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Influence of Three Specimen Fixation Modes on the Micro-tensile Bond Strength of Adhesives to Dentin

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The aim of this study was to investigate in how far the way the specimen is fixed to the testing device influences the micro-tensile bond strength of adhesives to dentin. Compared to a flat jig, a notched jig enables the specimen to be aligned easier and more accurately perpendicular to the interface, thereby concentrating better the tensile stress at the actual interface. A notched jig yielded a significantly higher bond strength and the graphs showed more uniform fracture curves. On the other hand, fixation of the specimen at their top and bottom guarantees a perfect perpendicular fixation to the interface, following the specimen's length-axis. The stress-time graphs revealed a completely different stress-distribution pattern. A failure closer to the dentin-composite interface was more often seen and the coefficient of variance was the lowest. Therefore, this completely newly designed top-bottom set-up produced the most reliable bond strength data.

Keywords: Specimen fixation, Micro-tensile bond strength, Dentin

INTRODUCTION

Since clinical trials are rather time-consuming and costly, relatively fast, simple and reliable *in vitro* techniques are still needed to screen new adhesives on their bonding effectiveness to tooth tissue. The micro-tensile bond strength (μTBS) test has been adopted by many research centers worldwide, mainly for its high versatility, while consuming less teeth, and also because of the more uniform stress distribution imposed at the interface, as compared to a conventional shear bond strength test[1-5].

Nevertheless, also the μTBS-test is sensitive to manipulation errors. Large number of variables such as the form and dimensions of the micro-specimens[6-12], the design of the jig and the way the specimens are fixed to the jig, the crosshead speed[13,14] as well as the operator who does the testing, will all to a certain degree influence the test results. Until now, there is still a lack of adequate knowledge and control of these variables and, therefore, test results cannot directly be compared when they originate from different laboratories.

Fixation of specimens in the testing apparatus requires careful manipulation and special test jigs such as a Bencor Multi-T device or a Ciucchi device[4]. These jigs should ideally assure that pure tensile forces are imposed to the tooth-biomaterial interface in order to obtain a homogeneous stress distribution at the true interface. In the original micro-tensile test set-up, micro-specimens are glued with quick-setting cyanoacrylate that covers the entire surface of both specimen ends. The glue should hold the specimen to the jig with a strength exceeding that of the tooth-biomaterial bond. Such a procedure is hard to standardize. In addition, a crucial factor in determining the validity of a bond strength test is that the micro-tensile load should be applied perpendicular to the interface and in a reproducible way[15-17]. This is not necessarily guaranteed when micro-specimens are just glued onto a flat device. Phrukkanon *et al.*[9,10] and Armstrong *et al.*[18] mounted the specimens passively in a jig by means of specially designed holders, providing support by embracing the specimens without any preloading stress. In the so-called Geraldeli's-jig[19], specimens were fixed in a groove prepared parallel to the tensile force axis.

The purpose of this study was to investigate in how far the way the specimen is fixed to the device influences the micro-tensile bond strength. The micro-tensile bond strength was determined of a three-step (OptiBond FL, Kerr, Orange, CA, USA) and a two-step (Scotchbond 1 XT, 3M ESPE, St. Paul, MN, USA) etch-and-rinse adhesive, as well as of a two-step self-etch adhesive (One Coat SE Bond, Coltène Whaledent, Altstätten, Switzerland) using a notched jig, a flat so-called Ciucchi's jig (control) and a newly developed 'top-bottom' design. A notched jig is a flat Ciucchi's jig, customly adapted by cutting a two-face groove parallel to the applied load. For the top-bottom test set-up, the trimmed specimen was mounted in the testing device at one end using the pin-chuck of the MicroSpecimen Former and at the other end with cyanoacrylate onto a custom-made horizontal table (Fig 1). We hypothesized that there is no difference in μTBS, irrespective of the specimen-device fixation method employed.

