©Operative Dentistry, 2010, **35-5,** 547-555

Microtensile Bond Strength of One- and Two-step Self-etching Adhesives on Sclerotic Dentin: The Effects of Thermocycling

C Xie • Y Han • XY Zhao
ZY Wang • HM He

Clinical Relevance

When compared with a two-step self-etching adhesive, the microtensile bond strength of a one-step self-etch adhesive bonding to both normal and sclerotic dentin was more affected by thermocycling.

◊Chao Xie, DDS, PhD, clinical instructor, Department of Oral Implantology, School of Stomatology, Fourth Military Medical University, Xi'an, Republic of China

◊Ying Han, DDS, PhD student, Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi'an, Republic of China

Xin-yi Zhao, DDS, PhD, professor and chair, Department of Dental Materials, School of Stomatology, Fourth Military Medical University, Xi'an, Republic of China

Zhong-yi Wang, DDS, professor, Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi'an, Republic of China

*Hui-ming He, DDS, PhD, professor, Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi'an, Republic of China

*Reprint request: 145 West Chang-le Road, Xi'an, 710032, Republic of China; e-mail: huiminghe@yahoo.cn

◊These authors contributed equally to this work and should be considered co-first authors.

DOI: 10.2341/10-025-L

SUMMARY

This study evaluated the effects of thermocycling on the microtensile bond strength (μTBS) of one- and two-step self-etch adhesives (SEAs) to sclerotic dentin. Two adhesives, Clearfil S³ Bond (S3), a one-step self-etch adhesive (1-SEA), and Clearfil SE Bond (SE), a two-step self-etch adhesive (2-SEA), were applied on cervical lesions in human premolars with sclerotic or normal dentin. After adhesive application, the lesions were restored and built up using a resin composite (Clearfil AP-X). After 24 hours in water storage, the restored teeth were sectioned into 0.7 x 0.7 mm composite-dentin beams. The beams were then aged with 0, 5,000 or 10,000 thermocycles. The use of two adhesives, two substrate types and three thermocycling regimens yielded 12 experimental groups of 14-19 beams each. The beams were subsequently subjected to μTBS testing at a crosshead

speed of 1 mm/minute and statistical analyses were computed with three-way ANOVA and Tukey's post hoc test at $p<0.05$. Three-way ANOVA showed statistically significant effects on bonding effectiveness by lesion type, adhesive system, thermocycling or combinations of the adhesive system and thermocycling ($p<0.05$). With sclerotic dentin, although S3 and SE provided comparable μTBS after 24 hours of water storage, S3 showed significantly lower μTBS than SE after thermocycling ($p<0.05$). Regardless of lesion type, the μTBS for S3 decreased significantly after 5,000 or 10,000 thermocycles, while the μTBS for SE showed a significant decrease only after 10,000 thermocycles. Regardless of the extent of thermocycling, the μTBS values for either SE or S3 bonded to sclerotic dentin were significantly lower than to normal dentin ($p<0.05$). The results suggested that thermocycling had a significant negative effect on the bond strength of the two SEAs tested. In contrast to 2-SEA, 1-SEA might not be a good choice for sclerotic dentin when seeking durability of the resin-dentin bond.

INTRODUCTION

A non-carious cervical sclerotic lesion is an abnormal substrate condition that is mostly observed in maxillary incisors and premolars where the highest stress contractions in the oral cavity occur.[1] Restoring a cervical lesion to relieve high stress and prevent further deterioration is advisable. Unfortunately, the mineral and matrix components of these lesions exhibit extensive compositional and structural differences when compared with normal dentin.[2] The surface of sclerotic dentin has been altered physiologically and pathologically, including partial or complete obstruction of the dentinal tubules with tube- or rod-like sclerotic casts,[3-4] the presence of an acid-resistant hypermineralized layer[3,5] and the presence of bacteria on the lesion surface.[5-6] Thus, cervical sclerotic dentin is considered a unique multilayered bonding substrate similar to a potential diffusion barrier to primer and resin-infiltration,[1] making it difficult to optimally acid-etch this bonding substrate.

The current adhesive strategy, which depends on micromechanical retention through the formation of intertubular hybrid layers and intratubular resin tags, appears less effective than normal when applied to hypermineralized sclerotic dentin.[7] This has been confirmed by many *in vitro* studies in which the bond strength to sclerotic dentin was significantly lower than to normal dentin.[6,8-10] However, Kusunoki and others[11] considered the multilayered bonding substrate of sclerotic dentin suitable for bonding. In their study, phosphoric acid-etching or citric acid-etching on sclerotic dentin promoted monomer diffusion into dentin and caused reduction of the monomer concentration at the adhesive interface, which might be detrimental to the bonding effectiveness of sclerotic dentin. Moreover, transmission electron microscopic (TEM) observations confirmed that the remaining hydroxyapatite within the submicron hybrid layer may have doubled as a receptor for additional chemical bonding, along with micromechanical interlocking through hybridization.[12] Therefore, self-etching adhesives (SEAs) using the smear layer as the bonding substrate may take advantage of this hybridized hypermineralized layer of sclerotic dentin to achieve better marginal adaptation[11] and bonding effectiveness[13] when compared to conventional etch-and-rinse adhesives.

