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Bonding Efficacy of Single-step Self-etch Systems to Sound Primary and Permanent Tooth Dentin

S Uekusa • K Yamaguchi • M Miyazaki
K Tsubota • H Kurokawa • Y Hosoya

Clinical Relevance

Single-step self-etch systems are capable of producing a predictable bond to primary dentin, although the bond strength was found to be lower than permanent dentin.

SUMMARY

Currently, there is little information regarding the bonding efficacy of single-step self-etch systems to primary tooth dentin. This study examined the microtensile bond strength of single-step self-etch systems (Clearfil tri-S Bond and One-Up Bond F Plus) to sound primary and permanent tooth dentin. Adhesives were applied to flat samples of primary and permanent tooth dentin, and resin composites were bonded according to the manufacturers' instructions. After 24 hours of storage in distilled water at 37°C, hour glass-shaped specimens were produced. They were subjected to microtensile testing at a crosshead speed of 1.0 mm/minute. The results were analyzed using 2-way analysis of variance (ANOVA) followed by the Tukey HSD post-hoc test (α=0.05). Field-emission scanning electron microscopy (FE-SEM) observations of the adhesive-treated dentin surfaces and the resin/dentin interface were also conducted. The bond strengths of primary tooth dentin were significantly lower than that of permanent tooth dentin for both self-etch systems: 44.7 ± 10.4 versus 54.3 ± 9.0 MPa for Clearfil tri-S Bond and 40.6 ± 9.9 versus 50.0 ± 8.7 MPa for One-Up Bond F Plus ($p<0.001$). There was no statistically significant interaction between the type of adhesive system and the dentin substrate ($p=0.957$). Although there was no statistically significant difference in the mean values among the different adhesive systems ($p=0.094$), there was a statistically significant difference in mean values among the different dentin substrates ($p<0.001$), which were lower for primary tooth dentin than for permanent tooth

Satoshi Uekusa, DDS, graduate student, Department of Operative Dentistry, Nihon University Graduate School of Dentistry, Tokyo, Japan

Kanako Yamaguchi, DDS, graduate student, Department of Operative Dentistry, Nihon University Graduate School of Dentistry, Tokyo, Japan

*Masashi Miyazaki, DDS, PhD, professor and chairperson, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Keishi Tsubota, DDS, PhD, junior instructor, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Hiroyasu Kurokawa, DDS, PhD, instructor, Department of Operative Dentistry, Nihon University School of Dentistry, Tokyo, Japan

Yumiko Hosoya, DDS, PhD, associate professor, Division of Pediatric Dentistry, Course of Medical and Dental Sciences, Department of Developmental and Reconstructive Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

*Reprint request: 1-8-13, Kanda-Surugadai, Chiyoda-Ku, Tokyo 101-8310, Japan; e-mail: miyazaki-m@dent.nihon-u.ac.jp

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dentin. The failure modes were also independent of the type of dentin but dependent on the adhesive systems, an equal distribution among the 3 types of failure for Clearfil tri-S Bond and cohesive failures in adhesives 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. Though the bond strengths to primary tooth dentin were lower than to permanent tooth dentin, excellent adaptation of the single-step self-etch systems to both dentin substrates was observed by FE-SEM. Further studies are required to determine the long-term clinical performance of these adhesive systems when applied to primary tooth dentin.

INTRODUCTION

The single-step self-etch system is one of the bonding techniques developed to simplify and shorten bonding procedures by combining the dentin conditioning, priming and bonding agent-application steps.[1] Simplification of the clinical procedure that is provided when using the single-step self-etch systems will be especially beneficial when treating potentially uncooperative patients, such as children. These adhesive systems form a continuous layer between the composite and dentin surface, which is simultaneously demineralized, followed by resin monomer penetration. Proper demineralization of the dentin substrate, uniform resin impregnation and sufficient mechanical strength of the cured adhesive resin are important factors required to create a high-quality resin/dentin interface for good dentin bonding.[2-3] However, there are few laboratory data discussing the bonding characteristics of the newly developed single-step self-etch systems to primary tooth dentin (primary dentin).

