©Operative Dentistry, 2010, **35-5,** 508-514

Laboratory Research

Influence of Storage Conditions of Adhesive Vials on Dentin Bond Strength

Y Shibuya-Chiba • M Iwasa • K Tsubota
M Miyazaki • H Hirose • JA Platt

Clinical Relevance

Short-term storage at low temperatures enhances the dentin bond strength of single-step self-etch adhesives.

SUMMARY

This study was carried out to examine the effect of storage conditions of adhesive vials on the dentin bond strength of single-step self-etch adhesive systems. The adhesive/resin composite combinations used were: Absolute 2/Ceram·X(AB), Adper Prompt L-Pop/Filtek Supreme(AP), Bond Force/Estelite Σ Quick(BF), Clearfil tri-S Bond/Clearfil AP-X(CT) and G-Bond/Gradia Direct(GB). Vials of adhesives were stored at 5°C, 23°C or 40°C. Specimens for the dentin bond strength tests were made after 0, 1, 2, 3, 4, 5 and 6 months. Labial bovine mandibular incisor dentin was wet ground with #600 SiC. The adhesives were applied according to the manufacturer's instructions. After adhesive light irradiation, resin composite cylinders were created (4 mm x 2 mm) and polymerized (n=10). Samples were stored in 37°C distilled water for 24 hours, then shear tested at a crosshead speed of 1.0 mm/minute. ANOVA and Dunnet tests were performed at a significance level of 0.05. Scanning electron microscopic (SEM) observations of the dentin surfaces were made. Bond strengths varied, with storage conditions ranging from 2.2 ± 1.4 to 9.3 ± 2.4 MPa for AB, 4.5 ± 1.5 to 13.3 ± 2.7 MPa for AP, 5.1 ± 1.9 to 5.1 ± 1.9 MPa for BF, 7.7 ± 1.9 to 19.7 ± 2.0 MPa for CT and 7.4 ± 1.3 to 15.7 ±

Yoko Shibuya-Chiba, DDS, Nihon University School of Dentistry, Department of Operative Dentistry, Tokyo, Japan

Mika Iwasa, DDS, Nihon University School of Dentistry, Department of Operative Dentistry, Tokyo, Japan

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

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

Hideharu Hirose, PhD, Nihon University School of Dentistry, Dental Materials, Tokyo, Japan

Jeffrey A Platt, DDS, MS, professor and director of the Dental Materials Division, Department of Restorative Dentistry, Indiana University School of Dentistry, Indianapolis, IN, USA

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

DOI: 10.2341/09-367-L

2.8 MPa for GB. With longer storage periods and higher temperatures, significant decreases in bond strength were found for all the adhesives. From SEM observation, the etching effect of the adhesives was weakened and the remaining smear layer was observed. The data suggests that the storage conditions of adhesive vials significantly affects the bond strengths of single-application self-etch adhesive systems.

INTRODUCTION

Conventional dentin bonding systems require sequential application of the conditioner, dentin primer and bonding agent in several clinical steps. To reduce technique sensitive factors that affect bond strength, a self-etch approach, involving either single or two-step application, has been developed.[1] The application methods of newly available adhesive systems have been simplified and manufacturers' instructions have been clarified to achieve optimum clinical results. Recently introduced single-step self-etch adhesives combine all three bonding steps into a single-application. Self-etch adhesives are applied to the tooth surface prior to resin composite placement to ensure maximum adhesion by improving monomer penetration into the hydrophilic dentin substrate and to improve wettability of the tooth surface by the resin components. In these adhesives, both the primer and bonding resin components are incorporated in a single vial along with water, ethanol or acetone.[2] Although these adhesives reduced the number of steps in the bonding procedure, relatively lower bond strengths were obtained when compared to two- and three-step adhesives.[3]