Currently, there are two kinds of SEA systems. The two-step SEAs (2-SEAs) have a separate priming step with hydrophilic monomers and a hydrophobic bonding step. The one-step SEAs (1-SEAs), also called "all-in-one" adhesives, combine the etching, priming and bonding procedures into one solution and a single step.[14] Compared with etch-and-rinse adhesives, these simplified SEAs exhibit several advantages, including less sensitivity to technique[15] and an optimally infiltrated hybrid layer,[16] as well as less postoperative sensitivity[17] when using normal dentin as the bonding substrate. Recent clinical studies using non-carious cervical sclerotic dentin as the bonding substrate have demonstrated that 1-SEAs show good clinical performance within one-year of observation[18-19] and acceptable clinical effectiveness with not less than two-years of clinical service.[20] With 2-SEAs, excellent clinical performance has been seen even after midterm (3–5 years) clinical service.[21-22] A common problem for both 1- and 2-SEAs is a progressive marginal deterioration that may cause discoloration.[18-22] Limited information can be found in the literature regarding the *in vitro* bonding effectiveness of SEAs to sclerotic dentin. Moreover, no studies have compared the bonding effectiveness between 1- and 2-SEAs with sclerotic dentin.

The durability of the bonding interface between a resin composite and tooth structure is an important factor with respect to the longevity of adhesive restorations. The most common *in vitro* aging methods evaluating bonding stability are thermocycling or water storage. Due to the ability to assess the results of thermal stresses and prolonged water exposure, thermocycling has been frequently used to simulate the natural aging process of bonding interfaces.[23] Previous studies have shown that thermocycling causes combined repetitive contraction/expansion stresses and accelerated chemical degradation at the resin-tooth interface,[24-25] which may eventually affect bond strength.[26] However, the effect of thermocycling has been found to be brand,[27-28] protocol[29-30] and cycle number dependent.[24] It should be noted that these results were mainly obtained using normal dentin[26-27,29-30] or caries-affected dentin[28] as the

bonding substrate. To date, there appears to have been no previous studies on the effects of thermocycling on the bonding effectiveness of current SEAs applied on sclerotic dentin.

The objective of this *in vitro* study was to investigate the effects of thermocycling on the microtensile bond strength (μTBS) on bonding of 1- and 2-SEAs to cervical sclerotic and normal dentin. The hypotheses tested were: 1) there is no difference in the bonding strengths of these adhesives to normal and sclerotic dentin, 2) there is no difference in bonding strengths between the 1- and 2-SEAs tested and 3) thermocycling does not affect bonding effectiveness.

METHODS AND MATERIALS

The teeth used in the current study were obtained from protocols approved by the Ethics Committee of the Fourth Military Medical University and with the informed consent of patients. Thirty human permanent premolars with natural wedge-shaped buccal cervical lesions were extracted for periodontal reasons and collected. All of the cervical lesions presented hard, smooth surfaces and were not carious. Another 30 sound human permanent premolars, extracted for orthodontic reasons, served as the controls. The teeth were stored in 0.5% chloramine T solution at 4°C for up to one month before use.

The natural cervical lesion teeth (Group N) were debrided, cleaned using a slurry of pumice and saline water with a rotating rubber cup, and the cervical lesions were inspected with a stereomicroscope (SMZ645; Nikon Co, Tokyo, Japan) to ensure no pumice and plaque remained on the bonding surface.[6] The intact teeth (Group A), which had no buccal cervical defects, were given wedge-shaped defects 4 mm wide and 3 mm deep in cervical dentin using a high-speed handpiece equipped with a fine diamond bur and water cooling. The wedge-shaped cavities of these artificial lesions were similar in shape to natural lesions.[6]

The adhesives were employed as described in Table 1. Teeth in Groups N and A were randomly divided into two subgroups with respect to the two adhesives used. S3 (Clearfil S^3 Bond, Kuraray Co, Osaka, Japan) was applied to a lesion for 20 seconds, gently air-dried for 20 seconds, then light-cured for 10 seconds using a halogen curing light unit (Spectrum 800, Dentsply Caulk Co, Milford, DE, USA). For the 2-SEA (Clearfil SE Bond, Kuraray Co), SE primer was applied on a lesion area for 20 seconds, gently air-dried of solvent for 20 seconds and two thin layers of SE bond were applied on the primed lesion, with each layer light-cured for 10 seconds. For both adhesives, the method of application followed the manufacturer's instructions.

After the bonding procedures, all the cervical cavities were restored by placing two layers of a resin composite (Clearfil AP-X, Kuraray Co). Each increment was placed and contoured with a hand instrument, then light-cured for 40 seconds. The surface of the resinous restoration and the surrounding enamel and cementum were then etched with the SE primer and treated with the SE bonding agent. The resin composite was built up in several increments to form a cylinder post,[6,8] which facilitated preparation of the subsequent beam-shaped specimen.

Following the recommendations of the International Organization for Standardization (ISO), all the test teeth were then stored in water at 37°C for 24 hours.[31] Upon completion of this procedure, all the restored teeth were sectioned buccolingually into ~0.7-mm thick, 6-mm long slabs, using a low-speed saw with a diamond-impregnated disk (Isomet, Buehler Ltd, Lake Bluff, IL, USA) with water cooling, which were then attached with sticky wax to plexiglass blocks for further sectioning to produce beam-shaped specimens with 0.7 x 0.7 mm^2 surfaces and 6 mm lengths. Care was taken to cut the slabs perpendicular to the resin-dentin interfaces.