Several reports have compared bond strength between primary and permanent tooth dentin (permanent dentin) with varying results.[4-6] Primary dentin has been assumed to be different from permanent dentin due to the variable amounts of mineral components, as well as morphological and structural differences. There might be substantial differences in the properties of primary dentin compared to permanent dentin.[7] The lower thickness of primary dentin compared to permanent dentin could be responsible for the lower bond strengths on primary dentin caused by the proximity of the adhesive to pulp. It has been suggested that primary dentin is more susceptible to acidic conditioners and, therefore, a shorter conditioning time in primary dentin treatment has been advocated.[8-9] However, other authors have tested different bonding systems and reported that the porous zone in primary dentin is not as distinct as that seen in permanent teeth. All of these factors might contribute to differences in dentin bond strength between primary and permanent dentin.[10]

This study examined the bonding ability of single-step self-etch systems to sound human primary and permanent dentin using measurements of microtensile bond strength. The adhesive-applied dentin surface and the resin/dentin interfaces were compared using FE-SEM. The null hypothesis to be tested was that the bond strengths of the single-step self-etch systems are not influenced by the type of dentin substrate, primary or permanent dentin.

METHODS AND MATERIALS

Materials Tested

The single-application self-etch adhesive systems used in this experiment, which both contained a combination of resin composites, were Clearfil tri-S Bond and Clearfil AP-X (TSB, Kuraray Medical, Tokyo, Japan) and One-Up Bond F and Palfique Estelite ∑(OBF, Tokuyama Dental, Tokyo, Japan). The ingredients and bonding procedures for TSB and DBF Plus are shown in Tables 1 and 2. All of 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.

Bond-strength Test

The buccal surfaces of caries-free human primary molars and premolars extracted and frozen in physiological saline soon after extraction were used. The teeth

Table 1: *Single-Step Self-Etch Systems Tested*

Code	Adhesive (Manufacturer)	Main Components	Lot #	Restorative Shade	Lot #
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 photoinitiator (aryl borate catalyst)	A: 004 B: 504	Palfique Estelite ∑ (A2)	J009

Abbreviations: MDP: 10-methacryloxydecyl di-hydrogen phosphate; bis-GMA: 2, 2bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]phenyl propane; HEMA: 2-hydroxyethyl methacrylate; MAC-10: 11-methacryloxy-1,1-undecandicarboxylic acid; MMA: metyl methacrylate.

Table 2: *Application Protocols of Single-step Self-etch Systems*

Adhesive	Application Protocol
Clearfil tri-S Bond (single bottle)	Dispense one drop of liquid into well. Apply to dentin for 20 seconds. Subject to a relatively strong stream of air to dry and light irradiation for 10 seconds.
One-Up Bond F Plus (2 bottles)	Mix equal amounts of the bond agents A and B until a pink homogenous liquid mixture is obtained. Apply to dentin for 10 seconds with agitation. Subject to a mild stream of air to dry and light irradiation for 10 seconds.

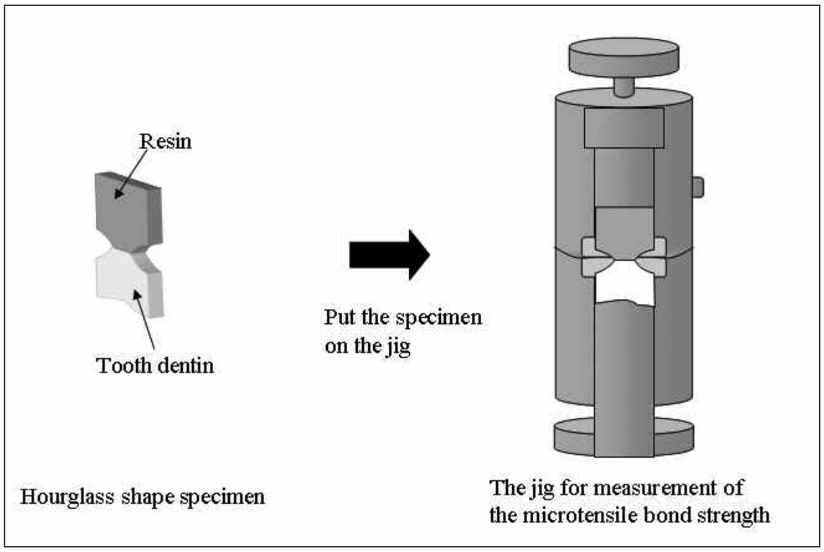

Figure 1. *Methods to measure the microtensile bond strength. An hour glass shape specimen was bonded to the jig with a cyanoacrylate adhesive and subjected to a tensile load.*

were collected under a protocol approved by the Ethics Committee of the Nihon University School of Dentistry, Japan. Informed consent was obtained from parents and patients before collecting the teeth. All teeth were used within 6 months of extraction. In order to obtain flat dentin surfaces, the buccal surfaces of the teeth were ground with a water-cooled air turbine using a 301 diamond bur (Shofu Inc, Kyoto, Japan), then they were abraded on wet 600-grit SiC paper to expose the middle dentin between the dentino-enamel junction and the pulp chamber wall. After ultrasonic cleaning with distilled water for 1 minute to remove excess debris, these surfaces were washed and dried with oil-free compressed air.