While the system becomes simpler, careful management of the products is still required to obtain an optimum bonding performance.[3] Since self-etch adhesives contain acidic functional monomers, methacrylate comonomers and water, acidified water can attack the ester bonds in monomers under acidic conditions.[4-5] Hydrolysis of the ingredients in the adhesive leads to a change in concentration of the components over the period of storage in a dental office. The reason for not achieving optimal bonding performance might be due to poor clinical procedures or a poor shelf life of the single-step self-etch adhesives.[6] Hydrolysis of the adhesive monomers changes the chemical composition and its mechanical properties. It has been reported that the storage stability of 10-methacryloyloxydecyl dihydrogen phosphate (MDP)-based adhesives exhibit deteriorated performance over time, even though they are stored according to the manufacturer's recommendations.[7]

There are many factors affecting the performance of adhesive systems, and material-related factors, such as storage conditions to extend shelf life, can affect the bonding performance of the materials. Manufacturers recommend keeping the adhesive systems in a lower temperature condition and using them before the expiration date. Proper storage conditions of single-step self-etch adhesives play an important role in gaining good bonding performance, but the influence of storage conditions of adhesive vials has not been well explored. The current study evaluated the shear bond strength to bovine dentin of single-step self-etch adhesives over six months at three different storage temperatures. The three storage environments employed in the current study were selected from among the available laboratory environments with fixed temperatures and according to previous related studies.[6-7] Field emission scanning electron microscopy (FE-SEM) observation of the resin/dentin interface was also conducted. The null hypothesis tested was that the dentin bond strength of single-step self-etch adhesives is not affected by the storage conditions of temperature and time.

METHODS AND MATERIALS

Materials Tested

The combinations of single-application self-etch adhesive systems and resin composites used in the current study were Absolute 2/Ceram·X (AB, Dentsply Sankin KK, Tokyo, Japan), Adper Prompt L-Pop/Filtek Supreme (AP, 3M ESPE, St Paul, MN, USA), Bond Force/Estelite Σ (BF, Tokuyama Dental Corp, Tokyo, Japan), Clearfil tri-S Bond/Clearfil AP-X (CT, Kuraray Medical Inc, Tokyo, Japan) and G-Bond/Gradia Direct (GB, GC Corp, Tokyo, Japan), as listed in Table 1. All adhesive systems were used in combination with the manufacturers' restorative resins.

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

Bond Strength Test

For each adhesive, three sets of the same lot number were used. All adhesive vials were kept in a refrigerator until the experiment. The bond strength specimen preparation was performed immediately after opening each vial, and the obtained dentin bond strength data were recorded as the baseline. One set of vials for each adhesive were then stored in the refrigerator at 5 ± 1°C, as recommended by the manufacturers, one set was stored in an air-conditioned laboratory room away from light at 23 ± 1°C and another set was stored in an environmental test chamber (SH-220S3, Tabai Espec Corp, Osaka, Japan) at 40 ± 1°C. The bond strength test specimens were repeated after 0, 1, 2, 3, 4, 5 and 6 months of storage.

Nine-hundred and fifty mandibular incisors extracted from two-to-three year old cattle and stored frozen for up to two weeks were used as a substitute for human

Table 1: *Materials Tested*

Code	Adhesive (Manufacturer)	Main Components	Lot #	Restorative (Shade)	Lot #
AB	Absolute 2 (Dentsply Sankin)	Methacrylate ester, fluoride compound, anhydrous silicic acid, acetone, water	420-004	Ceram·X (A2)	020135
AP	Adper Prompt L-Pop (3M ESPE)	Methacrylated phosphoric esters, bis-GMA, CQ, HEMA, stabilizer, polyalkenoic acid, water	252087	Filtek Surpeme (A2B)	3AU
BF	Bond Force (Tokuyama Dental)	Bis-GMA, phosphoric acid monomer, HEMA, TEGDMA, ethanol, CQ, water	012M	Estelite Σ (A2)	J279
CT	Clearfil Tri-S Bond (Kuraray Medical)	MDP, bis-GMA, HEMA, ethanol, water, colloidal silica, CQ, initiators, accelerators	00080A	Clearfil AP-X (A2)	00987A
GB	G-Bond (GC)	4-MET, UDMA, TEGDMA, water acetone, phosphoric acid monomer, silanated colloidal silica, initiator	0612201	Gradia Direct (A2)	0407051

