©Operative Dentistry, 2006, **31-6**, 710-718

Morphological Evaluation of 2- and 1-step Self-etching System Interfaces with Dentin

I Radovic • ZR Vulicevic • F García-Godoy

Clinical Relevance

This study investigated the interface of different self-etching systems with dentin and showed that the intensity of interaction was mostly dependent on the acidity of the primers/adhesives used.

SUMMARY

This *in vitro* study evaluated the resin-dentin interface formed by two 2-step and two 1-step self-etching adhesive systems under SEM. Class V cavities (4 x 2.5 x 1.5 mm) were prepared on the buccal surfaces of 25 extracted intact human third molars using a carbide bur in a high-speed handpiece. Four self-etching systems with corresponding resins were used: two 2-step systems, AdheSE (Ivoclar Vivadent) and Contax (DMG Hamburg), one 1-step/2 components system Futurabond NR (Voco) and a 1-step/1 component adhesive G-Bond (GC Corp). An etch and rinse system, PQ Clear (Ultradent), was used as a control. The teeth were thermocycled (500 cycles, 5°/55°C, 30 seconds dwell time), and 2 sections were made longitudinally through the restorations with a low-speed diamond saw, producing approximately 1-mm wide samples. The samples were polished with silicon carbide paper of increasing grit (400-1000), demineralized (6N HCl, 30 seconds), deproteinized (2.5% NaOCl, 10 minutes), left to air dry for 24 hours in a desiccator under low vacuum pressure, gold sputtered and viewed under SEM (JEOL-JSM-6460LV). In the AdheSE, Contax and Futurabond NR specimens, resin tag penetration into the tubules and lateral tags could be seen. G-Bond showed different interface morphology, with a tight, thin continuous junction and almost no resin penetration into tubules. PQ Clear samples exhibited the highest number of resin tags with numerous lateral tags. A clearly defined hybrid layer was seen in the Contax and PQ Clear specimens. Investigated self-etching systems showed similar interfacial morphology with dentin, except for the 1 step/1 component adhesive G-Bond. The degree of demineralization and interaction with dentin correlated with the acidity of the self-etching primers/adhesives. Fewer resin tags were formed with self-etching primers/adhesives than with the etch and rinse system.

*Ivana Radovic, DDS, MSc, clinical assistant, Clinic for Pediatric and Preventive Dentistry, Faculty of Dentistry, University of Belgrade, Serbia

Zoran R Vulicevic, DDS, MSc, PhD, professor, Clinic for Pediatric and Preventive Dentistry, Faculty of Dentistry, University of Belgrade, Serbia

Franklin García-Godoy, DDS, MSc, professor and associate dean for research, director, Bioscience Research Center, Biomaterials Research Center, and Clinical Research Center, College of Dental Medicine, Nova Southeastern University, Fort Lauderdale, FL, USA

*Reprint request: Dr Subotica 11, 11000 Beograd, Serbia: e-mail: ivana.radovic@iritel.com

DOI: 10.2341/05-145

INTRODUCTION

Since its beginnings, adhesive dentistry has been continuously challenged by a general trend to simplify clinical procedures. In an increasing attempt to accomplish this goal, the most common approach has been to shorten the adhesive system's application time and reduce the number of steps. However, it has been shown that, in general, simplification fails to result in improved features and durability,[1] regardless of whether the adhesive approach is etch and rinse or self-etch.[2-3]

Three-step etch and rinse systems have proven to be a gold standard.[4-5] Two-step versions, or so-called self-priming adhesives, resulted in increased technique sensitivity[6-7] compared to 3-step etch and rinse systems, since the priming and bonding steps have been joined into one, requiring different, more hydrophilic formulations of solvents and monomers. Although wet and dry bonding techniques have been described,[8-9] the true possibility for standardization of the etch and rinse procedure is questionable, since variables in the amount of wetness are difficult to define in precise terms as a recommendation for the clinician, especially if the wet bonding approach is used. There is a possibility for discrepancy[10-12] between depth of etch and depth of monomer infiltration, which influences degradation processes.[13-14] The above mentioned features have been recognized as primary drawbacks of the etch and rinse technique.

Self-etching systems were introduced and purported important advantages compared to etch and rinse systems. These systems are often described as using the most promising adhesive approach. The possibility for chemical interaction between functional monomers of some self-etching systems and tooth tissue has drawn new attention,[15] mostly in terms of potential benefits against hydrolytic degradation to which adhesive systems are exposed over a long period of clinical service.[16]

Conventional 2-step self-etching systems have been followed with simplified products. Since acidic monomers needed to be kept separate from water needed for ionization and subsequent demineralization, 1-step 2-component products were first introduced. Recently, new adhesives have been presented, where all the components for etching, priming and bonding are supplied in a single bottle. Considerable research has focused on 1-step adhesives, showing their potential shortcomings, such as incompatibility with chemical/dual cured composite resins.[17] Despite the fact that etching and resin infiltration occur simultaneously, which was believed to ensure that no discrepancies are possible between the 2 processes, nanoleakage was observed in some systems.[18-19] It was also shown that all-in-one adhesives are highly hydrophilic polymers which are permeable to water movement after polymerization.[20-23]

In addition to classification according to the number of clinical steps, self-etching systems are divided into mild, moderate and strong,[24-25] depending on etching aggressiveness. This classification is based on morphologic findings at the tooth-adhesive interface.