The adhesive was applied on the dentin surface according to the manufacturers' instructions and irradiated with the curing unit. A Teflon (Sanplatec Corp, Osaka, Japan) mold (height x diameter = 2.0 x 8.0 mm) was used to form and hold the resin composites to the dentin surface prior to curing for 40 seconds. The finished specimens were transferred to distilled water and stored at 37°C for 24 hours. After incubation, the specimens were sectioned at the widest section of the tooth crown using a diamond saw. The bonded slices were trimmed into an hour glass shape using a superfine diamond point under a constant water spray until a 1.0 x 1.0 mm bonded surface remained (Figure 1). Cross-sectional areas were individually measured to the nearest 0.01 mm for each specimen, using a digital caliper (500-151 CD-15C; Mitutoyo, 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), then subjected to a tensile load in an Instron testing machine (Type 4204; Instron Corp, Canton, MA, USA) at a crosshead speed of 1.0 mm/minute. 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 2.01; SPSS Inc, Chicago, IL, USA) using 2-way analysis of variance (ANOVA) in conjunction with the Tukey HSD post-hoc test. A probability (p) value of <0.05 was considered statistically significant.

Upon completion of the testing, the specimens were examined using an optical microscope (SZH-131; Olympus Ltd, Tokyo, Japan) at a magnification of 20x to define the location of the bond failure. The mode of failure for each specimen was then classified into 1 of 3 types: adhesive failure between restorative material and dentin, cohesive failure in adhesive resin and cohesive failure in dentin.

Scanning Electron Microscopy (SEM)

The treated dentin surface and restorative/dentin interface were observed by field-emission (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. For ultrastructural observation of the resin/dentin interface, bonded specimens were stored in distilled water at 37°C for 24 hours, then embedded in self-curing epoxy resin (Epon 812; Nisshin EM, Tokyo, Japan) at 37°C for 12 hours. The embedded specimens were vertically sectioned through the resin composite buildups and dentin, and the surfaces of the cut halves were polished with an Ecomet 4/Automet 2 (Buehler Ltd, Lake Bluff, IL, USA), using SiC papers of 600, 1,200 and 4,000-grit size, successively. The surface was finally polished with a special soft cloth and diamond paste (Buehler Ltd) with a grit size of 0.1 µm. All of the SEM specimens were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes and 100% for 2 hours), then transferred to a critical-point dryer for 30 minutes. These surfaces were then subjected to Argon-ion-beam etching (Type EIS-200ER, Elionix Ltd, Tokyo, Japan) for 30 seconds, with the ion beam (accelerating voltage = 1.0 kV; ion current density = 0.4 mA/cm^2) directed perpendicular to the polished surface. The surfaces were coated with a thin film of gold in a vacuum evaporator (Quick Coater Type SC-701; Sanyu Denshi Inc, Tokyo, Japan). The specimens were then observed using FE-SEM (ERA 8800 FE, Elionix Ltd).

RESULTS

The mean microtensile bond strengths and standard deviations for each adhesive system to primary and permanent dentin are listed in Table 3. The results of 2-way ANOVA showed that there was no statistically significant interaction between the type of adhesive system and the dentin substrate (p=0.957). Although there was no statistically significant difference in the mean values among the different adhesive systems (p=0.094), there was a statistically significant difference in the mean values among the different dentin substrates (p<0.001), which were lower for primary dentin (40.6 MPa for TSB and 44.7 MPa for OBF) than for permanent dentin (54.3 MPa for TSB and 50.0 MPa for OBF).

The failure modes were also independent of the type of dentin but dependent on the adhesive systems used. The fractured surfaces for most specimens using TSB showed an almost equal distribution among the 3 types of failure. However, OBF showed predominantly cohesive failure in the adhesives, followed by failure at the interface between dentin and the adhesives.