HEMA: 2-hydroxyethyl methacrylate, bis-GMA: 2,2bis[4-(2-hydrogen-3-methacryloyloxypropoxy)phenyl]propane; HEMA: 2-hydroxyethyl methacrylate; CQ: dl-camphorquinone MDP; 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA: triethyleneglycol-dimethacrylate; UDMA: di(methacryloxyethyl)trimethylhexamethylene diurethane.

teeth.[8-9] After removing the roots with a low-speed saw (Isomet, Buheler Ltd, Lake Bluff, IL, USA), the pulps were removed and the pulp chamber of each tooth was filled with cotton to avoid penetration of the embedding media. The labial surfaces of bovine incisors were ground on wet 240-grit SiC paper to a flat dentin surface. Each tooth was then mounted in cold-cure acrylic resin (Tray Resin II, Shofu Inc, Kyoto, Japan) to expose the flattened area, and they were placed in tap water to reduce the temperature rise from the exothermic polymerization reaction. The final finish was accomplished by grinding on wet 600-grit SiC paper. After ultrasonic cleaning with distilled water for one minute to remove excess debris, these surfaces were washed and then dried with oil-free compressed air.

A piece of double-sided adhesive tape, which had a 4-mm diameter hole, was firmly attached to define the adhesive area of the dentin for bonding. The adhesive was applied on the dentin surface according to the manufacturers' instructions (Table 2). Primed dentin surfaces were dried using oil-free compressed air, with an air pressure of 0.2 MPa for 10 seconds from 5 cm above the dentin surface using a three-way syringe. The dentin surfaces were then irradiated with the curing unit. A Teflon (Sanplatec Corp, Osaka, Japan) mold, 2.0 mm high and 4.0 mm in diameter, was used to form and hold the restorative resin onto the dentin surface. The resin composite was condensed into the mold and cured for 40 seconds. The finished specimens were transferred to distilled water and stored at 37°C for 24 hours.

Ten specimens per group were tested in a shear mode using a shear knife-edge testing apparatus in an Instron testing machine (Type 4204, Instron Corp, Canton, MA, USA) at a crosshead speed of 1.0 mm/minute. The shear bond strength values in MPa were calculated from the peak load at failure divided by the specimen surface area. After testing, the specimens were examined in an optical microscope (SZH-131, Olympus Ltd, Tokyo, Japan) at a magnification of 10x to define the location of the bond failure. The type of failure was determined based on the percentage of

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

Code	Application Protocol
AB	Dispense one drop of liquid into well. Apply to moist dentin for three seconds twice. Subject to a mild stream of air to dry for three seconds and light irradiate for 10 seconds.
AP	Squeeze the material from the reservoirs and mix applying a churning motion to the applicator for five seconds. Apply adhesive to dentin, rubbing in the solution for 15 seconds. Subject to a mild stream of air and apply a second coat of adhesive. Subject to a mild stream of air and light irradiate for 10 seconds.
BF	Dispense one drop of liquid into the well. Apply to dried dentin for 20 seconds. Subject to a mild stream of air to dry for five seconds, then blow the surface with strong air for five seconds. Perform light irradiation for 10 seconds.
CT	Dispense one drop of liquid into the well. Apply to dried dentin for 20 seconds. Subject to high pressure air for five seconds to dry and light irradiate for 10 seconds.
GB	Dispense one drop of liquid into the well. Apply dried dentin for 10 seconds. Dry thoroughly for five seconds under maximum air pressure. Light irradiate for 10 seconds.

substrate-free material as follows: adhesive failure, mixed failure (cohesive failure in composite and adhesive resin with partial adhesive failure), cohesive failure in dentin and cohesive failure in composite.