This *in vitro* study observed and compared the resin-dentin interfaces formed by 2-step and 1-step self-etching adhesive systems of different acidities under SEM.

METHODS AND MATERIALS

Twenty-five intact human third molars extracted for orthodontic reasons were used. The teeth, which originated from both men and women of different ages, were stored in formalin for no more than 6 months. Two weeks before starting the experiment, the teeth were rinsed thoroughly under running water and further stored in distilled water. Class V cavity preparations were placed on the buccal surfaces of the teeth using a carbide bur (US No 330, Brasseler USA, Savannah, GA, USA) in a high-speed handpiece, under water cooling. After every fifth preparation, the bur was replaced with a new one. The preparations were 4-mm long, 2.5-mm wide and 1.5-mm deep (measured from the dentino-enamel junction), with the occlusal margin in enamel and the gingival margin located 0.5 mm below the cementoenamel junction. The dimensions of each cavity were measured with a digital caliper, and the teeth were randomly divided into 5 groups (5 teeth per group) and restored with investigated adhesive systems and corresponding resin composites.

Group 1: 2-step self-etch system AdheSE, Tetric Ceram (Ivoclar Vivadent, Schaan, Liechtenstein)

Group 2: 2-step self-etch system Contax (DMG Hamburg, Hamburg, Germany), Grandio (Voco, Cuxhaven, Germany)

Group 3: 1-step 2-component self-etch system Futurabond NR, Grandio (Voco)

Group 4: 1-step 1-component self-etching adhesive G-Bond, Gradia direct (GC Corporation, Tokyo, Japan)

Group 5: PQ Clear, Amelogen (Ultradent, South Jordan, UT, USA), an etch and rinse adhesive system which was used in the control group

Adhesive systems were used according to the manufacturers' information (Table 1). Resin composite was placed in a single layer. Light curing was performed with an Astralis 10 curing light (Ivoclar Vivadent) using a "pulse" mode for 20 seconds. This mode is characterized by soft-start polymerization during the first 10 seconds (increased from 150 to 650 mW/cm^2) and pulsating between 650 and 1200 mW/cm^2 within the next 10 seconds. The intensity of the light was monitored periodically with a radiometer (Demetron/Kerr, Danbury, CT, USA). The restored teeth were left overnight in distilled water at room temperature and subjected to thermocycling (500 cycles, 5° to 55°C, 30 seconds dwell time). Following thermocycling, the

Table 1: *Adhesive Systems Used and Application Method*

Adhesive System	Etchant/Primer	Adhesive	Manufacturer	Batch #
AdheSE	Apply primer and, when thoroughly coated, brush into for 15 seconds (total reaction time: 30 seconds). Disperse excess amounts with a strong stream of air.	Apply bond, beginning at dentin. Disperse with a very weak stream of air. Light cure.	Ivoclar Vivadent	G 05739
Contax	Work primer into tooth structure for 20 seconds.	Work adhesive into primed tooth structure for 20 seconds, thin out, light cure.	DMG Hamburg	529426
Futurabond NR	Mix 1 drop of liquid A and 1 drop of liquid B for 5 seconds. Apply mixture to tooth and massage for 20 seconds. Dry with a faint air jet for 5 seconds. Light cure.		Voco	0482
G-Bond	Shake bottle well. Apply to tooth surfaces. Leave undisturbed for 10 seconds. Dry thoroughly for 5 seconds under maximum air pressure, to form a thin film. Light cure.		GC Corp	0405241
PQ Clear	Etch enamel and dentin for 15 seconds (UltraEtch). Rinse. Blow excess water off, without desiccating.	Apply adhesive and air thin. Maintain a high glossy surface with no dry spots. Light cure.	Ultradent Products	64QM

