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Microtensile bond strengths of single-step self-etch adhesive systems to bovine dentin

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Abstract: The purpose of this study was to examine the microtensile bond strength of a single-step self-etch adhesive system (Clearfil tri-S Bond and One-Up Bond F Plus) to bovine dentin. Adhesive was applied to a flat dentin surface, and resin composite was bonded according to the manufacturers' instructions. After 24 h storage in distilled water at 37°C, hourglass-shaped specimens were produced. These were subjected to microtensile bond strength testing at a cross-head speed of 1.0 mm/min. The results were analyzed using Student's *t*-test at a significance level of 0.05. Field-emission scanning electron microscopy (FE-SEM) observations of the fractured specimens and the adhesive-treated dentin surfaces were also conducted. The bond strength of Clearfil tri-S Bond was not significantly different from that of One-Up Bond F Plus, 41.1 ± 10.1 versus 42.3 ± 6.0 MPa. Mode of failure analysis for Clearfil tri-S Bond revealed an equal distribution between the three types of failure, and the predominant mode of failure was adhesive for One-Up Bond F Plus. FE-SEM observations of dentin to which adhesive had been applied revealed that the smear layer had been removed and the collagen fibers exposed. (J. Oral Sci. 49, 183-189, 2007)

Keywords: single-step self-etch system; dentin; bond strength; microtensile.

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Introduction

The development of a potentially useful bonding system requires an understanding of the bonding mechanism and characterization of the bonding interface between resin composites and tooth substrates. Bonding to a vital and wet dentin substrate remains a challenge in restorative dentistry due to the large amount of organic components, variation in intrinsic composition, the presence of fluid flow and odontoblastic processes in the tubules, the presence of a smear layer, and the inherent wetness of the surface (1). The mineral phase of the substrate needs to be partially removed, and resin monomers have to permeate into the demineralized collagen-rich layer and then polymerize (2). Several steps are required in order to obtain optimum bonding to the dentin substrate. Because bonding procedures have required multiple-step clinical approaches, the clinical success of these adhesive systems sometimes depends on technique-sensitive and material-related factors.

In an attempt to reduce technique sensitivity, self-etching primer systems have been developed. The self-etching primer is applied to the tooth surface, followed by solvent-free hydrophobic bonding resins (3). Recently, a more user-friendly single-step self-etching adhesive system has been introduced. The simplification of the clinical procedure resulting from the use of this system may be especially beneficial when treating potentially uncooperative patients, such as children. Uniform resin impregnation into partially demineralized dentin and sufficient mechanical strength of the cured adhesive resin are important factors that are required to create a high-quality bonding interface for durable dentin bonding (4,5). However, only limited information is available on the bonding ability of a single-step self-etch adhesive system to dentin.

It has previously been demonstrated that a wire-loop method of loading leads to smaller stress-concentration effects when three-dimensional finite-element analysis is employed. However, the true interface bond strength is significantly underestimated when the shear bond strength is determined by dividing the failure load by the cross-sectional area (6). Moreover, differences in diameter of the bonded area have been reported to affect the contact area between the knife-edge and the composite column, resulting in different stress distributions (7). Using a tensile test apparatus, including the microtensile test employed in the present study, might help to negate these issues (8).

The purpose of the present study was to examine the bonding characteristics of single-step self-etch systems to bovine dentin by means of microtensile bond strength testing. The adhesive-applied dentin surfaces and fractured specimens were observed using field emission scanning electron microscopy (FE-SEM).

Materials and Methods

Materials

The single-step self-etch adhesive systems used in this experiment were Clearfil tri-S Bond and Clearfil AP-X (TSB, Kuraray Medical, Tokyo, Japan) and One-Up Bond F and Estelite ∑ (OBF, Tokuyama Dental, Tokyo, Japan). The ingredients and bonding procedures for TBS and OBF are shown in Tables 1 and 2. The adhesive systems were used in combination with the manufacturers' suggested resin composites.

A curing unit (Optilux 501; sds Kerr, Danbury, CT, USA) was used and the power density (800 mW/cm^2) of the curing light was checked with a dental radiometer (Model 100, sds Kerr) before preparing the specimens.