Statistical Analysis

The statistical analysis was done to show how the bond strengths were influenced by storage temperature and its duration. The data for each group were subjected to ANOVA and Dunnet tests at a significance level of 0.05 within each adhesive system. The statistical analysis was carried out with the Sigma Stat software system (Ver 3.1, SPSS Inc, Chicago, IL, USA).

FE-SEM

For ultrastructure observation of the self-etch adhesive treated dentin surfaces by FE-SEM, the specimens were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes and 100% for two hours); they were then transferred to a critical-point dryer. The surfaces were coated in a vacuum evaporator, Quick Coater Type SC-701 (Sanyu Denshi Inc, Tokyo, Japan), with a thin film of Au. The specimens were observed in FE-SEM (ERA-8800FE, Elionix Ltd, Tokyo, Japan) at an accelerating voltage of 15 kV.

RESULTS

Results of the shear bond strength tests with the different storage conditions of adhesive vials are shown in Table 3. The dentin bond strengths varied with the different storage conditions and ranged from 9.3 to 2.2 MPa for AB, 13.3 to 4.5 MPa for AP, 18.4 to 5.1 MPa for BF, 19.7 to 7.7 MPa for CT and 15.7 to 7.4 for GB. The bond strengths of each bonding system were significantly lower (p<0.05) when the adhesive vials were stored for longer periods of time and this tendency was more pronounced for higher storage temperatures. The mode of failure seemed to have some relation to the bond strength data. The predominant mode of failure was mixed failure for specimens made with shorter storage periods of adhesive vials, and they changed to adhesive failure for specimens made with longer storage periods of adhesive vials. This tendency was more pronounced for higher storage temperatures.

Figure 1 shows the results of FE-SEM observations of dentin surfaces treated with adhesive vials stored in different conditions (baseline and six months storage at 40°C). The smear layer and smear plugs were removed for all the systems at the baseline stage. Demineralization of the dentin surface was less pronounced with longer storage times.

Table 3: *Shear Bond Strength (Mean (SD) in MPa) to Bovine Dentin*

AB	**Baseline**	**1 Month**	**2 Months**	**3 Months**	**4 Months**	**5 Months**	**6 Months**
5°C	9.3 (2.4)	9.0 (1.8)	7.8 (1.9)	7.8 (2.6)	7.6 (2.8)	7.6 (2.7)	7.5 (1.3)
23°C		6.6 (1.8)*	6.5 (1.6)*	5.1 (2.3)*	4.9 (2.4)*	4.7 (1.7)*	4.1 (1.4)*
40°C		4.7 (3.2)*	4.6 (1.0)*	3.6 (1.3)*	3.1 (1.1)*	2.9 (1.7)*	2.2 (1.4)*
AP	**Baseline**	**1 Month**	**2 Months**	**3 Months**	**4 Months**	**5 Months**	**6 Months**
5°C	13.3 (2.7)	13.0 (2.5)	12.0 (2.2)	11.9 (2.2)	11.6 (2.1)	11.6 (1.5)	11.0 (1.5)
23°C		12.5 (1.8)	12.3 (2.3)	11.8 (1.3)	12.0 (1.4)	11.3 (1.9)	9.2 (1.2)*
40°C		9.6 (3.4)	10.0 (2.0)	9.7 (2.9)	8.1 (1.5)*	6.5 (1.2)*	4.5 (1.5)*
BF	**Baseline**	**1 Month**	**2 Months**	**3 Months**	**4 Months**	**5 Months**	**6 Months**
5°C	18.4 (2.4)	18.2 (2.0)	18.3 (2.0)	18.0 (1.6)	17.3 (2.0)	16.5 (2.6)	16.2 (1.8)
23°C		17.9 (2.5)	17.0 (1.6)	17.0 (1.6)	15.4 (2.4)*	14.5 (1.6)*	14.0 (1.8)*
40°C		11.3 (2.6)*	8.4 (1.9)*	7.4 (1.7)*	6.5 (2.4)*	5.9 (1.1)*	5.1 (1.9)*
CT	**Baseline**	**1 Month**	**2 Months**	**3 Months**	**4 Months**	**5 Months**	**6 Months**
5°C	19.7 (2.0)	18.7 (1.8)	18.5 (1.9)	17.5 (2.5)	17.1 (2.0)	17.1 (1.4)	17.0 (2.1)
23°C		18.1 (2.7)	18.0 (2.3)	17.7 (2.5)	17.6 (2.2)	16.1 (1.8)*	15.7 (1.6)*
40°C		12.6 (2.5)*	10.6 (2.9)*	10.7 (2.4)*	10.6 (2.2)*	8.5 (1.4)*	7.7 (1.9)*
GB	**Baseline**	**1 Month**	**2 Months**	**3 Months**	**4 Months**	**5 Months**	**6 Months**
5°C	15.7 (2.8)	15.4 (2.0)	15.3 (1.9)	14.5 (1.4)	14.5 (1.5)	14.0 (1.8)	12.3 (1.6)*
25°C		15.1 (2.1)	14.7 (1.8)	14.1 (1.9)	13.3 (2.5)	12.7 (1.6)*	11.8 (1.8)*
40°C		13.1 (2.0)*	12.3 (1.9)*	11.2 (1.9)*	11.0 (2.4)*	9.6 (1.7)*	7.4 (1.3)*