All beams in each subgroup (S3 or SE) were further randomly divided into three groups according to three thermocycling regimens in which the beams were given 30-second dwell times alternately in two water baths, one at 5°C and the other at 55°C (ZLR Thermo-cycler; Senrida Co, Tianjin, China) for 0; 5,000 or 10,000 cycles.

The use of two substrate types, two adhesives and three thermocycling regimens produced 12 experimen-

Table 1: *Adhesive Systems Used*

Adhesive	Manufacturer	Type	Lot #	Components
Clearfil SE Bond (SE)	Kuraray, Okayama, Japan	2-SEA	Primer: 00453A Bond: 00623A	Primer: 10-MDP, HEMA, hydrophilic DMA, tertiary amine, water. Bond: 10-MDP, Bis-GMA, HEMA, hydrophobic DMA, tertiary amine, silanized colloidal silica, photoinitiator.
Clearfil S^3 Bond (S3)	Kuraray, Okayama, Japan	1-SEA	011170	10-MDP, HEMA, Bis-GMA, ethanol, water, photoinitiator, camphorquinone, silanized colloidal silica.

Abbreviations: 10-MDP, 10-Methacryloyloxydecyl; HEMA, 2-hydroxyethyl methacrylate; bis-GMA, bisphenol A diglycidyl methacrylate; DMA, dimethacrylate.

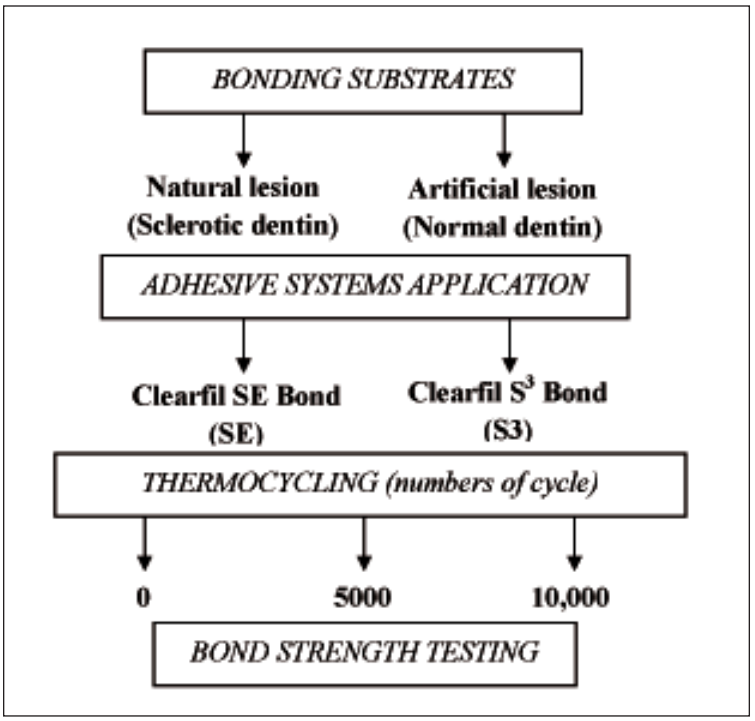

Figure 1: *Experimental design.*

tal groups with 14–19 beams for each. The experimental design is shown in Figure 1.

For each thermocycling regimen, individual resin-dentin beams were stressed under tension until failure using a universal testing machine (AGS-500; Shimadzu Co, Kyoto, Japan) at a crosshead speed of 0.5 mm/minute. The precise dimension of the cross-sectional area at the site of fracture was measured using a digital caliper. The μTBS was then calculated by dividing the load at failure by the cross-sectional area.

The fractured appearance of each beam was evaluated with a stereomicroscope (SMZ645; Nikon Co) at 50x magnification to identify the mode of failure and classified into three types: Type 1, adhesive failure with bond failure showing complete detachment at the resin-dentin bond interface; Type 2, cohesive failure with the bond failure entirely within the restorative resin or dentin and Type 3, mixed failure with the bond failure showing as a combination of cohesive and adhesive failure modes.

Three-way ANOVA was used to statistically analyze the influence of lesion type, adhesive system, thermocycling and interactions among these three factors. The Tukey's post hoc test was then used for multiple comparisons (α=0.05), the failure mode data analyzed using the Chi-squared test (α=0.05) and all analyses were processed using SPSS 13.0 software (SPSS, Chicago, IL, USA).

RESULTS

The mean cross-sectional area of the fractured beams ranged from 0.47 to 0.51 mm^2 and no differences among the experimental groups were detected (p>0.05). Three-way ANOVA showed that the three factors of lesion type, adhesive system and thermocycling had significant main effects (p<0.001). There was also a significant interaction between the adhesive system and thermocycling regimen (p=0.019).

The overall μTBS values of the experimental groups are shown in Table 2. In terms of the adhesive system, S3 and SE provided comparable μTBS after 24 hours of water storage without thermocycling when bonding to either sclerotic or normal dentin (p>0.05). However, after thermocycling, the μTBS for S3 was significantly lower than for SE (p<0.05), except for the artificial lesion groups subjected to 5,000 thermocycles (p>0.05).