Microtensile bond strength test

Mandibular incisors extracted from 2 to 3-year-old cattle and stored frozen (-20°C) for up to 2 weeks were used as a substitute for human teeth (9-11). After removing the roots with a low-speed diamond saw (Isomet 1000; Buehler Ltd., Lake Bluff, IL, USA), the pulps were removed, and the labial surfaces of the teeth were ground on wet 600-grit SiC paper. After ultrasonic cleaning for 3 min in distilled water to remove the excess debris, the surfaces were washed and dried with oil-free compressed air.

The adhesive was applied to the dentin surface according to the manufacturers' instructions and irradiated with the curing unit. A Teflon (Sanplatec Corp., Osaka, Japan) mold (height × diameter = 2.0 × 8.0 mm) was used to form and hold the resin composites to the dentin surface prior to curing for 40 sec. The finished specimens were transferred to distilled water and stored at 37°C for 24 h. After incubation, the specimens were sectioned at the widest part of the tooth using a diamond saw. The bonded

Table 1 Single-step self-etch systems tested

Code	Adhesive (Manufacturer)	Main components	Lot No.	Restorative	Lot No.
TSB	Clearfil tri-S Bond (Kuraray Medical)	MDP, bis-GMA, HEMA, initiator ethanol, water, stabilizer, filler hydrophobic dimethacrylate	040219	Clearfil AP-X (A2)	00841A
OBF	One-Up Bond F Plus (Tokuyama Dental)	MAC-10, HEMA, MMA, multifunctional methacrylic monomer, fluoroaluminosilicate glass, water photo initiator, aryl borate catalyst	A: 551F-2 B: 551F-2	Estelite ∑ (A2)	J009

Table 2 Application protocols of single-step self-etch systems

Adhesive	Application Protocol
TSB (single bottle)	Dispense one drop of liquid into well. Apply to dentin for 20 sec. Subject to a relatively strong stream of air to dry and light irradiation for 10 sec.
OBF (two bottles)	Mix equal amounts of the bond agents A and B until a pink homogenous liquid mixture is obtained. Apply to dentin for 10 sec with agitation. Subject to a mild stream of air to dry and light irradiation for 10 sec.

slices were trimmed into an hourglass shape with a superfine diamond point under a constant water spray until a 1.0 × 1.0 mm bonded surface remained. Cross-sectional areas were individually measured to the nearest 0.01 mm for each specimen using a digital caliper (500-151 CD-15C; Mitsutoyo, Tokyo, Japan). Fifteen specimens for each group were attached to a microtensile apparatus with a cyanoacrylate adhesive (ZAPIT; Dental Ventures of America Inc., Corona, CA, USA) and then subjected to tensile loading in a universal testing machine (Type 4204; Instron Corp., Canton, MA, USA) at a cross-head speed of 1.0 mm/min (Fig. 1). The tensile strength (MPa) was calculated from the peak load at failure divided by the original cross-sectional area at the smallest section.

Statistical analysis was carried out with the Sigma Stat software system (Ver. 3.1; SPSS Inc., Chicago, IL, USA) using one-way analysis of variance (ANOVA) in conjunction with Student's *t*-test. A probability (*P*) value of < 0.05 was considered to indicate statistical significance.

After testing, the specimens were examined using an optical microscope (SZH-131; Olympus Ltd., Tokyo, Japan) at a magnification of ×20 to define the location of the bond failure. The mode of failure for each specimen was then classified into one of three types: adhesive failure between restorative material and dentin; cohesive failure in adhesive resin; and cohesive failure in dentin.

FE-SEM

The treated dentin surface and fractured specimens after the bond strength test were observed by FE-SEM. Sample preparation involved treatment of the dentin surfaces with adhesives according to each manufacturer's instructions, followed by rinses with acetone and water. All of the SEM specimens were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 min, 75% for 20 min, 95% for 20 min and 100% for 2 h) and then transferred to a critical-point dryer for 30 min. The surfaces were coated in a vacuum evaporator (Quick Coater SC-701; Sanyu Denshi Inc., Tokyo, Japan), with a thin film of Au. The specimens were then observed using FE-SEM (ERA 8800FE, Elionix Ltd., Tokyo, Japan).

Results

The microtensile bond strengths to bovine dentin and fracture modes after microtensile testing are listed in Table 3. There was no statistically significant difference in the mean values between TSB and OBF ($P = 0.695$), 41.1 ± 10.1 versus 42.3 ± 6.0 MPa.