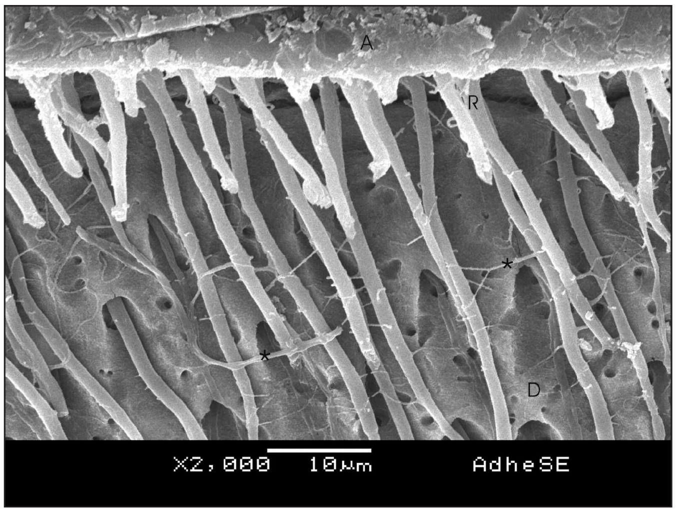

Figure 1. *SEM micrograph of a moderate 2-step 2-component adhesive AdheSE (Ivoclar Vivadent) interface with dentin. Resin tag formation with corresponding lateral tags (asterisks) is visible. Hybrid layer is not clearly visible under this magnification. A: adhesive. R: resin tag. D: dentin.*

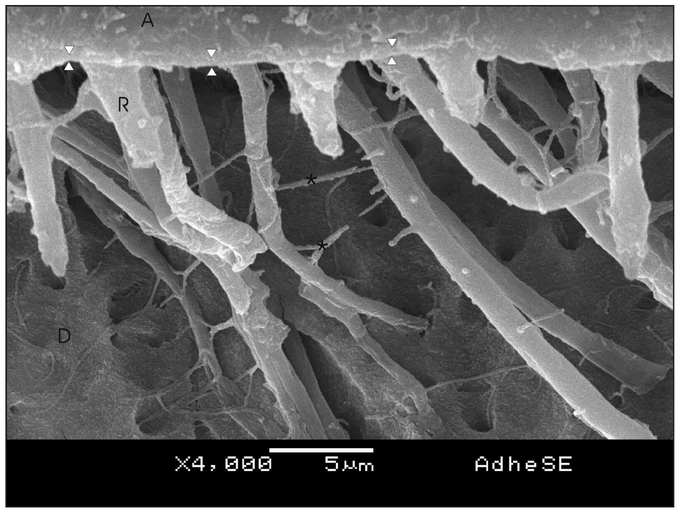

Figure 2. *SEM resin-dentin interface of AdheSE. A shallow hybrid layer of approximately 1 µm could be seen with some specimens at higher magnifications (between arrowheads). Resin tags show lateral branches (asterisks). A: adhesive. R: resin tag. D: dentin.*

teeth were sectioned longitudinally through the restorations in a buccolingual plane with a low speed diamond saw (Isomet/Buehler Ltd, Lake Bluff, IL, USA). Two sections were made per tooth, producing approximately 1-mm wide samples that were polished with silicon carbide paper of increasing grit (400, 600, 800 and 1000) under running water. After thorough rinsing, the samples were demineralized for 30 seconds with 6N HCl, rinsed again, deproteinized with 2.5% NaOCl for 10 minutes to enable examination of the interface, then left to air dry in a desiccator under low vacuum pressure (270 mbar) for 24 hours. Following the drying procedure, the samples were sputter-coated with gold (Bal-Tec SCD 005 Sputter Coater) and viewed under SEM (JEOL-JSM-6460LV), operating at 20 kV under various magnifications. Representative pictures of the interfaces were taken primarily along the axial walls of the preparations in areas where dentin tubules predominantly run perpendicularly to the interface, thus facilitating adhesive penetration.

RESULTS

Where AdheSE was used, SEM micrographs in Group 1 showed characteristic reverse cone-shaped tags about

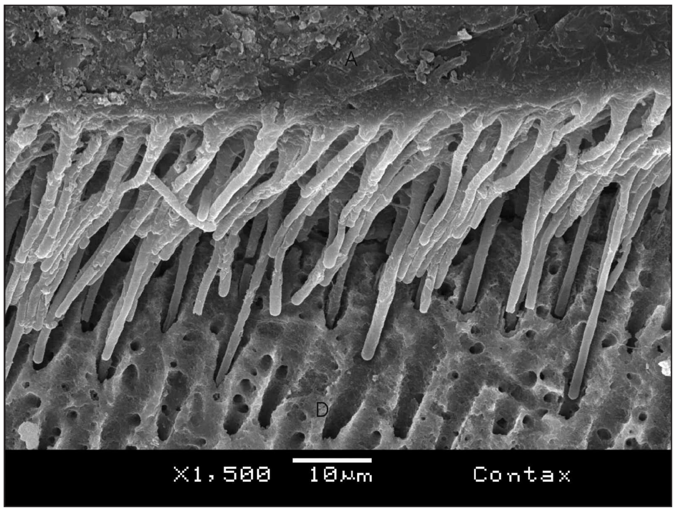

Figure 3. *SEM micrograph of a moderate 2-step 2-component adhesive Contax (DMG Hamburg) interface with dentin. Numerous resin tags are visible. Lateral tags are fewer in number. A: adhesive. D: dentin.*