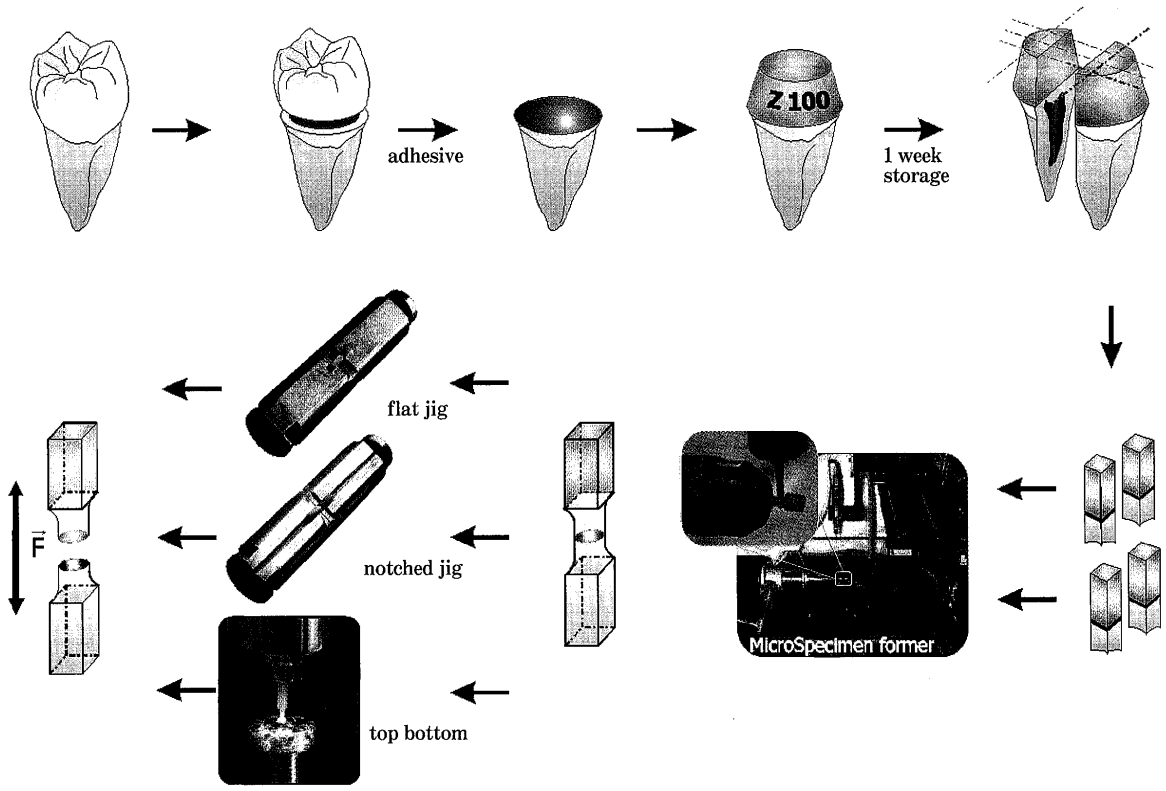

Fig. 1 Schematic study design.

Table 1 Adhesives used in this study

Adhesive	Composition[1] [lot number]	Application
OptiBond FL (Kerr, Orange, CA, USA)	Etching: 37.5% phosphoric acid, silica thickener [3-1084] Primer: HEMA, GPDM, PAMM, ethanol, water, photo initiator [212652] Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler, photo initiator [301335]	Etch for 15 sec; rinse for 15 sec; gently air dry for 5 sec; scrub the surface for 15 sec with primer; apply a thin coat of bonding agent and light cure for 30 sec.
Scotchbond 1 XT (3M ESPE, St. Paul, MN, USA)	Etching: 35% phosphoric acid [4BT] Primer and adhesive: dimethacrylates, HEMA, polyalkenoic acid copolymer, 5 nanometer silane treated colloidal silica, ethanol, water, photo initiator	Etch for 15 sec; rinse for 10 sec; blot excess water; apply 2-3 consecutive coats of adhesive for 15 sec with gentle agitation; gently air thin for 5 sec and light cure for 10 sec.
One Coat SE Bond (Coltène Whaledent, Alstätten, Switzerland)	Primer: water, HEMA, glycerol mono- and dimethacrylate, acrylamidosulfonic acid, polyalkenoate methacrylized [NA599] Bond: HEMA, glycerol mono- and dimethacrylate, UDMA, polyalkenoate methacrylized [NA599]	Apply primer and rub in for 30 sec; air dry lightly; apply bond and rub in for 20 sec; air dry lightly and light cure for 30 sec.