SD: standard deviation, N=10
Values with the asterisks in each adhesive system are significantly different from the baseline value (Dunnett test, p*<0.05).*

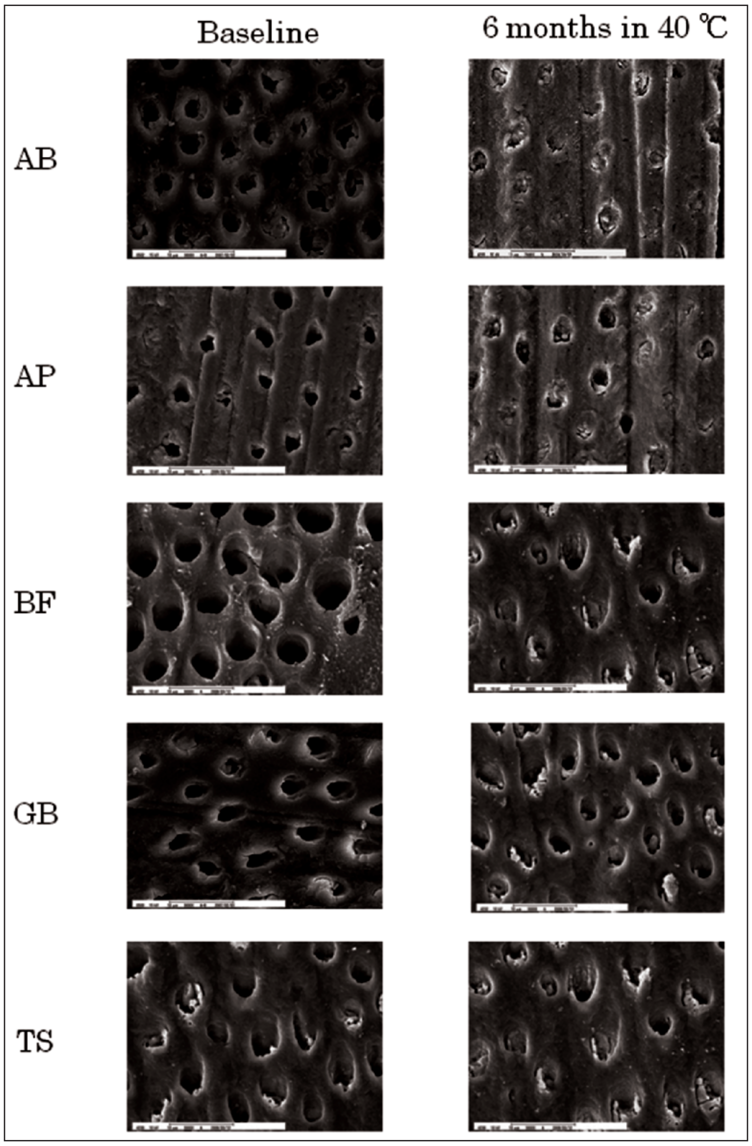

Figure 1. *SEM observation of adhesive dentin surfaces treated with different storage conditions of adhesive. After storage for a longer duration in higher storage temperature, slight surface alterations of the dentin surface with the remaining smear layer were observed for all the adhesives tested.*