The failure modes differed among the adhesive systems used. The fractured surfaces of most specimens in which TSB was used showed an almost equal distribution between the three types of failure (Fig. 2). However, OBF predominantly showed failure at the interface between the dentin and the adhesive. FE-SEM observations revealed partial remnants of adhesive resin (Fig. 3).

Representative SEM views of the treated dentin surfaces are shown in Fig. 4. The smear layer was removed, and dentinal tubules were partially open for both of the adhesive systems. Remnants on the treated dentin surfaces were more pronounced for TSB than for OBF, indicating that the etching effect was more pronounced in the latter.

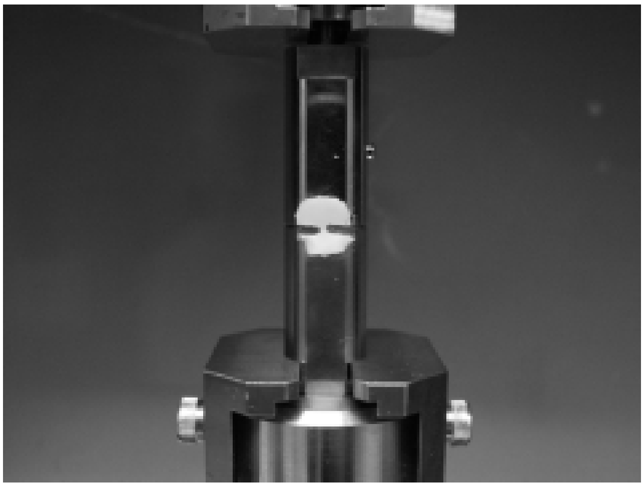

Fig. 1 Microtensile bond strength measurement jig used in this study. An hourglass-shaped specimen was bonded to the jig with a cyanoacrylate adhesive and subjected to tensile loading at a cross-head speed of 1.0 mm/min.

Table 3 Microtensile bond strength (Mean (SD) in MPa) to bovine dentin

Code	Bond strength	Mode of fracture		
		A	R	D
TSB	41.1 (10.1)	5	4	6
OBF	42.3 (6.0)	13	1	1

SD: standard deviation, n = 15.
No significant difference was found for the bond strengths ($P > 0.05$).
Failure mode: A, adhesive failure; D, cohesive failure in dentin; R, cohesive failure in adhesive.

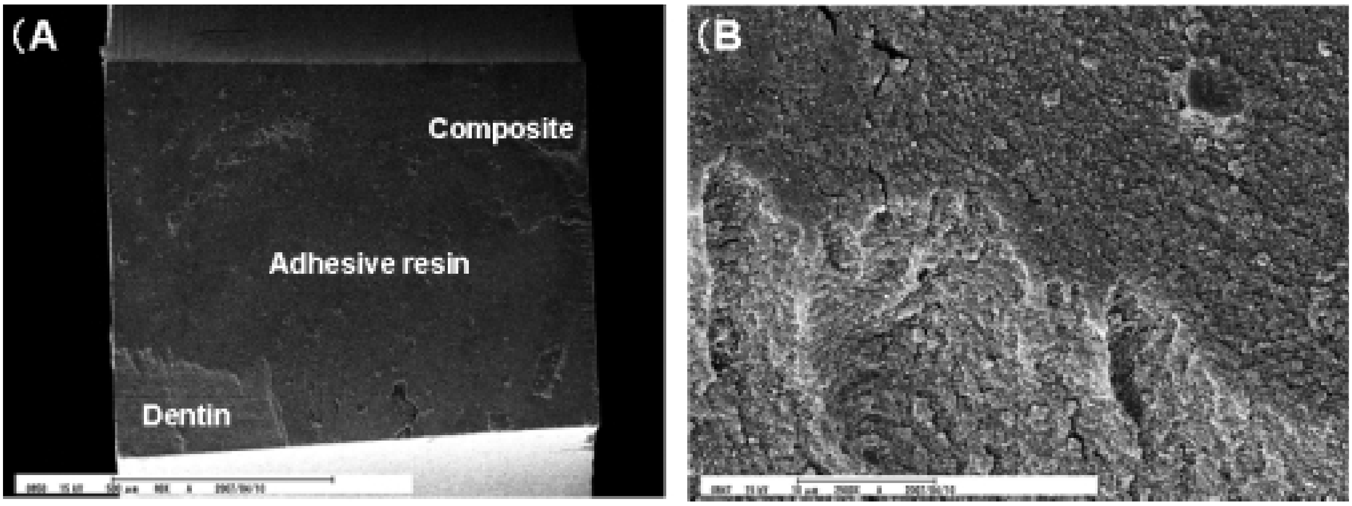

Fig. 2 Representative SEM observations of the fractured surface of TSB after microtensile bond strength testing. The fractured surfaces showed an almost equal distribution between the three types of failure.