Multiple comparisons revealed significant differences in μTBS among the three thermocycles (p<0.05). With increased thermocycling, the μTBS for S3 and SE decreased significantly. The influence of thermocycling on the bond strength to either sclerotic or normal dentin was most pronounced for S3, which showed significant decreases in bond strength after 5,000 thermocycles (34.5 MPa) and 10,000 thermocycles (23.4 MPa, Table 3). However, SE showed a decreasing, but not statistically significant, bond strength after 5,000 thermocycles (43.8 MPa); after 10,000 thermocycles (32.7 MPa), the μTBS for SE decreased significantly (p<0.05, Table 3).

The lesion type was found to have a significant effect

Table 2: *Microtensile Bond Strengths (MPa) of Each Experimental Group*

Lesion Type	Adhesive System	Thermocycles		
		0	5000	10000
Natural Lesion	One-step Self-etch (S3)	41.7 (8.9)^{a,C}	25.5 (8.6)^{a,B}	14.9 (5.9)^{a,A}
	Two-step Self-etch (SE)	40.8 (11.9)^{a,B}	35.0 (11.4)^{b,B}	25.5 (8.4)^{b,A}
Artificial Lesion	One-step Self-etch (S3)	60.7 (12.5)^{b,C}	45.8 (10.7)^{c,B}	32.3 (8.7)^{b,A}
	Two-step Self-etch (SE)	61.5 (15.5)^{b,B}	53.2 (13.0)^{c,A,B}	42.0 (14.2)^{c,A}

All values are mean (SD); S3 = Clearfil S3 Bond; SE = Clearfil SE Bond. Different superscript lower case letters (analysis in column) and different superscript upper case letters (in row) indicate statistically significant differences (p<0.05, ANOVA and Tukey's tests).

Table 3: *Microtensile Bond Strengths (MPa) for Each Adhesive System at Each Thermocycling Period*

Adhesive System	Thermocycles		
	0	5000	10000
S3	50.9 (14.4)[c]	34.5 (13.9)[b]	23.4 (11.4)[a]
SE	50.5 (17.1)[c]	43.8 (15.1)[c]	32.7 (13.8)[b]

All values, mean (SD); same superscript letters indicate no significant differences of MTBS (p>0.05).

Table 4: *Microtensile Bond Strengths (MPa) for Each Adhesive System Bonding to Different Lesions*

Lesion Type	Adhesive System	
	S3	SE
Natural lesion	27.3 (13.4)[a]	33.7 (12.3)[a]
Artificial lesion	46.3 (15.8)[b]	52.6 (16.1)[b]

All values, mean (SD); same superscript letters indicate no significant differences of MTBS (p>0.05).

on bond strength among the adhesive systems employed ($p<0.001$). Regardless of the thermocycling regimen, the mean μTBS achieved by S3 bonded to sclerotic dentin (27.3 MPa) was significantly lower than to normal dentin (46.3 MPa) when all bond strength values were pooled ($p<0.05$). Similarly, the mean μTBS of pooled data from SE to sclerotic dentin (33.7 MPa) was significantly lower than to normal dentin (52.6 MPa, $p<0.05$, Table 4).

The percentages of failure modes for each experimental group are shown in Figure 2. For all tested beams, adhesive failure was the most prevalent observed fracture mode, followed in occurrence by mixed failure. Though the percentage of adhesive failures increased for either S3 or SE with increased thermocycles, irrespective of the lesion type, there were no significant differences among failure modes in each experimental group ($p>0.05$).