DISCUSSION

A large number of intact extracted teeth are required for conducting bond strength tests; however, it is difficult to obtain enough extracted human teeth in Japan. It has been reported that adhesion to the superficial layer of dentin showed no significant differences between human and bovine dentin, and the dentin bond strength decreased with the depth of dentin because of the lower density of dentinal tubules.[8] Because differences in tubule diameters and the number of lateral branches may have some effect on dentin bond strength, bovine superficial dentin was used as a substitute for human dentin in the current study, as reported in previous studies.[8-9] Experimental approaches to the measurement of adhesive bond strengths in dentistry have consisted primarily of tensile or shear bond strength determinations performed within a defined area. Although the testing procedures used are apparently similar, the results presented in different studies may differ tremendously.[10] Care should be taken when drawing conclusions from the bond strength data, since there are many factors that affect the bond values.

The etching effect of self-etch adhesives 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 the simultaneous demineralization and resin penetration.[11] Single-step self-etch adhesives have to contain water and water-soluble hydrophilic monomers, such as 2-hydroxyethyl methacrylate (HEMA), so that the acidic monomer can dissociate and penetrate into the hydrophilic dentin.[12] Water is an essential component in adhesives to generate the hydrogen ions required for effective dissolution and demineralization. Protons in solutions derived from the acidic monomer interact with the mineral component of the tooth substrate.[13]

From the results of the current study, different trends in dentin bond strengths were observed for self-etch adhesives stored in different temperature conditions. When the storage temperature of the adhesive vials increased to 40°C, bond strength degradation occurred for all the materials tested. When compared to Absolute, tri-S Bond and G-Bond exhibited relatively stable bond strengths when the adhesive vials were stored at room temperature. The degradation tendency of the adhesives was related to the temperature and storage durations that are likely related to dissociation of the phosphoric or carboxylic acids in functional monomers. It has been reported that the functional monomer MDP has an ability to resist degradation longer than 4-MET under higher temperature conditions.[14] Since MDP has a longer carbonyl chain in its structure, this monomer shows a more hydrophobic nature that is relatively hydrolysis stable. Because the aromatic group of 4-MET is hydrophobic, it is also difficult to solvate in water. This monomer is soluble in acetone and moderately soluble in ethanol. When 4-MET is put into an ethanol solution, the etching potential of the self-etch adhesive should decrease, since the carboxylic acid in 4-MET is esterified.[15] However, the methacryloxy ester portion in 4-MET would be hydrolyzed because of the dissociation of carboxylic acid in 4-MET.[5] For Adper Prompt, dentin bond strength was relatively stable, even for storage in higher temperature conditions. This adhesive has two different fluid-containing chambers that were mixed with each other just before application. Compared to one-vial adhesives, degradation of adhesive seems to be retarded and bond strength degradation was not evident during the first three months of storage in higher temperature conditions.

The degree of dissociation of acidic functional monomers is dependent on the pKa value of those monomers and it is directly related to the etching effect of the tooth surface by them. It has been reported that the demineralization potential of an acidic functional monomer is greater than that with a carboxylic acid, because of the pKa values of these acids.[16] For adhesive systems using carboxylic acid, such as 4-MET, hydrolysis of the methacryloyl ester portion of the acidic monomer might occur and, in addition, loss of function might occur via esterification of the carboxyl group of the acidic functional monomer. Single-step self-etch adhesive contains water, acidic functional monomers and methacrylate monomers, such as HEMA, TEGDMA and bis-GMA in the same vial. Under such acidic conditions, ester bonds in resin monomers are easily attacked, then free phosphoric or carboxylic acid can be found. This kind of hydrolysis may lead to a decrease in the concentration of acidic functional monomers over the duration of storage in higher temperature conditions. It has been reported that the shelf life stability problem of adhesives is not limited to acidic functional monomers; HEMA is also prone to degradation.[17] This monomer acts as a wetting agent that helps resin monomers diffuse into the dentin substrate and it has an ability to react with collagen fibrils. Hydrolysis of the monomers changes their chemical composition, leading to loss of their clinical function with prolonged storage.