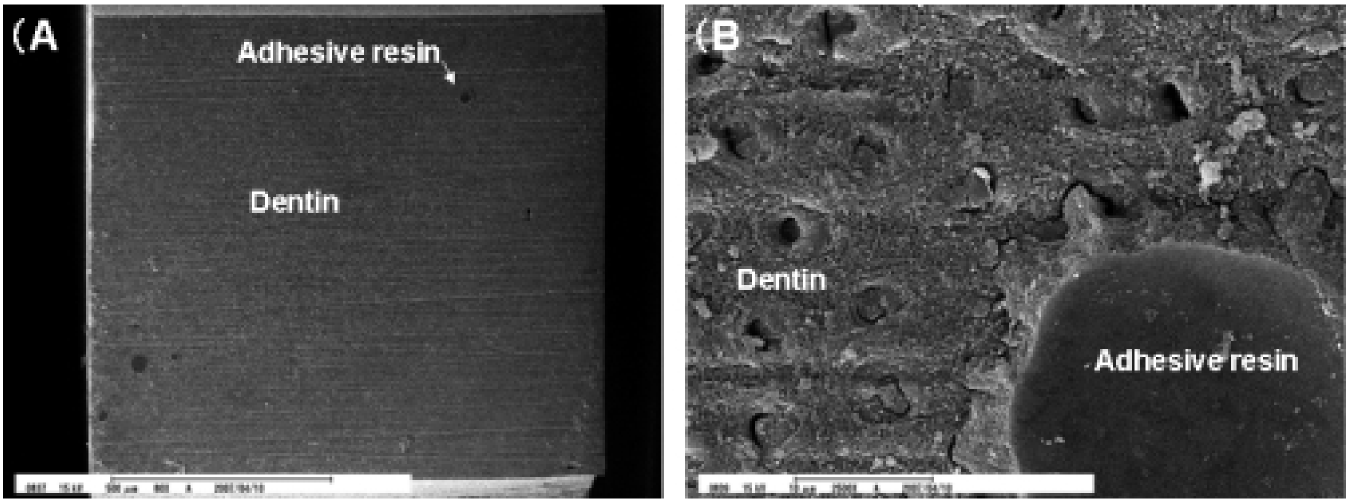

Fig. 3 Representative SEM observations of the fractured surface of OBF after microtensile bond strength testing. Bonding failure between the dentin and the adhesive is apparent (A). At higher magnification (B), remnants of adhesive resin are evident on the dentin surface.

Discussion

Large numbers of intact, extracted teeth are required for conducting bond strength tests, but it is difficult to obtain sufficient numbers of extracted human teeth in Japan. It has been reported that adhesion to the superficial layer of dentin does not differ significantly between human and bovine dentin, and that the dentin bond strength decreases with dentin depth due to the lower density of dentinal tubules (11). Since differences in tubule diameter and the number of their lateral branches may have some effect on dentin bond strength (12,13), bovine superficial dentin was used as a substitute for human dentin in this study, as has been the case in previous studies (10,11). Measured bond strength values depend on the bonding system used, the site on the tooth, and the type of tooth structure (14-16). Care should be taken when drawing conclusions from bond strength data because there are numerous factors that can affect bond values (17).

Much of the research related to dentin bonding has attempted to assess the integrity and strength of the interfacial bond. Experimental approaches in dentistry that measure adhesive bond strengths consist primarily of tensile or shear bond strength determinations performed within a defined area (17). The resistance against initiation of crack growth and propagation depends on the geometry and the load configuration of the specimen. The use of the microtensile bond strength test to evaluate bond strength of adhesive systems has been widely accepted (18-20).

Smaller specimens contain a lower number of initial defects and have more homogeneous stress distributions; hence, bond strengths as well as adhesive failure between the adhesive and dentin can be examined more accurately. This phenomenon reflects the characteristics of the microtensile test, resulting from stress concentration at the bonded interface (21). Bond strength values can be used for comparing the effectiveness of bonding systems; however, they cannot be related directly to what might happen clinically.