Figure 2 shows the representative SEM observations of the treated dentin surfaces. The smear layer and plugs were removed, and dentinal tubules were opened for both dentin substrates. Remnants on the treated dentin surfaces were more pronounced for primary than permanent dentin, indicating demineralization was more pronounced in primary dentin than in permanent dentin for both systems.

The representative SEM micrographs of the dentin/resin interface after argon-ion-beam etching are shown in Figure 3. For both adhesive systems, excellent adaptation to both dentin substrates was observed. A hybrid layer with a low resistance to argon-ion etching was not observed. No apparent morphological differences in the resin-dentin interface were observed for both

Table 3: *Microtensile Bond Strength (Mean (SD) in MPa) to Human Primary and Permanent Dentin*

Code	Tooth	μ-TBS	Tukey Group*	Mode of Fracture		
				A	R	D
TSB	Primary	44.7 (10.4)	a	4	3	3 = 10
	Permanent	54.3 (9.0)	b	4	4	2 = 10
OBF	Primary	40.6 (9.9)	a	2	7	1 = 10
	Permanent	50.0 (8.7)	b	2	7	1 = 10

SD: standard deviation, n=15.
**: Matching letters indicate no significant difference (p>0.05).*
Failure mode: A, adhesive failure; D, cohesive failure in dentin; R, cohesive failure in adhesive.

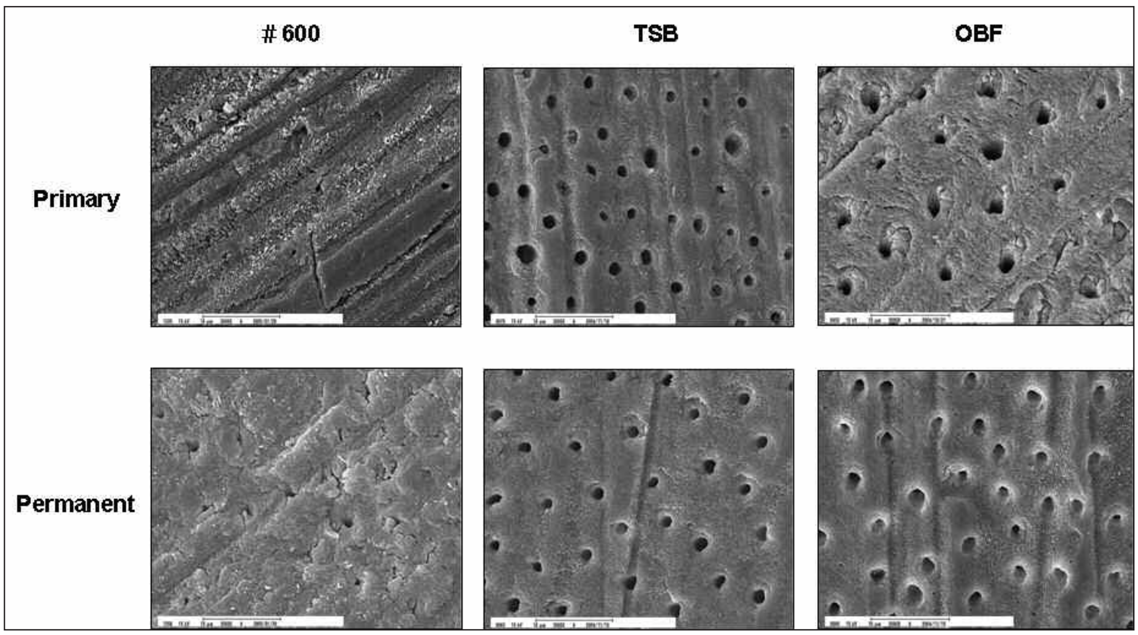

Figure 2. *SEM observations of the treated dentin surfaces of Clearfil tri-S Bond (TSB) and One-Up Bond F Plus (OBF). The smear layer and plugs were removed, and dentinal tubules were opened and remnants on the treated dentin surfaces were more pronounced for primary than for permanent dentin.*