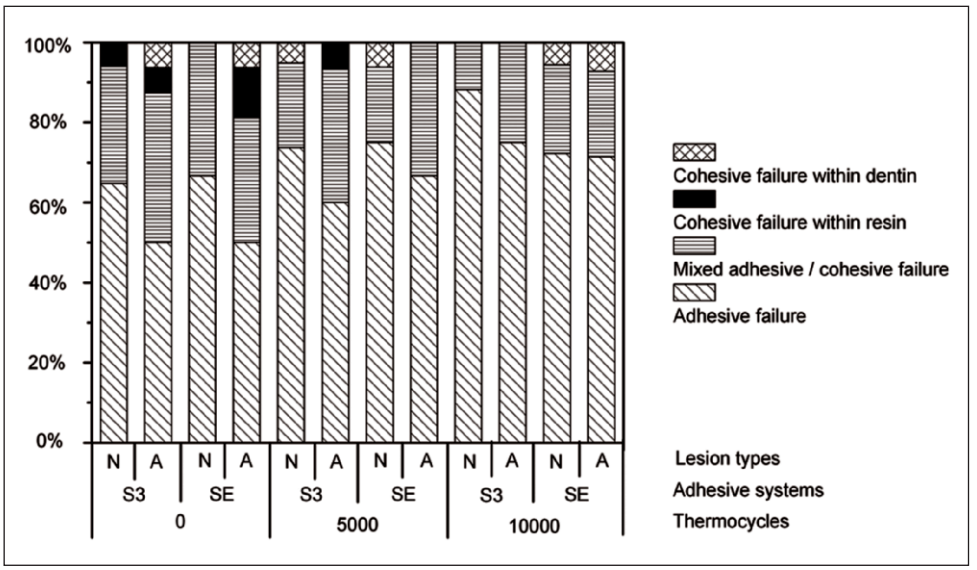

Figure 2: *Classification of failure mode.*

DISCUSSION

The most frequently used tests to evaluate the bonding effectiveness of adhesive systems are bond strength tests, such as shear bond and microtensile bond strengths. Many studies have used an improved microtensile test method to evaluate bond strengths of current adhesive systems to compromised tooth structure (for example, caries-affected dentin or sclerotic dentin).[6,8-9] Though the μTBS test is more complicated, operator-sensitive and time-consuming, its major advantage is ascribed to the measurement of the bond strength of relatively small specimens (<1 mm^2 cross-sectional area). Thus, this is considered a conservative testing method, because more specimens can be produced from one tooth, thereby reducing the number of teeth required for experimental processing.[32] For cervical wedge-shaped lesions, this technique made it possible to evaluate differences of regional bond strengths at disparate parts of the cervical lesion surface.[6,8-9]

Regional bond strengths have been predicted to vary with the diverse dentinal tubule orientation of cervical lesions. Yoshiyama and others[8] evaluated the influence of bonding location on the bond strength to cervical sclerotic dentin and found no significant difference in the mean bond strengths between gingival and occlusal sites of wedge-shaped cavities. A similar study conducted by Kwong and others[6] also concluded that the μTBS among different locations (gingival vs occlusal vs apex) of the cervical sclerotic lesion was not significantly different when SEAs were used, showing results that are comparable to a previous study in which the bond strengths of a SEA to sound dentin were not influenced by tubule orientation.[33] The results of a preliminary experiment reconfirmed this point. Therefore, the parameter of bonding location was not tracked, and the data obtained from different parts of the cervical sclerotic dentin pooled to simplify statistical processing.

In the current study, the μTBS obtained from SEAs (SE and S3) bonding to sclerotic dentin were significantly lower than the values from bonding to normal

dentin, regardless of the thermocycling regimen (Table 4). Therefore, the first null hypothesis, that there is no difference in bond strength to normal and sclerotic dentin, was rejected, a result that was in agreement with several previous studies in which a significant drop in bond strengths was reported for sclerotic dentin when compared with normal dentin.[6-8] Here, the mean μTBS after 24 hours of water storage for sclerotic dentin and normal dentin were 41.2 MPa and 61.1MPa, respectively, when the data from SE and S3 were pooled; these results were similar to those reported by Kwong and others,[6] in which the mean μTBS produced by a SEA, Clearfil Liner Bond 2V, was 48.7 and 65.8 MPa for sclerotic and normal dentin, respectively. These μTBS values, however, were higher than those from other studies,[8,13,34] irrespective of what kind of adhesive system was used or how the bonding surface was treated. One explanation for these results might be that the cross-sectional areas (≈0.49 mm^2) of the beam-shaped specimen in the current study were smaller than in those studies where areas ranged from 0.69-1 mm^2. The smaller adhesive interface areas used in the microtensile test may have reduced the risk for critical size defects at and near the interface and may have been less likely to experience premature failures due to the more homogeneous stress distribution at the interface.[35]

The results of the current study showed that thermocycling caused a significant decrease in the μTBS of two SEAs bonded to either sclerotic or normal dentin. An exception was found, however, for SE, which showed decreasing, but not statistically significant, bond strengths after 5,000 thermocycles. Thus, the third hypothesis, that thermocycling does not affect bonding effectiveness, had to be rejected. Thermocycling generates mechanical stresses at the tooth-biomaterial interface through differences in the thermal contraction/expansion coefficient between the restorative materials and tooth tissue[24-25] and also accelerates the hydrolytic degradation of hydrophilic components in the adhesive systems and the collagen fibrils at the base of the hybrid layer.[26] Due to these two common aging effects, thermocycling imposes a negative effect on bond strength.[26] There are several factors affecting thermocycling, including storage medium, number of cycles, temperature setting, dwell time and intervals between baths, which make it relatively difficult to directly compare these experimental results. Of all the related factors, however, the number of cycles is usually arbitrarily set and considered to be the most influential.[24] According to the ISO standard, 500 thermocycles in water at temperatures between 5°C and 55°C is considered an appropriate test for aging dental materials; however, previous research based on this standard showed no effect of thermocycling on the bond strength of a SEA bonding to flat dentin surfaces.[36] Furthermore, in a study by Omar and others,[28] the effect of thermocycling on the bond strength of two SEAs and a conventional three-step adhesive to both intact and caries-affected dentin showed that 3,000 thermocycles did not significantly affect the bond strength of two SEAs. In contrast, Saboia and others[23] found that extensive thermocycling (6,000 cycles) had a significantly negative influence on the bond strength of a two-step etch-and-rinse adhesive. This negative influence was also examined in another study, in which 5,000 thermocycles dramatically reduced the bond strength of three one-bottle (etch-and-rinse) adhesives and one SEA.[37] The current results were in agreement with the negative effect of thermocycling. From the results listed above, it was concluded that thermocycling had a negative effect on bond strength after a large number of thermocycles.[24] Based on the hypothesis that thermocycling might occur 20-50 times a day, it is estimated that 10,000 thermocycles corresponds to ≈1 year of *in vivo* functioning.[38] In the current study, the bonded surfaces were subjected to 5,000 or 10,000 thermocycles to mimic approximately one-half or a full year of clinical function, which could be considered reasonable aging times.