The dentin bonding system results in the formation of three distinct layers, each layer showing different mechanical properties. The elastic modulus of the successive layers across a resin-dentin bonding area has been determined using a nano-indentation technique, and a gradient of elastic modulus has been observed (22). The more elastic layer might have a strain capacity sufficient to conserve the dentin bond (23). When failure occurs in an adhesive area, the material at the crack tip will deform plastically to some extent. At the crack-tip end, a plastic zone will form. The relationship between the size of the crack-tip plastic-zone and the size of the material determines the fracture process (24). The crack resistance is a function of the plastic behavior of the adhesive at the site of the crack tip and of the fracture characteristics (25). In thin specimens, where the plastic-zone size is not small compared to the thickness of the adhesive, plane stress develops. In these cases, higher stress intensity can be applied before crack propagation occurs. When the plastic-zone size is small compared to the adhesive thickness, yielding in the direction of the thickness cannot take place freely, but is restrained by the surrounding elastic materials. The path of the fracture placed under tension will pass through the weakest area in the bulk of the adhesive area or interface. It is speculated that an initial crack site is produced in the bonded area of the specimen. The crack might be initiated and eventually propagate along a bonded interface with inherent flaws. Smaller specimens contain a lower number of initial defects and show more homogeneous stress distributions, therefore exhibiting greater bond strengths as well as adhesive failure between the bonding agent and dentin (26). The elastic modulus of the material is not affected appreciably by strain rate, but the plastic deformation is sensitive to this factor (27).

To create stable bonding to dentin, a self-etch adhesive should penetrate beyond the smear layer into the underlying dentin. Single-step self-etch adhesive systems rely on partial demineralization of the dentin surface by acidic monomers to remove the smear layer and expose collagen fibrils for penetration by resin monomers (Fig. 4). The etching effect of the self-etch adhesive is related to the acidic functional monomers that interact with the mineral component of the tooth substrate, and create a continuum between the tooth surface and the adhesive by simultaneous demineralization and resin penetration. The single-step system has to contain water as well as water-soluble hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA), so that the acidic monomer can dissociate and penetrate into the hydrophilic dentin. The depth of the demineralization during adhesive application depends on the type of acidic monomers, their concentration, and the duration of application and composition of the dentin. The adhesive TSB contains MDP, while OBF contains MAC-10 as an acidic functional monomer (Table 1). In a