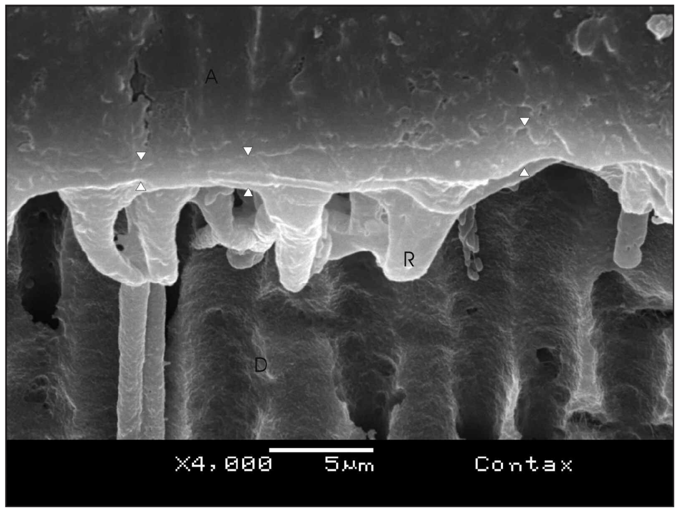

Figure 4. *SEM resin-dentin interface formed by Contax. A hybrid layer ranging from 1-2 μm is visible (between arrowheads). A: adhesive. R: resin tag. D: dentin.*

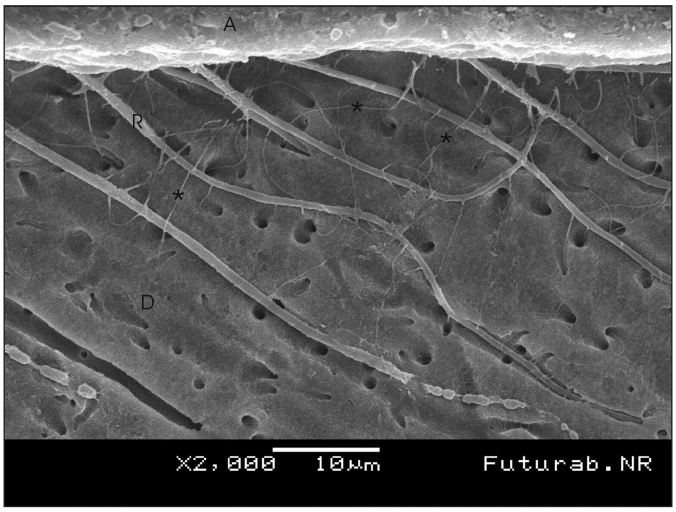

Figure 5. *SEM micrograph of a moderate 1-step 2-component adhesive Futurabond NR (Voco) interface with dentin. Resin tags and lateral branches (asterisks) are scattered and thinner. A: adhesive. R: resin tag. D: dentin.*

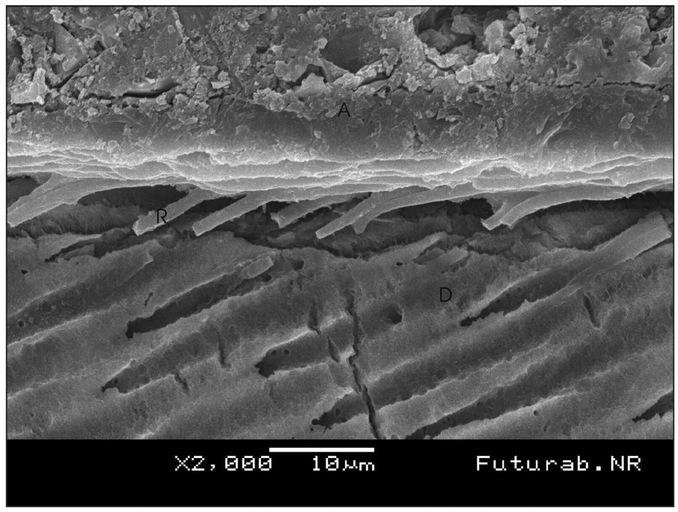

Figure 6. *SEM resin-dentin interface formed by Futurabond NR. Hybrid layer formation is not visible. A: adhesive. R: resin tag. D: dentin.*

20-60 μm long. Corresponding adhesive lateral branches were numerous (Figure 1). A hybrid layer was not clearly recognizable in this group, although with some specimens, at higher magnifications, a shallow zone of ± 1 μm could be seen at the transition between the adhesive layer and demineralized dentin (Figure 2). In Group 2, where Contax was used, there were similar findings regarding the shape and length of the resin tags (Figure 3), but lateral tags appeared slightly fewer in number. On the other hand, a hybrid layer was more clearly defined and ranged between 1 and 2 μm (Figure 4). In the Futurabond NR specimens, resin tags and their lateral branches were more scattered and thinner (Figure 5). There was no evidence of hybrid layer formation in this group (Figure 6). In Group 4, where G-Bond was used, minimal formation of tags was seen, indicating that most of the dentin tubules remained obstructed by smear plugs (Figure 7). The resin tags were non-uniform, irregular in shape and, unlike previous groups, no lateral tags were noticed. On one specimen, voids were noticed in deeper portions of a resin tag (Figures 8 and 9). The junction between adhesive and dentin appeared tight, thin and continuous, but a distinct hybrid layer was not detected (Figure 10). In Group 5, where the etch and rinse system PQ Clear was used, an extensive penetration of adhesive into