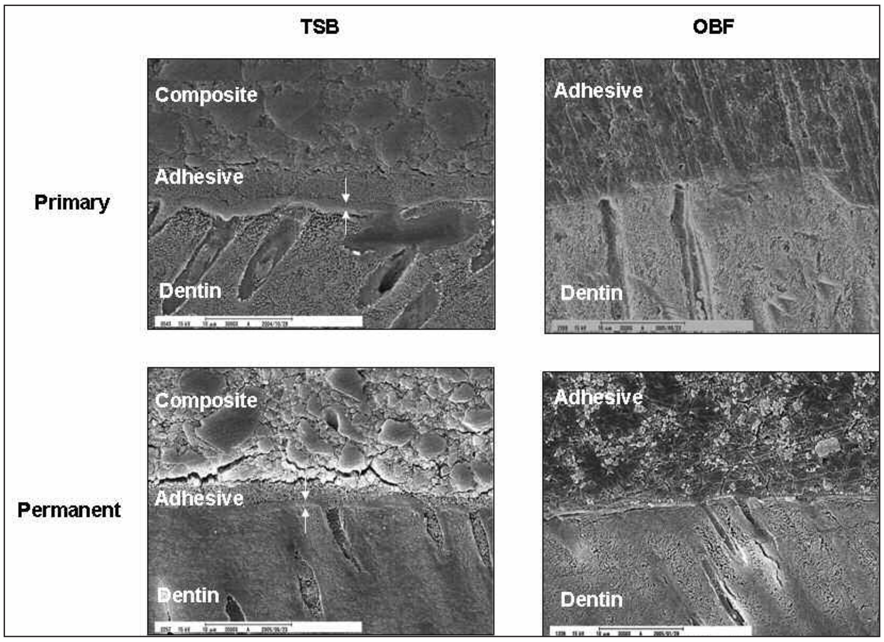

Figure 3. *SEM micrographs of the dentin/resin interface after argon-ion-beam etching. Excellent adaptation to both dentin substrates and no apparent morphological differences in the resin-dentin interface were observed for both dentin substrates.*

dentin substrates, irrespective of the type of adhesive systems used.

DISCUSSION

Previous studies on the suitability of newly developed adhesive systems for primary teeth have been complicated by the limited bonding area available for producing specimens. In this study, use of the microtensile bond-strength test has enabled the authors to evaluate bond strength on small areas of primary dentin. Obtaining multiple specimens from a single tooth is considered advantageous in cases where teeth are difficult to obtain. Each microtensile specimen is interpreted as a separate experimental unit, regardless of whether it is obtained from the same or different teeth.[11] The small adhesive interface used in the microtensile test contains fewer defects compared with larger interfaces, resulting in higher recorded bond strengths compared with other test methods that use larger surface areas.[12] Resistance against the initiation of crack growth and propagation depends on the geometry and load configuration of the specimen. Smaller specimens contain less initial defects and more homogeneous stress distributions; hence, bond strengths and adhesive failure between the bonding agent and dentin can be more accurately examined. Bond strength values can be used as a comparison of the effectiveness of bonding systems; however, they cannot be related directly to what might happen clinically. Care must be taken when comparing results from different testing procedures, because inappropriate conclusions might be drawn as a result of differences in stress-strain fields.[13]

Based on the results from this study, the microtensile bond strengths of primary dentin were lower than permanent dentin, and there were statistically significant differences. The differences in chemical composition and structural components between primary and permanent dentin might be related to differences in the microtensile bond strengths obtained. Due to the reduced mineral content of primary dentin compared to permanent dentin, a different effect of acidic primers on primary dentin has been suggested.[14] FE-SEM observation of the dentin surfaces to which single-step self-etch adhesive had been applied revealed that demineralization was slightly more pronounced in primary than in permanent dentin. The dentinal tubule and diameter of primary dentin have been reported to be greater than for permanent dentin, indicating a decrease in the dentin substrate available for bonding with adhesives. As peritubular dentin, which was demineralized rapidly during acid treatment, was thicker for primary than for permanent dentin, further decreases in the available bonding substrate might occur.