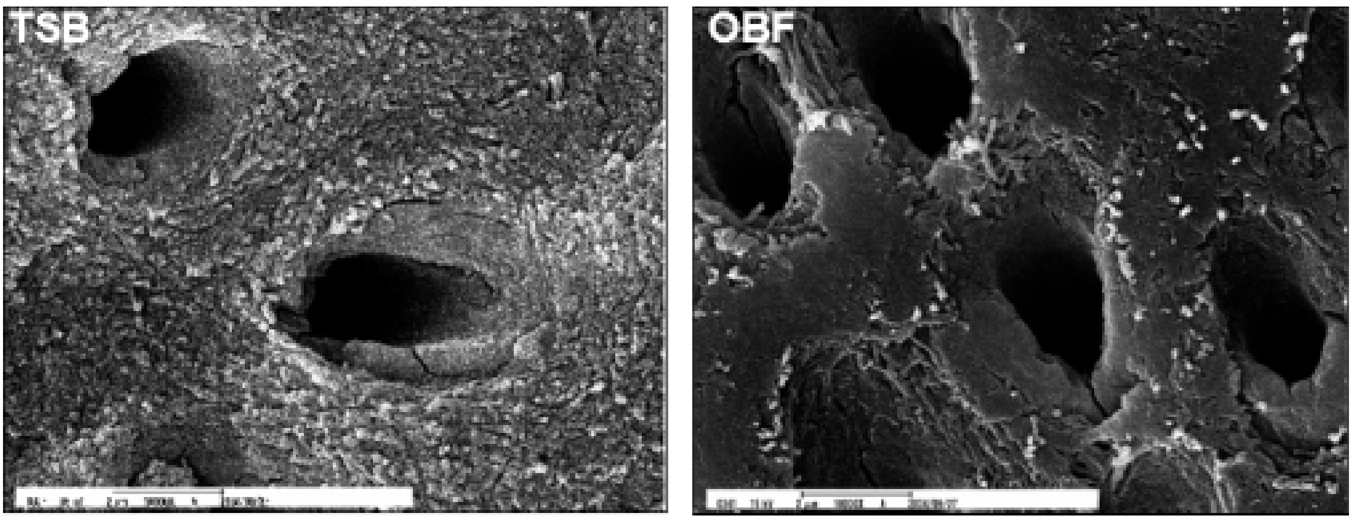

Fig. 4 SEM observations of dentin surfaces treated with TSB and OBF. The smear layer and plugs are removed, and the dentinal tubules opened. Opening of dentinal tubules on the treated dentin surfaces was more pronounced for OBF (A) than for TSB (B) (original magnification; ×10,000).

previous study (28) performed to compare the chemical bonding efficacy of functional monomers, MDP was reported to have a high chemical bonding potential to hydroxyapatite within a clinically reasonable application time. Furthermore, the calcium salt of MDP was highly insoluble, and consequently able to resist ultrasonic cleaning. According to the adhesion-decalcification concept (29), the less soluble the calcium salt of the acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate. The complex fracture modes observed in this study might indicate the stronger ability of this material to bond chemically to tooth structures.

Solvents such as water are also included in the self-etch adhesive, as they play an important role in the demineralization of dentin. For TSB, the adhesive-applied dentin surface should be air-dried in order to evaporate the solvents, and this can result in a thin adhesive layer. By contrast, the adhesive OBF is not strongly air dried, leading to a thicker adhesive layer (~ 60 μm). Although the applied adhesive was thicker than that of TSB, remaining solvents such as water do not appear to be an obstacle to the polymerization of OBF. This is presumably due to the excellent polymerization ability of the dye-sensitized photopolymerization system employed in this adhesive. The initiator system of OBF contains a dye-sensitizer, a co-initiator and a borate derivative. The energy transfer reaction from the dye-sensitizer to the co-initiator takes place upon light irradiation, making the co-initiator enter an excited state. The polymerizable radical species is then formed by reaction of the borate derivative with the activated co-initiator containing hydrogen ions derived from the dye-sensitizer as well as acidic functional monomers. However, air drying is essential to obtain adequate dentin bond strengths for TSB. It has been demonstrated that the bond strength should theoretically be proportional to the strength of the adhesive resin that infiltrates into the demineralized dentin. Consequently, adhesive resins have lower tensile strengths than resin-infiltrated demineralized dentin (30). As the adhesive resin might be the weakest component of the adhesive interface, the mechanical strength of cured monomers could reflect the quality of the bonding interface (31,32).

Further research on the single-step self-etch restorative system is needed to determine its long-term bonding ability to primary and permanent tooth dentin, and clinical performance.

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References

1. Pashley DH, Ciucchi B, Sano H, Carvalho RM, Russell CM (1995) Bond strength versus dentine structure: a modelling approach. Arch Oral Biol 40, 1109-1118
2. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, Yoshida Y, Inoue S, Suzuki K (2005) Technique-sensitivity of contemporary adhesives. Dent Mater J 24, 1-13
3. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 28, 215-235
4. Miyazaki M, Onose H, Moore BK (2002) Analysis of the dentin-resin interface by use of laser Raman spectroscopy. Dent Mater 18, 576-580
5. Miyazaki M, Onose H, Iida N, Kazama H (2003) Determination of residual double bonds in resin-dentin interface by Raman spectroscopy. Dent Mater, 19, 245-251
6. DeHoff PH, Anusavice KJ, Wang Z (1995) Three-dimensional finite element analysis of the shear bond test. Dent Mater 11, 126-131
7. Van Noort R, Noroozi S, Howard IC, Cardew G (1989) A critique of bond strength measurements. J Dent 17, 61-67
8. Goracci C, Sadek FT, Monticelli F, Cardoso PE, Ferrari M (2004) Influence of substrate, shape, and thickness on microtensile specimens' structural integrity and their measured bond strengths. Dent Mater 20, 643-654
9. Nakamichi I, Iwaku M, Fusayama T (1983) Bovine teeth as possible substitutes in the adhesion test. J Dent Res 62, 1076-1081
10. Fowler CS, Swartz ML, Moore BK, Rhodes BF (1992) Influence of selected variables on adhesion testing. Dent Mater 8, 265-269
11. Schilke R, Bauβ O, Lisson JA, Schuckar M, Geurtsen W (1999) Bovine dentin as a substitute for human dentin in shear bond strength measurements. Am J Dent 12, 92-96
12. Ferrari M, Davidson CL (1996) In vivo resin-dentin interdiffusion and tag formation with lateral branches of two adhesive systems. J Prosthet Dent 76, 250-253

