronas DG. Effects of time after extraction and depth of dentin on resin dentin adhesives. J Am Dent Assoc 1986; 113: 285-287.

10) Rossomando KJ, Wendt SL Jr. Thermocycling and dwell times in microleakage evaluation for bonded restorations. Dent Mater 1995; 11: 47-51.

11) Dominici JT, Eleazer PD, Clark SJ, Staat RH, Scheetz JP. Disinfection/sterilization of extracted teeth for dental student use. J Dent Educ 2001; 65: 1278-1280.

12) Kumar M, Sequeira PS, Peter S, Bhat GK. Sterilization of extracted human teeth for educational use. Indian J Med Microbiol 2005; 23: 256-258.

13) Recommended infection-control practices for dentistry, 1993. Centers for Disease Control and Prevention. MMWR Recomm Rep 1993; 42(RR 8):1-12.

14) Titley KC, Chernecky R, Rossouw PE, Kulkarni GV. The effect of various storage methods and media on shear-bond strengths of dental composite resin to bovine dentine. Arch Oral Biol 1998; 43: 305-311.

15) Yoo HM, Oh TS, Pereira PN. Effect of saliva contamination on the microshear bond strength of one-step self-etching adhesive systems to dentin. Oper Dent 2006; 31: 127-134.

16) Bishara SE, Ajlouni R, Oonsombat C. Evaluation of a new curing light on the shear bond strength of orthodontic brackets. Angle Orthod 2003; 73: 431-435.

17) Goodis HE, Marshall GW Jr, White JM, Gee L, Hornberger B, Marshall SJ. Storage effects on dentin permeability and shear bond strengths. Dent Mater 1993; 9: 79-84.

18) Gwinnett AJ, Yu S. Effect of long-term water storage on dentin bonding. Am J Dent 1995; 8:109-111.

19) Sengun A, Ozer F, Ceylan N. Bonded surface area and bond strength: A micro-shear test. J Dent Res 2002; 81: 281-290

20) Usumez A, Ozturk AN, Usumez S, Ozturk B. The efficiency of different light sources to polymerize resin cement beneath porcelain laminate veneers. J Oral Rehab 2004; 31: 160-175.

21) Chuang SF, Chang LT, Chang CH, Yaman P, Liu JK. Influence of enamel wetness on composite restorations using various dentine bonding agents. Part II: Effects on shear bond strength. J Dent 2006; 34: 352-361.

22) Erickson RL. Surface interaction of dentin adhesive materials. Oper Dent 1992; 5: 81-94.

23) Perinka L, Sano H, Hosoda H. Dentin thickness, hardness, and Ca-concentration *versus* bond strength of dentin adhesive. Dent Mater 1992; 8:229-233.

24) Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion testing of dentin bonding agents: a review. Dent Mater 1995; 11: 117-125.

25) Phrukkanon S, Burrow MF, Tyas MJ. Effect of cross-sectional surface area on bond strengths between resin and dentin. Dent Mater 1998; 14:120-128.

26) Tantbirojn D, Cheng YS, Versluis A, Hodges JS, Douglas WH. Nominal shear or fracture mechanics in the assessment of composite-dentin adhesion? J Dent Res 2000; 79: 41-48.

27) Sano H, Sonoda H, Shono T, Takatsu T, Ciucchi B, Carvalho R, Pashley DH. Relationship between surface area for adhesion and tensile bond strength: Evaluation of a micro-tensile bond test. Dent Mater 1994; 10: 236-240.

28) Hosoya Y, Nishiguchi M, Kashiwabara Y, Horiuchi A, Goto G. Comparison of two dentin adhesives to primary *vs.* permanent bovine dentin. J Clin Pediatr Dent 1997; 22: 69-76.

29) Malferrari S, Finger WJ, Garcia-Godoy F. Resin bonding efficacy of Gluma 2000 to dentine of primary teeth: an *in vitro* study. Int J Paediatr Dent 1995; 5: 73-79.

30) Almuammar MF, Schulman A, Salama FS. Shear bond strength of six restorative materials. J Clin Pediatr Dent 2001; 25: 221-225.

31) Fujisawa S, Kadoma Y. Effect of phenolic compounds on the polymerization of methyl methacrylate. Dent Mater 1992; 8: 324-326.

32) Silverstone LM. The histopathology of enamel lesions produced *in vitro* and their relation to enamel caries, Vol. 1, Thesis, University of Bristol, England, 1967.

33) Williams VD, Svare CW. The effect of five-year storage prior to bonding on enamel/composite bond strength. J Dent Res 1985; 64: 151-154.

34) Fejerskov O, Ekstrand J, Burt BA. Fluoride in Dentistry, 2nd ed, Munksgaard, Copenhagen, 1996, p.252.

35) Bancroft JD, Stevens A. Theory and practice of histological techniques, 3rd ed, Churchill Livingstone, Edinburgh, 1990, p.312.