[1]Composition as provided by the respective manufacturer: Bis-GMA = Bisphenol-glycidyl methacrylate; GPDM = Glycerol phosphate dimethacrylate; HEMA = Hydroxyethylmethacrylate; PAMM = Phthalic acid monoethyl methacrylate; TEGDMA = Triethylene glycol dimethacrylate; UDMA = Urethane dimethacrylate.

MATERIALS AND METHODS

Specimen preparation

For this study, non-carious and non-restored third molars (gathered following informed consent approved by the Commission for Medical Ethics of the Catholic University of Leuven) were stored in 0.5% chloramine in water at 4℃ and used within 1 month after extraction. First, all teeth were mounted in gypsum blocks in order to ease manipulation. The occlusal third of the molar crowns was removed to expose mid-coronal dentin by means of a

water-cooled slow-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Dentin surfaces were verified for absence of enamel and/or pulp tissue using a stereomicroscope (Wild M5A, Heerbrug, Switzerland). A standard smear layer was created by removing a standard thin layer of the dentin surface using a water-cooled, high-speed medium-grit (100 μm) diamond bur (842, Komet, Lemgo, Germany) mounted in a MicroSpecimen Former (University of Iowa, Iowa City, IA, USA), providing in this way a constant bur pressure. A new bur was used for each tooth. All specimens were randomly divided into three groups and subjected to a bonding treatment strictly according to the manufacturers' instructions (Table 1). After adhesive treatment, the surfaces were built up with the micro-hybrid resin composite Z100 (3M ESPE, St. Paul, MN, USA) in five layers to a height of 5−6 mm[16]. Each layer was light-cured for 40 sec using an Optilux 500 light-curing unit (Demetron/Kerr, Danbury, CT, USA) with a regularly controlled light-output of 500 mW/cm^2. After storage of the teeth for seven days in 0.5% chloramine at 37℃, they were sectioned perpendicular to the bonding surface using minimal pressure on the water-cooled slow-cutting Isomet saw. Per tooth, four rectangular sticks of about 1.85×1.85 mm wide and 8−9 mm long were prepared (Fig. 1)[20]. Pressure and speed of the saw were recorded and standardized. The specimens were then mounted in the pin-chuck of the MicroSpecimen Former and trimmed at the tooth-biomaterial interface to a cylindrical hour-glass shape with a diameter of 1.1 mm using a cylindrical extra-fine grit (15 μm) diamond bur (835 KREF, Komet, Lemgo, Germany) in a water-cooled high-speed hand piece. The diameter of each specimen was measured to the nearest 0.001 mm using a stereomicroscope at a magnification of 20x (400-NRC, Leitz, Germany). A bonding surface of about 1 mm^2 was obtained.

Micro-tensile bond strength test

The micro-specimens were fixed with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Ohtawara, Japan) onto a flat Ciucchi's jig, onto a notched jig or in a top-bottom design (Fig 1).

The μTBS of the specimens was determined in a universal testing machine (Instron 5848 Micro Tester, High Wycombe, Bucks, UK) at a crosshead speed of 1 mm/min using a load cell of 500N. We calculated the μTBS of each specimen in MPa, by dividing the imposed force (in N) at the time of fracture by its cross-sectional bond area (in mm^2). All specimens were maintained moist throughout the whole preparation and test procedure. One operator carried out all procedures to ensure standardization.