As described previously, methacrylate monomers are susceptible to hydrolysis in aqueous solutions. Not only the ester-groups of acrylates hydrogel, but also the phosphate and carboxyl groups in acidic functional monomers might be vulnerable to hydrolysis in the presence of water.[18] On the other hand, the hydrolysis of resin monomers would decrease the concentration of water in the adhesive solution. A decrease in water concentration results in less ionization of the acidic functional monomers that remain in the solution, thereby lowering the ability to etch tooth substrate. This phenomenon was shown in FE-SEM pictures of adhesive-treated dentin surfaces (Figure 1).

When considering the durability of dentin bonding, the presence of exposed collagen without resin infiltration is questioned, since this layer is thought to be susceptible to proteolytic degradation.[19] These monomers should be sufficiently supplied to infiltrate into demineralized dentin, including the exposed collagen fibril network and should establish a hybrid layer.[20] Adequate bonding of adhesive materials to dentin not only depends on adequate penetration of the adhesive into dentin, but also on the mechanical properties of the cured adhesive. Hypothetically, stronger resins might lead to stronger bonding to dentin.[21] The strength of cured adhesive resin is dependent on the composition, degree of conversion and length of polymer chains. Unreacted resin monomer remaining in adhesive resins may alter their mechanical properties. Thus, evaluation of the mechanical properties of adhesive resin is of importance to the durability of bonding to dentin.[22-23]

The adhesives in the current study were cured by a free radical polymerization reaction and photoinitiator, such as dl-camphorquinone (CQ).[24] CQ requires coinitiators for an effective polymerization process to occur, and a tertiary amine photoreductant is employed. Tertiary amine interacts with an activated triplet state CQ to form an intermediate excited complex followed by the production of reactive radicals for polymerization. There is a possibility that the polymerization ability of resin monomer is affected by acidic moieties, since tertiary amines in adhesive resins might be neutralized by acidic functional monomers in self-etch adhesives. It has been reported that adhesive functional monomers affect the polymerization of BPO/amine or even CQ/amine catalysts, resulting in poor polymerization and reduced mechanical properties.[25] To improve polymerization in the presence of acidic monomers, accelerators, such as aromatic sulfinic acid sodium salts, have been incorporated together with initiator systems.[26]

Considering the findings of the current study, the benefits to using single-step self-etch adhesives in terms of simplifying the clinical procedure might be negated by the storage conditions of adhesive vials, which can lead to an alteration in bond strength. The dentin bond strengths of single-step self-etch adhesives decreased when their storage temperature was increased and their storage periods were prolonged. This tendency of measured dentin bond strength values was due to degradation of the etching effect on the dentin substrate by self-etch adhesives during their storage period. Chemical reactions have been attributed to deterioration of the adhesive over time and are accelerated by higher temperatures. It seems that temperatures above room temperature may be above the tolerance limit for adhesive performance to a dentin substrate. Adhesive formulations are important, because acidic functional monomers are the key components of single-step self-etch adhesives, they provide for demineralization and functioning in polymerization of the polymer network, both of which are critical to the final bonding performance to tooth substrate.

CONCLUSIONS

Some single-step self-etch adhesives claim to be able to be stored at room temperature. However, the results of the current study indicate that all of the self-etch adhesives tested show their best dentin bond strength if the adhesive vials are stored at 5°C for short time periods.