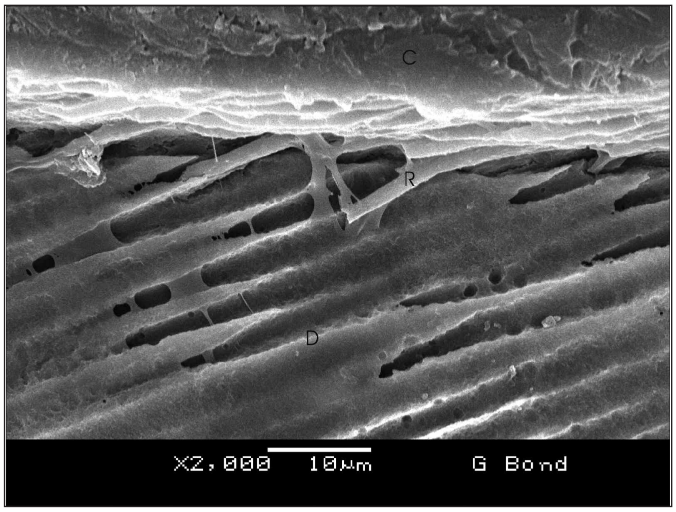

Figure 7. *SEM micrograph of a mild 1-step 1-component adhesive G-BOND (GC Corp) interface with dentin. Resin tags formation is minimal. C: composite resin. R: resin tag. D: dentin.*

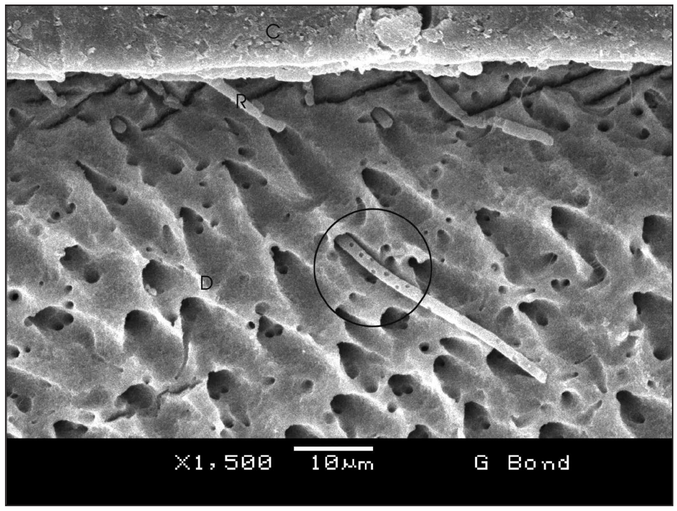

Figure 8. *SEM resin-dentin interface formed by G-Bond. Voids can be seen in the resin tag (circle) C: resin composite. R: resin tag. D: dentin.*

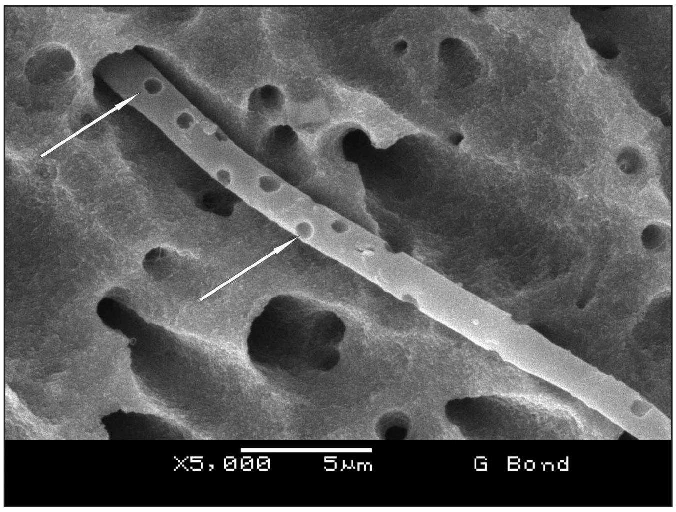

Figure 9. *A larger magnification of the area in circle on Figure 8. Voids in the resin tag (arrows) are a result of water droplet evaporation during specimen preparation for SEM.*