Failure analysis

Two independent evaluators analyzed all specimens quantitatively and qualitatively using a stereomicroscope at a magnification of 50x (Wild M5A, Heerbrug, Switzerland). Failures were recorded as either 'cohesive in dentin', 'mixed failure' or 'cohesive in resin'.

Statistical analysis

Two-way analysis of variance (ANOVA) and Scheffe multiple comparisons test were used to determine statistical differences in μTBS between the three fixation modes and the three adhesives used. The results were analyzed at a significance level of 0.05. All statistics were performed using the Statistica software package (Stat Soft, Tulsa, OK, USA).

RESULTS

The mean μTBS and the ratio of the standard deviation over the mean are summarized per adhesive and fixation mode in Table 2 and presented in Figure 2. Specimens tested with a notched jig consistently yielded higher values than samples fixed onto a flat surface or following a top-bottom design ($p<0.0001$).

Table 2 Micro-tensile bond strength values (in MPa) and coefficient of variation (CV = standard deviation/mean) for each adhesive per fixation method employed

Adhesive	Flat jig		Notched jig		Top-bottom	
	mean	CV	mean	CV	mean	CV
OptiBond Fl	38.86$^{(a,b)}$	38.36%	43.33$^{(a)}$	33.67%	32.88$^{(a,b)}$	31.02%
	(n=16)		(n=16)		(n=18)	
Scotchbond 1 XT	27.76$^{(a,b,c)}$	43.84%	35.90$^{(a,b)}$	36.34%	28.91$^{(a,b,c)}$	24.97%
	(n=15)		(n=16)		(n=19)	
One Coat SE Bond	26.10$^{(a,c)}$	47.23%	35.86$^{(a,b)}$	42.59%	14.37$^{(c)}$	34.09%
	(n=15)		(n=16)		(n=18)	

Means with the same superscript are not significantly different (two way ANOVA and Scheffe multiple comparisons test); n = total number of specimens

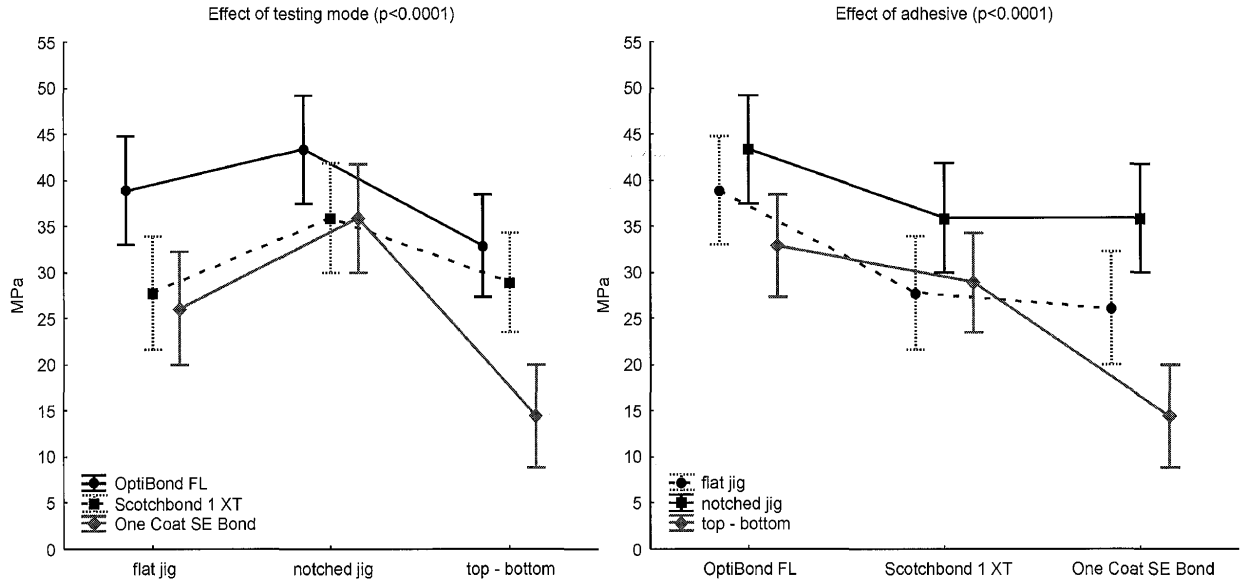

Fig. 2 Effect of adhesive and fixation method on micro-tensile bond strength values (in MPa).