To create a stable bonding to dentin, the self-etch adhesive should penetrate beyond the smear layer into the underlying dentin. Current dentin-bonding systems rely on partial demineralization of the dentin surface by acidic monomers to remove the smear layer and expose collagen fibrils to penetration by resin monomers. The presence of the smear layer could affect penetration of the self-etch adhesive, due to neutralization of the acid monomers by buffering components.[15] However, another report suggested bond strength of the self-etch system was not affected by smear-layer thickness.[16] The smear layer is a porous substrate through which acidic functional monomers can diffuse to the basal portion of the layer and react with the mineralized dentin underneath. The depth to which the resin monomers can penetrate into dentin depends on the acidity of the adhesives, and there is no correlation between bond strength and thickness of the resin/dentin interaction zone. The thickness of this layer depends on the adhesive systems used, but variations in thickness have been observed between specimens prepared from different dentin substrates using the same adhesive system.[17-18]

The application of acidic self-etch adhesive should be sufficient to remove the smear layer and partially, but not excessively, demineralize the intertubular dentin. If the depth of demineralization is too aggressive to be replaced by resin monomers, a weak zone that allows nanoleakage[19] might be left at the bottom of the bonding interface.[20] Theoretically, single-step self-etch adhesives prevent discrepancies between the depth of demineralization and the ability of the resin monomer to penetrate the same depth of mineral removal. 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 and water-soluble hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA), so that the acidic monomer can dissociate and penetrate into hydrophilic dentin. The depth of demineralization during adhesive application depends on the type of acidic monomers, their concentration, the duration of application and the composition of the dentin. The adhesive TSB contains MDP, while OBF contains MAC-10 as an acidic functional monomer (Table 1). From a previous study[21] that compared the chemical bonding efficacy of functional monomers, MDP has been 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, was able to resist ultrasonic cleaning. According to the adhesion-decalcification concept,[22-23] the less soluble the calcium salt of the acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate. The higher and more stable bonding performance of MDP might be reflected in the thin, but clear presence of a resin/dentin interaction zone observed in the FE-SEM images of the MDP-based adhesive system TSB.

It has been demonstrated that bond strength should theoretically be proportional to the strength of the adhesive resin that infiltrates into the demineralized dentin.[24] Consequently, adhesive resins have lower tensile strengths than resin-infiltrated demineralized dentin.[25] Since 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.[26-27] The layers of adhesive and the resin/dentin interaction zone might act as strain-absorbing layers in an adhesive system.[28] The dentin/resin interface is comprised of several layers of materials with differing elastic properties. The elastic moduli of the successive layers across a resin/dentin bonding area was determined by the ultrasonic technique,[29] and a gradient of elastic modulus was observed from the relatively stiff dentin through to a more flexible hybrid layer and through the bonding agent layer to the stiffer resin composite. An elastic resin, together with the resin-impregnated dentin, might relieve stresses induced by the polymerization shrinkage of resin composites, thereby improving the marginal integrity and retention of the restoration.[30] Adequate bonding of the resin composite to dentin depends not only on penetration of the adhesive resin into dentin to create a resin/dentin interaction zone, but also on the mechanical properties of the cured adhesive. Further studies are required to determine the mechanical properties of adhesive systems that produce a thin adhesive layer.

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 was not strongly air dried, leading to a thicker adhesive layer (~60-80 µm). Although the applied adhesive was thicker than that of TSB, the remaining solvents, such as water, do not appear to be an obstacle for the polymerization of OBF. This is presumably caused by the excellent polymerization ability of the dye-sensitized photopolymerization system employed in this adhesive. OBF's initiator system contains dye-sensitizer, co-initiator and the borate derivative. The energy transfer reaction from the dye-sensitizer to the co-initiator takes place by light irradiation to form an excited state of the co-initiator. Following this, the polymerizable radical species is formed by reaction of the borate derivative with the activated co-initiator containing hydrogen ions derived from the dye-sensitizer and acidic functional monomers.[31] However, air drying is essential for obtaining adequate dentin bond strengths for TSB. From the FE-SEM pictures of the resin/dentin interface, the difference in adhesive thickness was obvious.

In the microtensile bond-strength test, the applied force is transmitted through the body of the dentin and resin, so that partial cohesive failure, rather than interfacial failure, often occurs. The dentin-bonding system comprises 3 materials, with each layer showing different mechanical properties. Crack resistance is a function of the plastic behavior of the material at the site of the crack tip and fracture characteristics. When placed under tension, the fracture will pass through the weakest areas in the bulk of the adhesive area or interface.[32] The hardness and elastic modulus of the primary dentin were lower than that of permanent dentin.[7] Dentin is a mineralized tissue that lies between enamel and pulp and consists of a hydrated matrix of type I collagen reinforced with hydroxyapatite crystallites.[33] The microstructure of dentin is dominated by the presence of dentinal tubules that transverse the entire dentin thickness and occupy ~10% of the total dentin volume.[34] Dentinal tubules are oriented in dentin from the pulp chamber to the dentin/enamel junction. The density of tubules increases from ~4vol% near the dentin/enamel junction to ~16vol% near the pulp chamber.[35] The shear strength of dentin differs in the central and cusp areas and is dependent on dentinal tubule orientation in the central area.[36] However, tubule orientation has no appreciable effect on the elastic behavior of normal dentin, as intertubular dentin governs the elastic behavior of the dentin substrate.[37] Any increase in the elastic modulus is offset by the decrease in the volume fraction of the matrix phase.