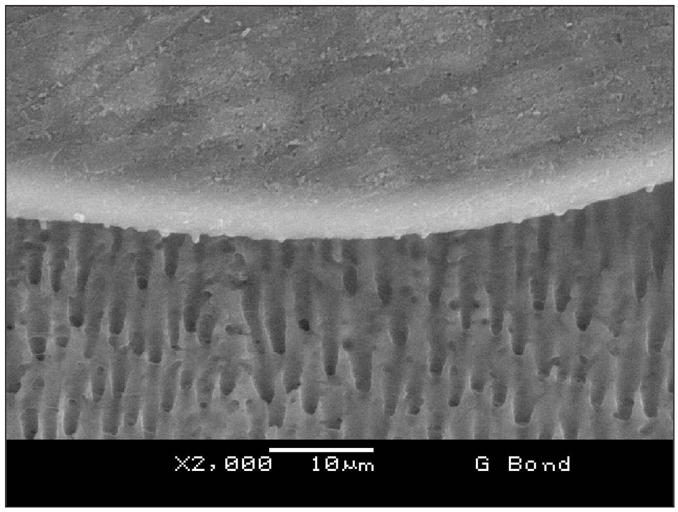

Figure 10. *SEM resin-dentin interface formed by G-Bond. The resin composite lies directly on the dentin; neither the adhesive layer nor the hybrid layer are visible at the transition.*

dentinal tubules was noticeable, forming prominent tags with numerous, fine branching lateral tags (Figure 11). Hybrid layer formation was also seen and ranged between 2-2.5 µm (Figure 12).

DISCUSSION

In order to examine the micromorphology of the interface adhesive system with dentin, adhesives are most commonly applied on flat occlusal surfaces of dentin discs. This method allows for the standardization of dentin depth chosen in the specific experiment and the direction of the dentin tubules. In this study, the authors chose to observe the interface formed in Class V cavities for 2 reasons. The first reason was that the samples that were prepared in this way are more likely to represent the clinical situation involving the difficulties in achieving the suggested width of the adhesive layer for each system, along with the higher C factor and, therefore, higher contraction stress. The other rationale was to make the correlation with microleakage investigations of the same adhesive systems more relevant.

The most widely used method for artificial aging, thermocycling, was performed. The number of cycles (500) was chosen based on the current ISO standard.[26] Nevertheless, there is a need to question the ability of this number of cycles to even partly simulate the complexity of aging processes *in vivo*, since it was concluded that approximately 10,000 cycles correspond to not more than 1 year of clinical service.[27]

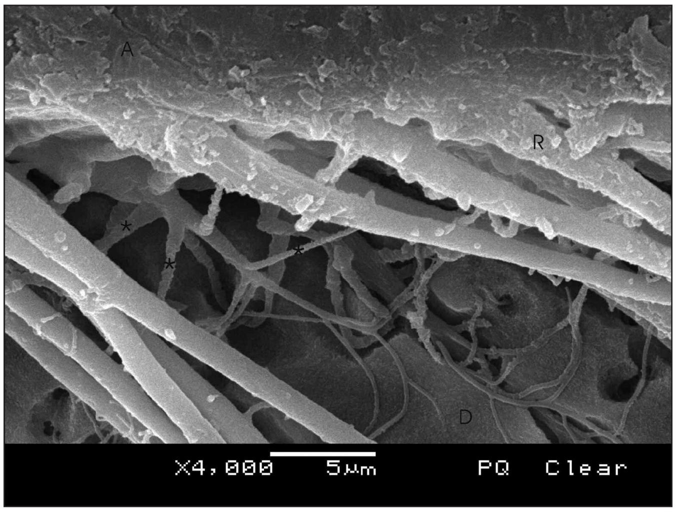

Figure 11. *SEM micrograph of a 2-step etch and rinse adhesive system PQ Clear (Ultradent) interface with dentin. The formation of resin tags and numerous and fine branching lateral tags (asterisks) is clearly visible A: adhesive layer. R: resin tag. D: dentin.*

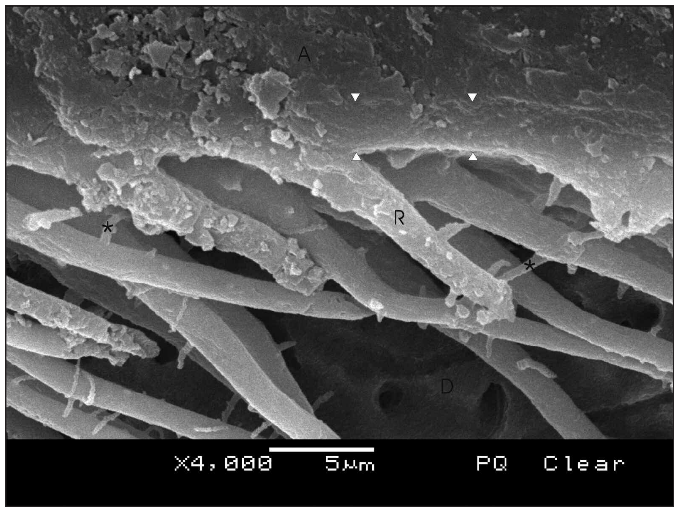

Figure 12. *SEM resin-dentin interface formed by PQ Clear. Resin tags show lateral branches (asterisks). Hybrid layer formation can be seen, ranging from 2-2.5 µm (between arrowheads). A: adhesive layer. R: resin tag. D: dentin.*