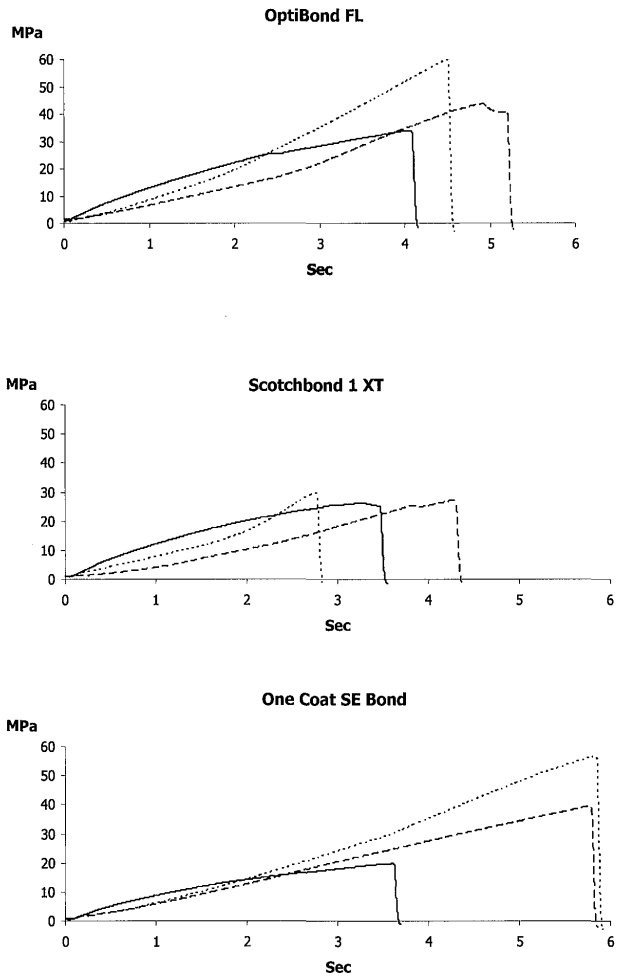

Fig. 3 Stress-time graphs of representative specimens of each adhesive with the solid line representing specimens tested using a top-bottom set-up, the dotted line representing specimens tested using a notched jig, and the dashed line representing specimens tested using a flat jig.

Table 3 Failure patterns of μTBS specimens as analyzed through stereomicroscopy

Adhesive	Fixation mode	Cohesive in dentin	Mixed failure*	Cohesive in resin	Total (n)
OptiBond Fl	flat jig	6	5	5	16
	notched jig	6	5	5	16
	top-bottom	5	13	0	18
Scotchbond 1 XT	flat jig	2	13	0	15
	notched jig	4	11	1	16
	top-bottom	0	19	0	19
One Coat SE Bond	flat jig	1	14	0	15
	notched jig	2	14	0	16
	top-bottom	0	18	0	18

*mixed failure = interfacial failure and cohesive failure in resin/dentin and interfacial failure.

The standard deviation/coefficient of variation, very useful parameters to estimate the experiment's precision, was the smallest for the top-bottom design. OptiBond FL showed significantly higher μTBS for the three fixation modes ($p<0.0001$), while an extreme aberration was seen for One Coat SE Bond in the top-bottom set-up.

If specimens were attached to a flat jig, the graphs revealed a more irregular pattern and the maximum stresses were built up more slowly. The top-bottom design resulted in a completely different, convex, stress-time graph (Fig. 3).