In the current study, S3 exhibited μTBS comparable to SE after 24 hours of water storage, which was consistent with a previous investigation where the differences in μTBS between 1- and 2-SEAs were not significant,[39] although the bonding substrates were different from the current study. In a recent study, evaluation of the bonding effectiveness to enamel and dentin of nine 1-SEAs showed that a 2-SEA, SE, served as the control, producing higher dentin bond strengths than 1-SEAs. However, the differences between SE and some of the 1-SEAs, including S3, were not statistically significant, and the results also showed that S3 exhibited the best bonding effectiveness among all 1-SEAs tested.[40] In contrast, other *in vitro* studies have concluded that both etch-and-rinse adhesives and 2-SEAs produced better bond strengths than 1-SEAs.[41-42] The reason for these conflicting results may be due to differences in the SEAs, testing conditions, operational factors and bonding substrates used in these studies.[37] In the current work, however, after 5,000 and 10,000 thermocycles, the mean μTBS values for S3 were about 21% and 28%, respectively, lower than the corresponding values for SE, with the result being statistically significant ($p<0.05$, Table 3). Thus, the second hypothesis had to be partially rejected.

In the current study, the effect of thermocycling on the bond strength to either sclerotic or normal dentin was more pronounced for S3, which was attributed to several factors. First, TEM observations have shown that the adhesive layer of SE was much thicker (≈50 μm) than S3 (≈10 μm).[43] The thinner adhesive layer

was considered to be a sign of suboptimal polymerization[44] and low degrees of monomer conversion[45] and, due to relatively higher degrees of conversion, 2-SEA SE may be less permeable than S3.[44] Second, the 2-SEA SE contains a special functional monomer, 10-MDP, having two hydroxyl groups that may have chelated calcium ions of the dentin[46] and retained residual hydroxyapatite around collagen fibrils, thereby preventing collagen fibrils from hydrolysis (for example, degradation of the bonding interface).[47] Third, previous studies have shown that 1-SEAs contain higher concentrations of acidic monomers to properly etch the dentin surface,[48] which renders them more susceptible to water sorption and thereby affecting their long-term durability.[49] Fourth, 1-SEAs contain high water concentrations to improve the ionization of acidic monomers, but an increasing water concentration inevitably reduces the resin concentration and compromises bond strength.[43] Finally, as a hydrophilic 1-SEA, S3 has a HEMA-containing composition with a hydrophilic nature, which may act as permeable membranes and have a high proclivity for osmosis, absorbing significant amounts of water and forming osmosis-induced droplets.[40] However, SE can create a hydrophobic coating that prevents the adhesive layer from being a permeable membrane after polymerization,[50] and thus it does not exhibit phase separation or osmosis-induced droplets.[40] For these reasons, it was speculated that a more hydrophobic adhesive formulation, such as etch-and-rinse or 2-SEAs, could be less affected by water-mediated aging *in vitro* tests.[23]

Although these *in vitro* aging results for 1-SEAs in both this and other studies are disappointing, several current clinical studies have demonstrated acceptable clinical performances for 1-SEAs and showed no performance or retention differences between 1- and 2-SEAs.[19] In these clinical studies,[18-20] cervical dentin was roughened before applying an adhesive, a step considered to be an effective mechanical treatment contributing to the improvement of the sclerotic dentin-resin bond.[51] This type of preparation was not included in the current study. Further studies are required to focus on the bonding durability of SEAs bonded to sclerotic dentin using different adhesion protocols, including surface treatment and increasing the acid conditioning times.

The failure modes of the adhesive systems tested were more frequently partial adhesive failures combined with partial mixed failures when occurring before thermocycling. Adhesive failures between the bonding resin and dentin were most commonly observed after thermocycling, with the increased percentage of adhesive failures related to bond strength reduction after thermocycling. This finding agreed with results from another *in vitro* study.[37]

CONCLUSIONS

Within the limitations of the current study, it was concluded that the μTBS values of 1- or 2-SEA bonded to sclerotic dentin were significantly lower than to normal dentin and that thermocycling had a significantly negative effect on the long-term durability of the resin-dentin bond, although the effect was adhesive-dependent. Additionally, there were similar initial μTBS values between 1- and 2-SEA before thermocycling; however, the 1-SEA was more prone to *in vitro* water-mediated aging, which resulted in bond strengths inferior to 2-SEA after thermocycling.

Acknowledgements

The authors thank Dr Kang Biao for extensive technical assistance in microtensile testing and Dr Yunming Li for assistance in statistical analysis. This study was supported by a grant from the National Natural Science Foundation of China (30470433).