Table 2: *pH Values of Self-etching Primers/Adhesives**

Component	pH value
AdheSE primer	1.5
Contax primer	1.3
Futurabond NR (mixture A+B)	1.4
G-Bond	2

**Information from the manufacturers.*

It is well known that specimen preparation procedures for scanning electron microscopy induce artifacts. Perdigão and others[28] and Carvalho and others[29] investigated 3 possible alternative techniques to critical point drying that had most commonly been used. Among hexamethyldisilazane (HMDS) drying, Peldri II drying and air drying, HMDS was found to be a good alternative for critical point drying in preparing the dentin specimens for SEM. In the latter study, it was also shown that all investigated forms of specimen preparation caused significant shrinkage artifacts. In this study, air drying was performed, bearing in mind that the exact dimensions of morphology features seen under SEM have been influenced by shrinkage that occurred due to specimen preparation.

The pH values of self-etching primers or adhesives (in the case of Futurabond NR and G-Bond) are shown in Table 2. The degree of demineralization and interaction with dentin correlates with the acidity of the self-etching primers/adhesives, as observed in previous investigations.[24-25] Table 3 lists the composition of the adhesive systems used.

AdheSE primer contains hydrolytically stable monomers, water as a solvent and no solvent in the adhesive. In terms of bond strength[30-31] and morphology,[3] such a hydrophobic bonding layer applied after the self-etching primer may explain the favorable results obtained with this adhesive. In the latter study, under TEM, it was shown that AdheSE completely dissolved the dentin smear layer and created a 2 µm-thick hybrid layer within the intertubular dentin, with some nanoleakage observed within the hybrid layer, while the adhesive layer was completely devoid of water trees. The shallower, less pronounced hybrid layer seen in this study (Figure 2) may be attributed to the SEM methodology used. As mentioned previously, the method of drying the specimens could have caused excessive shrinkage, thus underestimating the width of the hybrid layer.[32] Shrinkage induced by vacuum in the SEM chamber causes additional difficulties in identifying hybrid layers that are shallow in nature. Numerous resin tags and lateral tags indicate that the smear layer was sufficiently dissolved by the self-etching primer, correlating with the TEM findings of Frankenberger and Tay.[3]

So far, no studies have been published that describe either the Contax or Futurabond NR relationship with tooth tissue. Contax primer contains maleic acid, which is known to remove the smear layer.[33] In a study by Breschi and others,[34] maleic acid, with a pH of 1.4, was shown to demineralize dentin to a depth from 4.8 to 5.5 µm and the ability of this organic acid to dissolve the mineral phase of dentin is related to the pH value. Although maleic acid in the Contax adhesive system is the active component of the self-etching primer that dissolves the smear layer and incorporates it into a hybrid layer, the pH value of 1.3 might explain why the hybrid layer was easier to recognize in this group. The manufacturer states in the instructions that "it is not necessary to thin out the primer." However, on the

Table 3: *Composition of Adhesive Systems Used*

Adhesive System	Etchant/Primer		Adhesive
AdheSE	(primer) Phosphonic acid acrylate Bis-acrylamide Water Initiators Stabilizers		(adhesive) Dimethacrylates Hidrohy-ethil methacrylate Highly dispersed silicon dioxide Initiators Stabilizers
Contax	(primer) Water Maleic acid Sodium fluoride		(adhesive) Hydrophilic, acidic Bis-GMA-based resin matrix Water Additives, catalysts
Futurabond NR	Liquid A: Polyfunctional adhesive monomers (Methacroyl-Phosphorus-Acid-Ester, Methacroyl-Carbon-Acid-Ester), Dimethacrylates, Functionalized SiO_2-nano-particles, Initiators Liquid B: Ethanol, Water, Hydrophilic adhesive monomers, Fluorides		
G-Bond	4-MET Phosphate ester Urethane-dimethacrylate Acetone Water Silica fine powder Catalyst		
PQ Clear	35% orthophosphoric acid	Methacrylate resins Ethanol solvent Silicate fillers (filler content 8%) Camphorquinone Initiator (proprietary)	

specimens used in this study, the primer that was applied in this manner pooled in the corners of the cavity. For this reason, the specimens were dried gently with a weak stream of air to evaporate water, while ensuring that the surface of the cavities remained coated with primer.