The results of this investigation reject the null hypothesis that microtensile bond strength does not differ between primary and permanent dentin. The microtensile bond strengths of single-step self-etch systems were found to be lower for primary dentin than for permanent dentin. Further research is needed to determine the long-term bonding ability and clinical performance of single-step self-etch restorative systems.[38]

CONCLUSIONS

Within the limitations of this *in vitro* study, the following conclusions can be drawn:

1. The microtensile bond strengths of single-step self-etch adhesives (Clearfil tri-S Bond and One-Up Bond F Plus) to primary dentin were significantly lower than those obtained for permanent dentin ($p<0.001$).
2. There was no statistically significant difference in mean microtensile bond strengths among the different adhesive systems ($p=0.094$).
3. From the SEM observations of adhesive-applied dentin surfaces, the smear layer and plugs were removed and the dentinal tubules were opened with the application of both adhesive systems. This effect was similar for both types of dentin substrate.
4. For TSB, formation of a thin resin/dentin interaction zone was observed, which was more easily distinguished for Clearfil tri-S Bond than for One-Up Bond F Plus.

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References

1. 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 *Operative Dentistry* **28(3)** 215-235.
2. Miyazaki M, Onose H & Moore BK (2002) Analysis of the dentin-resin interface by use of laser Raman spectroscopy *Dental Materials* **18(8)** 576-580.
3. Miyazaki M, Onose H, Iida N & Kazama H (2003) Determination of residual double bonds in resin-dentin interface by Raman spectroscopy *Dental Materials* **19(3)** 245-251.
4. el-Kalla IH & García-Godoy F (1998) Bond strength and interfacial micromorphology of compomers in primary and permanent teeth *International Journal of Pediatric Dentistry* **8(2)** 103-114.
5. Hosoya Y, Kawashita Y, Yoshida M, Suefuji C & Marshall GW Jr (2000) Fluoridated light-activated bonding resin adhesion to enamel and dentin: Primary vs permanent *Pediatric Dentistry* **22(2)** 101-106.
6. Burrow MF, Nopnakeepong U & Phrukkanon S (2002) A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin *Dental Materials* **18(3)** 239-245.
7. Hosoya Y & Marshall GW (2005) The nanohardness and elastic modulus of sound deciduous canine dentin and young premolar dentin-preliminary study *Journal of Materials Science: Materials in Medicine* **16(1)** 1-8.
8. Nör JE, Feigal RJ, Dennison JB & Edwards CA (1996) Dentin bonding: SEM comparison of the resin-dentin interface in primary and permanent teeth *Journal of Dental Research* **75(6)** 1396-1403.
9. Hosoya Y, Kawashita Y, Marshall GW Jr & Goto G (2001) Influence of Carisolv for resin adhesion to sound human primary dentin and young permanent dentin *Journal of Dentistry* **29(3)** 163-171.
10. Koutsi V, Noonan RG, Horner JA, Simpson MD, Matthews WG & Pashley DH (1994) The effect of dentin depth on the permeability and ultrastructure of primary molars *Pediatric Dentistry* **16(1)** 29-35.
11. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA & Tay F (1999) The microtensile bond test: A review *Journal of Adhesive Dentistry* **1(4)** 299-309.
12. Baran GR, McCool JI, Boberick KG & Zhang HQ (1999) Size effect in resin/glass composite flexure strengths *Journal of Oral Rehabilitation* **26(10)** 775-780.
13. Loguerico AD, Barroso LP, Grande RH & Reis A (2005) Comparison of intra- and intertooth resin-dentin bond strength variability *Journal of Adhesive Dentistry* **7(2)** 151-158.
14. Sumikawa DA, Marshall GW, Gee L & Marshall SJ (1999) Microstructure of primary tooth dentin *Pediatric Dentistry* **21(7)** 439-444.
15. Kenshima S, Reis A, Uceda-Gomez N, Tancredo L de L, Filho LE, Nogueira FN & Loguercio AD (2005) Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin *Journal of Adhesive Dentistry* **7(2)** 117-126.
16. Tay FR, Sano H, Carvalho R, Pashley EL & Pashley DH (2000) An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin *Journal of Adhesive Dentistry* **2(2)** 83-98.