(Received 1 February 2010; Accepted 13 April 2010)

References

1. Tay FR & Pashley DH (2004) Resin bonding to cervical sclerotic dentin: A review *Journal of Dentistry* **32(3)** 173-196.
2. Karan K, Yao X, Xu C & Wang Y (2009) Chemical profile of the dentin substrate in non-carious cervical lesions *Dental Materials* **25(10)** 1205-1212.
3. Kwong SM, Tay FR, Yip HK, Kei LH & Pashley DH (2000) An ultrastructural study of the application of dentine adhesives to acid-conditioned sclerotic dentine *Journal of Dentistry* **28(7)** 515-528.
4. Sakoolnamarka R, Burrow MF, Prawer S & Tyas MJ (2000) Micromorphological investigation of non-carious cervical lesions treated with demineralizing agents *Journal of Adhesive Dentistry* **2(4)** 279-287.
5. Tay FR, Kwong SM, Itthagarun A, King NM, Yip HK, Moulding KM & Pashley DH (2000) Bonding of a self-etching primer to non-carious cervical sclerotic dentin: Interfacial ultrastructure and microtensile bond strength evaluation *Journal of Adhesive Dentistry* **2(1)** 9-28.
6. Kwong SM, Cheung GS, Kei LH, Itthagarun A, Smales RJ, Tay FR & Pashley DH (2002) Microtensile bond strengths to sclerotic dentin using a self-etching and a total-etching technique *Dental Materials* **18(5)** 359-369.
7. El-din AK, Miller BH & Griggs JA (2004) Resin bonding to sclerotic, non-carious, cervical lesions *Quintessence International* **35(7)** 529-540.
8. 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 *Journal of Dental Research* **75(6)** 1404-1413.
9. Eliguzeloglu E, Omurlu H, Eskitascioglu G & Belli S (2008) Effect of surface treatments and different adhesives on the hybrid layer thickness of non-carious cervical lesions *Operative Dentistry* **33(3)** 338-345.

10. Lopes GC, Baratieri CM, Baratieri LN, Monteiro S Jr & Cardoso VLC (2004) Bonding to cervical sclerotic dentin: Effect of acid etching time *Journal of Adhesive Dentistry* **6(1)** 19-23.

11. Kusunoki M, Itoh K, Hisamitsu H & Wakumoto S (2002) The efficacy of dentine adhesive to sclerotic dentine *Journal of Dentistry* **30(2-3)** 91-97.

12. 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.

13. Karakaya S, Unlu N, Say EC, Ozer F, Soyman M & Tagami J (2008) Bond strengths of three different dentin adhesive systems to sclerotic dentin *Dental Materials Journal* **27(3)** 471-479.

14. Moszner N, Salz U & Zimmermann J (2005) Chemical aspects of self-etching enamel-dentin adhesives: A systematic review *Dental Materials* **21(10)** 895-910.

15. Toledano M, Osorio R, de Leonardi G, Rosales-Leal JI, Ceballos L & Cabrerizo-Vilchez MA (2001) Influence of self-etching primer on the resin adhesion to enamel and dentin *American Journal of Dentistry* **14(4)** 205-210.

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. Unemori M, Matsuya Y, Akashi A, Goto Y & Akamine A (2004) Self-etching adhesives and postoperative sensitivity *American Journal of Dentistry* **17(3)** 191-195.

18. Kurokawa H, Miyazaki M, Takamizawa T, Rikuta A, Tsubota K & Uekusa S (2007) One-year clinical evaluation of five single-step self-etch adhesive systems in non-carious cervical lesions *Dental Materials Journal* **26(1)** 14-20.

19. Turkun LS (2005) The clinical performance of one- and two-step self-etching adhesive systems at one year *Journal of the American Dental Association* **136(5)** 656-664; quiz 683.

20. Kubo S, Yokota H, Yokota H & Hayashi Y (2009) Two-year clinical evaluation of one-step self-etch systems in non-carious cervical lesions *Journal of Dentistry* **37(2)** 149-155.

21. Peumans M, Munck J, Van Landuyt K, Lambrechts P & Van Meerbeek B (2005) Three-year clinical effectiveness of a two-step self-etch adhesive in cervical lesions *European Journal of Oral Sciences* **113(6)** 512-518.

22. Burrow MF & Tyas MJ (2007) Clinical evaluation of three adhesive systems for the restoration of non-carious cervical lesions *Operative Dentistry* **32(1)** 11-15.

23. Saboia VP, Silva FC, Nato F, Mazzoni A, Cadenaro M, Mazzotti G, Giannini M & Breschi L (2009) Analysis of differential artificial aging of the adhesive interface produced by a two-step etch-and-rinse adhesive *European Journal of Oral Sciences* **117(5)** 618-624.

24. Amaral FLB, Colucci V, Palma-Dibb RG & Corona SAM (2007) Assessment of *in vitro* methods used to promote adhesive interface degradation: A critical review *Journal of Esthetic and Restorative Dentistry* **19(6)** 340-353; discussion 354.

25. 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.

26. Yang B, Adelung R, Ludwig K, Bossmann K, Pashley DH & Kern M (2005) Effect of structural change of collagen fibrils on the durability of dentin bonding *Biomaterials* **26(24)** 5021-5031.

27. Visintini E, Mazzoni A, Vita F, Pasquantonio G, Cadenaro M, Di LR & Breschi L (2008) Effects of thermocycling and use of ElectroBond on microtensile strength and nanoleakage using commercial one-step self-etch adhesives *European Journal of Oral Sciences* **116(6)** 564-570.

28. Omar H, El-Badrawy W, El-Mowafy O, Atta O & Saleem B (2007) Microtensile bond strength of resin composite bonded to caries-affected dentin with three adhesives *Operative Dentistry* **32(1)** 24-30.