The results from light-microscopy failure analysis are summarized in Table 3. For Scotchbond 1 XT and One Coat SE Bond, most failures were recorded as 'mixed', including interfacial failure and areas

that failed partially 'adhesively' between tooth and resin, and parts of 'cohesive' failure in resin or tooth. OptiBond FL tended to fail more cohesively in dentin or resin, a pattern commonly associated with higher bond strengths and mechanically 'stronger' adhesives. No significant difference in failure pattern could be detected between the specimens attached to a notched jig and a flat jig, while the top-bottom design resulted in a significantly higher failure at the actual interface (Chi-square $p=0.0204$).

DISCUSSION

In this study, the influence of specimen fixation to the μTBS-testing device was evaluated. Three adhesives representing three different classes of adhesives were tested, while the same composite was used for all groups. Care was taken that the adhesives were applied to standardized tooth substrates and strictly according to their respective manufacturer's instructions (Table 1). In order to bond consistently to about the same dentin depth, a standard thin layer of the dentin surface was removed using a medium-grit diamond bur. In this way, the orientation of the tubuli was perpendicular to the surface and regional effects on the μTBS were minimized. This procedure also resulted in a uniform, clinically relevant smear layer. Using the MicroSpecimen Former, the tooth-resin interface was constricted cylindrically to about 1 mm^2, as recommended by Sano *et al.*[3], Shono *et al.*[4] and Phrukkanon *et al.*[9]. Doing so, the tensile stress imposed to the tiny and relatively fragile μTBS samples was minimized and standardized.

As expected, among the different adhesives, OptiBond FL presented with the highest bond strength values[21]. With both jig designs, this 'stronger' adhesive revealed more cohesive failures. As cohesive fractures of dentin are not encountered clinically and as fracture within one of the two substrates does not represent the actual interfacial bond strength, the top-bottom design is a more representative test set-up because this set-up resulted in more failures at the interface itself.

Attaching specimens to a notched jig resulted in significantly higher μTBS-values. Compared to a flat jig, this simplified and self-aligning specimen fixation protocol demonstrated a more linear and uniform stress-time graph (Fig. 3). The more standardized test set-up of a notched jig also reduced the coefficient of variation. This experiment's precision parameter was the lowest when specimens were fixed in the top-bottom set-up. As we also recorded in this set-up significantly more failures at the interface, we think that this must be attributed to a perfectly perpendicular alignment of the micro-specimens to the interface and to stress imposed following the specimen's length-axis.

In the original micro-tensile bond strength test, specimens are fixed to a jig with cyanoacrylate glue that covers the entire surfaces of both ends. This glue consists of a gel that is put first on the jig, and of which hardening is fastened by a spray, the hardener. Until today, no fully detailed specimen fixation protocol has yet been described. Compared to a flat jig, a notched jig facilitated the application of glue. Only a small amount of glue was needed and a better and more rapid positioning of the specimen enabled us to put hardener on the jig before the specimen was positioned. In addition, the potential impact of the glue on the bond strength was reduced because there was less risk that the glue contaminated the interface. In this way, compared to a flat jig, a notched jig resulted in less fault registrations and in more uniform results. The influence of the glue was even more obvious when testing following the top-bottom design. Even before the test was started, some stress was recorded that must be ascribed to the hardening of the glue. As setting of the glue generated more stress in dentin and/or resin in the top-bottom set-up, lower bond strengths and convex stress-time graphs were recorded in this newly designed set-up, as compared to the concave graphs recorded with both jig designs. The stress was built up immediately after loading and slowed down to reach the maximum stress, at which the specimen was fractured. In addition, in more than 50% of the top-bottom tests, a difference between 'stress at maximum load' and 'stress at break' was seen. Also, the extremely low bond strengths of the 'weaker adhesive' One Coat SE Bond in the top-bottom design may be explained by the stress generated by the setting of the glue.

CONCLUSION

Attaching the specimens onto a Ciucchi's jig customly adapted with a vertical notch eased manipulation and fixation of the micro-specimens to the testing device, and lead to higher bond strengths. In the top-bottom set-up, the tensile stress is concentrated perfectly perpendicular to the interface and parallel with the specimen's length-axis. This resulted in a higher number of failures at the true interface.