13. Ferrari M, Cagidiaco MC, Kugel G, Davidson CL (1996) Dentin infiltration by three adhesive systems in clinical and laboratory conditions. Am J Dent 9, 240-244
14. Yoshiyama M, Sano H, Ebisu S, Tagami J, Ciucchi B, Carvalho RM, Johnson MH, Pashley DH (1996) Regional strengths of bonding agents to cervical sclerotic root dentin. J Dent Res 75, 1404-1413
15. Burrow MF, Sano H, Nakajima M, Harada N, Tagami J (1996) Bond strength to crown and root dentin. Am J Dent 9, 223-229
16. Ogata M, Okuda M, Nakajima M, Pereira PN, Sano H, Tagami J (2001) Influence of the direction of tubules on bond strength to dentin. Oper Dent 26, 27-35
17. Miyazaki M, Oshida Y, Xirouchaki L (1996) Dentin bonding system. Part I: literature review. Biomed Mater Eng 6, 15-31
18. Uekusa S, Yamaguchi K, Miyazaki M, Tsubota K, Kurokawa H, Hosoya Y (2006) Bonding efficacy of single-step self-etch systems to sound primary and permanent tooth dentin. Oper Dent 31, 569-576
19. Perdigão J, Gomes G, Gondo R, Fundingsland JW (2006) In vitro bonding performance of all-in-one adhesives. Part I – microtensile bond strengths. J Adhes Dent 8, 367-373
20. Harnirattisai C, Senawongse P, Tagami J (2007) Microtensile bond strengths of two adhesive resins to discolored dentin after amalgam removal. J Dent Res 86, 232-236
21. Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM (1995) Adhesion testing of dentin bonding agents: a review. Dent Mater 11, 117-125
22. Hosoya Y, Tay FR (2007) Hardness, elasticity, and ultrastructure of bonded sound and caries-affected primary tooth dentin. J Biomed Mater Res B Appl Biomater 81, 135-141
23. Van Meerbeek B, Willems G, Celis JP, Roos JR, Braem M, Lambrechts P, Vanherle G (1993) Assessment by nano-indentation of the hardness and elasticity of the resin-dentin bonding area. J Dent Res 72, 1434-1442
24. Wakasa K, Yamaki M, Matsui A (1995) Calculation models for average stress and plastic deformation zone size of bonding area in dentine bonding systems. Dent Mater J 14, 152-165
25. Oshida Y, Miyazaki M (1996) Dentin bonding system. Part II: effect of crosshead speed. Biomed Mater Eng 6, 87-100
26. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay F (1999) The microtensile bond test: a review. J Adhes Dent 1, 299-309
27. Yamaguchi K, Miyazaki M, Takamizawa T, Tsubota K, Rikuta A (2006) Influence of crosshead speed on micro-tensile bond strength of two-step adhesive systems. Dent Mater 22, 420-425
28. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van Meerbeek B (2004) Comparative study on adhesive performance of functional monomers. J Dent Res 83, 454-458
29. Yoshioka M, Yoshida Y, Inoue S, Lambrechts P, Vanherle G, Nomura Y, Okazaki M, Shintani H, Van Meerbeek B (2002) Adhesion/decalcification mechanisms of acid interactions with human hard tissues. J Biomed Mater Res 59, 56-62
30. Sano H, Takatsu T, Ciucchi B, Russell CM, Pashley DH (1995) Tensile properties of resin-infiltrated demineralized human dentin. J Dent Res 74, 1093-1102
31. Takahashi A, Sato Y, Uno S, Pereira PN, Sano H (2002) Effects of mechanical properties of adhesive resins on bond strength to dentin. Dent Mater 18, 263-268
32. Yamada M, Miyazaki M, Moore BK (2004) Influence of interchanging adhesive resins and self-etching primers on the mechanical properties of adhesive resins. Oper Dent 29, 532-537