29. Amaral FLB, Colucci V, Souza-Gabriel AE, Chinelatti MA, Palma-Dibb RG & Corona SAM (2008) Adhesion to Er:YAG laser-prepared dentin after long-term water storage and thermocycling *Operative Dentistry* **33(1)** 51-58.

30. Hasegawa T, Retief DH, Russell CM & Denys FR (1995) Shear bond strength and quantitative microleakage of a multipurpose dental adhesive system resin bonded to dentin *Journal of Prosthetic Dentistry* **73(5)** 432-438.

31. International Organization for Standardization 1994 Dental Materials—Guidance on Testing on Adhesion to Tooth Structure Geneva, Switzerland ISO TR 11405.

32. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA & Tay F (1999) The microtensile bond test: A review *The Journal of Adhesive Dentistry* **1(4)** 299-309.

33. Phrukkanon S, Burrow MF & Tyas MJ (1999) The effect of dentine location and tubule orientation on the bond strengths between resin and dentine *Journal of Dentistry* **27(4)** 265-274.

34. Lopes GC, Baratieri CM, Baratieri LN, Monteiro S Jr & Cardoso VLC (2004) Bonding to cervical sclerotic dentin: Effect of acid etching time *Journal of Adhesive Dentistry* **6(1)** 19-23.

35. Pashley DH, Sano H, Ciucchi B, Yoshiyama M & Carvalho RM (1995) Adhesion testing of dentin bonding agents: A review *Dental Materials* **11(2)** 117-125.

36. Nikaido T, Kunzelmann KH, Chen H, Ogata M, Harada N, Yamaguchi S, Cox CF, Hickel R & Tagami J (2002) Evaluation of thermal cycling and mechanical loading on bond strength of a self-etching primer system to dentin *Dental Materials* **18(3)** 269-275.

37. Helvatjoglu-Antoniades M, Koliniotou-Kubia E & Dionyssopoulos P (2004) The effect of thermal cycling on the bovine dentine shear bond strength of current adhesive systems *Journal of Oral Rehabilitation* **31(9)** 911-917.

38. Gale MS & Darvell BW (1999) Thermal cycling procedures for laboratory testing of dental restorations *Journal of Dentistry* **27(2)** 89-99.

39. Knobloch LA, Gailey D, Azer S, Johnston WM, Clelland N & Kerby RE (2007) Bond strengths of one- and two-step self-etch adhesive systems *Journal of Prosthetic Dentistry* **97(4)** 216-222.

40. Van Landuyt KL, Mine A, De Munck J, Jaecques S, Peumans M, Lambrechts P & Van Meerbeek B (2009) Are one-step adhesives easier to use and better performing? Multifactorial assessment of contemporary one-step self-etching adhesives *Journal of Adhesive Dentistry* **11(3)** 175-190.

41. Perdigão J, Gomes G, Gondo R & Fundingsland JW (2006) *In vitro* bonding performance of all-in-one adhesives. Part I—microtensile bond strengths *Journal of Adhesive Dentistry* **8(6)** 367-373.

42. Sadek FT, Goracci C, Cardoso PE, Tay FR & Ferrari M (2005) Microtensile bond strength of current dentin adhesives measured immediately and 24 hours after application *Journal of Adhesive Dentistry* **7(4)** 297-302.

43. Finger WJ, Shao B, Hoffmann M, Kanehira M, Endo T & Komatsu M (2007) Does application of phase-separated self-etching adhesives affect bond strength? *Journal of Adhesive Dentistry* **9(2)** 169-173.

44. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di LR, Prati C, Biasotto M, Contardo L & Breschi L (2005) Degree of conversion and permeability of dental adhesives *European Journal of Oral Sciences* **113(6)** 525-530.

45. Nunes TG, Ceballos L, Osorio R & Toledano M (2005) Spatially resolved photopolymerization kinetics and oxygen inhibition in dental adhesives *Biomaterials* **26(14)** 1809-1817.

46. 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.

47. Inoue S, Koshiro K, Yoshida Y, De Munck J, Nagakane K, Suzuki K, Sano H & Van Meerbeek B (2005) Hydrolytic stability of self-etch adhesives bonded to dentin *Journal of Dental Research* **84(12)** 1160-1164.

48. Tay FR & Pashley DH (2001) Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers *Dental Materials* **17(4)** 296-308.

49. Suppa P, Breschi L, Ruggeri A, Mazzotti G, Prati C, Chersoni S, Di LR, Pashley DH & Tay FR (2005) Nanoleakage within the hybrid layer: A correlative FEISEM/TEM investigation *Journal of Biomedical Materials Research. Part B, Applied Biomaterials* **73(1)** 7-14.

50. Tay FR, Suh BI, Pashley DH, Prati C, Chuang SF & Li F (2003) Factors contributing to the incompatibility between simplified-step adhesives and self-cured or dual-cured composites. Part II Single-bottle, total-etch adhesive *Journal of Adhesive Dentistry* **5(2)** 91-105.

51. Camargo MA, Roda MI, Marques MM & de Cara AA (2008) Microtensile bond strength to bovine sclerotic dentine: Influence of surface treatment *Journal of Dentistry* **36(11)** 922-927.