REFERENCES

1) Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion testing of dentin bonding agents: A review. Dent Mat 1995; 11: 117-125.
2) Pashley DH, Carvalho RM, Sano H, Nakajima M, Shono Y, Fernandes CA, Tay F. The Microtensile bond test: a review. J Adhes Dent 1999; 1: 299-309.
3) Sano H, Shono T, Sonoda H, 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.
4) Shono Y, Terashita M, Pashley EL, Brewer PD, Pashley DH. Effects of cross-sectional area on resin-enamel tensile bond strength. Dent Mater 1997; 13: 290-296.
5) Sudsangiam S, van Noort R. Do dentin bond strength tests serve a useful purpose? J Adhes Dent 1999; 1: 57-67.
6) Goracci C, Sadek F, Monticelli F, Cardoso P, Ferrari M. Influence of substrate, shape, and thickness on microtensile specimens' structural integrety and their measured bond strengths. Dent Mat 2004; 20: 643-654.
7) Hosoya Y, Kawada E, Ushigome T, Oda Y, Garcia-Godoy F. Micro-tensile bond strength of sound and caries-affected primary tooth dentin measured with original designed jig. J Biomed Mater Res 2005; 77B: 241-248.
8) Oshita S, Nara Y, Tanaka H. Mutual relation between specimen form and micro-tensile bond strength. Jpn J Conserv Dent 2004; 47: 587-607.
9) Phrukkanon S, Burrow MF, Tyas M. Effect of cross-sectional surface area on bond strengths between resin and dentin. Dent Mater 1998; 14: 120-128.
10) Phrukkanon S, Burrow MF, Tyas M. The influence of cross-sectional shape and surface area on the microtensile bond test. Dent Mater 1998; 14: 212-221.
11) Shono Y, Ogawa T, Terashita M, Carvalho RM, Pashley EL, Pashley DH. Regional measurement of resin-dentin bonding as an array. J Dent Res 1999; 78: 699-705.
12) Stamatacos-Mercer C, Hottel T. The validity of reported tensile bond strength utilizing non-standardized specimen surface areas. An analysis of *in vitro* studies. Am J Dent 2005; 18: 105-108.
13) Reis A, de Oliveira Bauer JR, Louguercio AD. Influence of crosshead-speed on resin-dentin microtensile bond strength. J Adhes Dent 2004; 6: 275-278.
14) Yamaguchi K, Miyazaki M, Takamizawa T, Tsubota K, Rikuta A. Influence of crosshead speed on micro-tensile bond strength of two-step adhesive systems. Dent Mat 2006; 12: 420-425.
15) El Zohairy A, de Gee A, de Jager N, van Ruijven L, Feilzer A. The influence of specimen attachment and dimension on micro-tensile strength. J Dent Res 2004; 83: 420-424.
16) Van Noort R, Noroozi S, Howard IC, Cardew G. A critique of bond strength measurements. J Dent 1989; 17: 61-67.
17) Zheng L, Pereira P, Nakajima M, Sano H, Tagami J. Relationship between adhesive thickness and microtensile bond strength. Oper Dent 2001; 26: 97-104.
18) Armstrong S, Jessop J, Vargas M, Zou Y, Qian F, Campbell J, Pashley D. Effects of exogenous collegenase and cholesterol esterase on the durability of the resin-dentin bond. J Adhes Dent 2006; 8: 151-160.
19) Perdigão J, Geraldeli S, Carmo A, Dutra H. *In vivo* influence of residual moisture on microtensile bond strengths of one-bottle adhesives. J Esthet Restor Dent 2002; 14: 31-38.
20) De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Suzuki K, Lambrechts P. Four-year water degradation of a resin-modified glass-ionomer adhesive bonded to dentin. Eur J Oral Sci 2004; 112: 73-83.
21) Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonoccore memorial lecture: adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003; 28: 215-235.