Clinicians using these simplified systems must be aware that storage conditions of adhesive vials can influence bond strengths.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research (C) (20592237) from the Japan Society for the Promotion of Science (JSPS). This project was also supported in part by the Sato Fund and a grant from the Dental Research Center, Nihon University School of Dentistry.

(Received 15 December 2009; Accepted 16 March 2010)

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. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P & Van Meerbeek B (2007) Systematic review of the chemical composition of contemporary dental adhesives *Biomaterials* **28(26)** 3757-3785.
3. 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 *Dental Materials Journal* **24(1)** 1-13.
4. Nishiyama N, Suzuki K, Yoshida H, Teshima H & Nemoto K (2004) Hydrolytic stability of methacrylamide in acidic aqueous solution *Biomaterials* **25(6)** 965-969.
5. Nishiyama N, Tay FR, Fujita K, Pashley DH, Ikemura K, Hiraishi N & King NM (2006) Hydrolysis of functional monomers in a single-bottle self-etching primer—correlation of 13C NMR and TEM findings *Journal of Dental Research* **88(5)** 422-426.
6. Fujita K & Nishiyama N (2006) Degradation of single bottle type self-etching primer effectuated by the primer's storage period *American Journal of Dentistry* **19(2)** 111-114.
7. Sadr A, Ghasemi A, Shimada Y & Tagami J (2007) Effects of storage time and temperature on the properties of two self-etching systems *Journal of Dentistry* **35(3)** 218-225.
8. Schilke R, Bauβ O, Lisson JA, Schuckar M & Geutsen W (1999) Bovine dentin as a substitute for human dentin in shear bond strength measurements *American Journal of Dentistry* **12(2)** 92-96.
9. Fowler CS, Swartz ML, Moore BK & Rhodes BF (1992) Influence of selected variables on adhesion testing *Dental Materials* **8(4)** 265-269.
10. Miyazaki M, Oshida Y & Xirouchaki L (1996) Dentin bonding system. Part I: Literature review *Bio-Medical Materials & Engineering* **6(1)** 15-31.
11. 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.
12. 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.
13. 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.
14. Salz U, Zimmermann J, Zeuner F & Moszner N (2005) Hydrolytic stability of self-etching adhesive systems *Journal of Adhesive Dentistry* **7(2)** 107-116.
15. Fujita K, Ma S, Li R, Li J, Ikemi T & Nishiyama N (2007) Effect of solvent type on the degradation of 4-MET *Dental Materials Journal* **26(6)** 792-799.
16. Fujita K & Nishiyama N (2006) 13C NMR analysis of the etching efficacy of acidic monomers in self-etching primers *Journal of Dentistry* **34(2)** 123-133.
17. Aida M, Odaki M, Fujita K, Kitagawa T, Teshima I, Suzuki K & Nishiyama N (2009) Degradation-stage effect of self-etching primer on dentin bond durability *Journal of Dental Research* **88(5)** 443-448.
18. Ma S, Nakajima KF & Nishiyama N (2009) Effects of storage temperature on the shelf life of one-step and two-step self-etch adhesives *Operative Dentistry* **34(4)** 472-480.
19. Hashimoto M (2010) A review—Micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **92(1)** 268-280.
20. Wang Y, Spencer P & Walker MP (2007) Chemical profile of adhesive/caries-affected dentin interfaces using Raman microspectroscopy *Journal of Biomedical Materials Research Part A* **81(2)** 279-286.
21. 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.
22. 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.
23. 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.
24. Jakubiak J & Rabek JE (1999) Photoinitiators for visible light polymerization *Polimery* **44(7-8)** 447-461.
25. Sanares AM, Itthagarun A, King NM, Tay FR & Pashley DH (2001) Adverse surface interactions between one-bottle light-cured adhesives and chemical-cured composites *Dental Materials* **17(6)** 542-556.
26. Sideridou I, Tserki V & Papanastasiou G (2002) Effect of chemical structure on degree of conversion in light-cured dimethacrylate-based dental resins *Biomaterials* **23(8)** 1819-1829.