17. Vargas MA, Cobb DS & Denehy GE (1997) Interfacial micromorphology and shear bond strength of single-bottle primer/adhesives *Dental Materials* **13(5)** 316-324.

18. Prati C, Chersoni S, Mongiorgi R & Pashley DH (1998) Resin-infiltrated dentin layer formation of new bonding systems *Operative Dentistry* **23(4)** 185-194.

19. Sano H, Takatsu T, Ciucci B, Horner JA, Matthews WG & Pashley DH (1995) Nanoleakage: Leakage within the hybrid layer *Operative Dentistry* **20(1)** 18-25.

20. Sato M & Miyazaki M (2005) Comparison of depth of dentin etching and resin infiltration with single-step adhesive systems *Journal of Dentistry* **33(6)** 475-484.

21. 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 *Journal of Dental Research* **83(6)** 454-458.

22. Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, Lambrechts P, Vanherle G & Okazaki M (2001) Adhesion to and decalcification of hydroxyapatite by carboxylic acids *Journal of Dental Research* **80(6)** 1565-1569.

23. 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 *Journal of Biomedical Materials Research* **59(1)** 56-62.

24. Pashley DH, Ciucchi B, Sano H, Carvalho RM & Russell CM (1995) Bond strength versus dentine structure: A modeling approach *Archives of Oral Biology* **40(12)** 1109-1118.

25. Sano H, Takatsu T, Ciucchi B, Russell CM & Pashley DH (1995) Tensile properties of resin-infiltrated demineralized human dentin *Journal of Dental Research* **74(4)** 1093-1102.

26. Takahashi A, Sato Y, Uno S, Pereira PN & Sano H (2002) Effects of mechanical properties of adhesive resins on bond strength to dentin *Dental Materials* **18(3)** 263-268.

27. Yamada M, Miyazaki M & Moore BK (2004) Influence of interchanging adhesive resins and self-etching primers on the mechanical properties of adhesive resins *Operative Dentistry* **29(5)** 532-537.

28. Kemp-Scholte CM & Davidson CL (1990) Complete marginal seal of Class V resin composite restorations effected by increased flexibility *Journal of Dental Research* **69(6)** 1240-1243.

29. Watanabe T, Miyazaki M, Inage H & Kurokawa H (2004) Determination of elastic modulus of the components at dentin-resin interface using the ultrasonic device *Dental Materials Journal* **23(3)** 361-367.

30. Perdigão J, May KN Jr, Wilder AD Jr & Lopes M (2000) The effect of depth of dentin demineralization on bond strengths and morphology of the hybrid layer *Operative Dentistry* **25(3)** 186-194.

31. Miyazaki M, Iwasaki K & Onose H (2002) Adhesion of single application bonding systems to bovine enamel and dentin *Operative Dentistry* **27(1)** 88-94.

32. Wiskott HWA, Nicholls JI & Belser UC (1995) Stress fatigue: Basic principles and prosthodontic implications *International Journal of Prosthodontics* **8(2)** 105-116.

33. Marshall GW Jr, Marshall SJ, Kinney JH & Balooch M (1997) The dentin substrate: Structure and properties related to bonding *Journal of Dentistry* **25(6)** 441-458.

34. Garberoglio R & Brännström M (1976) Scanning electron microscopic investigation of human dentinal tubules *Archives of Oral Biology* **21(6)** 355-362.

35. Pashley DH (1996) Dynamics of the pulpo-dentin complex *Critical Reviews in Oral Biology and Medicine* **7(2)** 104-133.

36. Watanabe LG, Marshall GW Jr & Marshall SJ (1996) Dentin shear strength: Effects of tubule orientation and intratooth location *Dental Materials* **12(2)** 109-115.

37. Kinney JH, Balooch M, Marshall GW & Marshall SJ (1999) A micromechanics model of the elastic properties of human dentine *Archives of Oral Biology* **44(10)** 813-822.

38. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005) A critical review of the durability of adhesion to tooth tissue: Methods and results *Journal of Dental Research* **84(2)** 118-132.