Futurabond NR is a new adhesive system, which, unlike its previous version (Futurabond, Voco), is applied in a single layer. The other changes include replacing the water/acetone solvent base with the water/ethanol combination and adding nanofillers. Futurabond NR consists of 2 types of polyfunctional monomers that need to be mixed with water immediately before use. Water and ethanol are components of liquid B. According to the manufacturer's information, adhesion is explained as a mixture of retentive micromechanical interaction and chemical adhesion due to the Ca-complexing character of adhesive monomers. The pH value of Futurabond is in the range of AdheSE and Contax primers. The interface morphology can be described as similar to AdheSE and Contax, although it was not as uniform, and resin tags were more scattered and thinner, while a hybrid layer could not be seen (Figure 5). The simplified application system in 1 clinical step might serve as a possible explanation for this variation.

In the case of G-Bond, the pH value of 2 may explain the shallow interaction zone with dentin. This adhesive also differs from the other groups by how it is used: besides the fact that it is extremely simplified, it should be dried under maximum air pressure before polymerization, thus forming a very thin layer. Theoretically, this should evaporate all remaining acetone and water. However, voids seen in the resin tag (Figure 9) represent remnants of water in the form of droplets, which were trapped in the adhesive before polymerization. During the desiccation procedure, water evaporated, leaving voids. These voids seem to correlate with the droplets seen on samples of the experimental 1-step/1-component adhesive (marked as Exp-Ac) in a study by Van Landuyt and others.[35] In their investigation, the number of water droplets was significantly reduced after strong air drying, although they did not completely disappear. In this study, the fact that adhesive was placed in cavity preparations may serve as an additional explanation why water could not be completely eliminated, even though the adhesive was strongly dried. The role that water remnants and oxygen inhibition play in the long-term performance of such thin adhesive layers needs to be determined. It was recently suggested that the morphological feature seen at the interface of mild self-etching systems with dentin should be termed as the nano interaction zone.[36-37] These abstracts reported a very thin (less than 300 nm depth) hybrid layer formed with G-Bond, viewed under TEM, with the hydroxyapatite crystals mostly remaining within the hybrid layer. Since the pH value of G-Bond is high, it is speculated that the amount of hydroxyapatite remaining around the collagen fibers is sufficient to enable the additional chemical interaction.[4] Although the SEM method used in this study does not allow for detailed observation of such a shallow interaction zone, it is reasonable to expect a certain degree of chemical interaction, bearing in mind that this adhesive contains 4-MET monomer, which was proven to form ionic bonds with hydroxyapatite.[15] From the clinical standpoint, it can be speculated that an additional chemical interaction may be beneficial for the long-term quality of the tooth restoration interface formed by this adhesive.

The limitations of conventional scanning electron microscopy compared to more sophisticated methods of examining tooth-restoration interfaces have been recognized and described.[38] However, regardless of the method used (SEM or TEM), no correlation between the hybrid layer width and bond strength of the adhesive systems could be determined.[39-40] This finding was also confirmed for the adhesive systems investigated in this study (personal unpublished results), all of which showed satisfactory values of microtensile bond strength to dentin, even when nearly no micromechanical interlocking could be observed by the SEM, as in the case of G-Bond.

From a clinical standpoint, long resin tags and a thick hybrid layer do not necessarily imply superior behavior towards adhesive systems in which these features are less pronounced. One of the most frequently investigated self-etching adhesive systems, Clearfil SE bond (Kuraray), forms a submicron hybrid layer and short resin tags.[41] However, in a 2-year clinical study, this mild 2-step self-etching adhesive had shown a retention rate that was not statistically different from a 2-step etch and rinse adhesive. No post-operative sensitivity was recorded for either of 2 adhesive systems.[42]

It can be speculated that the adhesives investigated in this study can obtain reliable adhesion *in vivo*, as well. Nevertheless, for the strong clinical recommendation, the results should be complimented with long-term clinical evaluation. The SEM method can, however, provide basic information which points to possible specific features of the interface that need to be examined more closely

CONCLUSIONS

All investigated self-etching systems showed similar interfacial morphology with dentin, except for the 1-step/1-component adhesive G-Bond. The degree of demineralization and interaction with dentin correlated with the acidity of the self-etching primers/adhesives. Less resin tags were formed in all self-etching systems than in the etch and rinse system.

Acknowledgements

The authors thank the manufacturers for their generous donation of all materials used in this study. Special thanks go to Mr Milos Bokorov, University of Novi Sad, Serbia and Montenegro, for kind assistance with the SEM.

(Received 3 November 2005)

References

1. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry *Operative Dentistry* **Supplement 6** 119-144.
2. Inoue S, Vargas MA, Abe Y, Yoshida Y, Lambrechts P, Vanherle G, Sano H & Van Meerbeek B (2001) Microtensile bond strength of eleven contemporary adhesives to dentin *Journal of Adhesive Dentistry* **3(3)** 237-245.
3. Frankenberger R & Tay FR (2005) Self-etch vs etch-and-rinse adhesives: Effect of thermo-mechanical fatigue loading on marginal quality of bonded resin composite restorations *Dental Materials* **21(5)** 397-412.
4. 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.
5. 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.
6. Unterbrink GL & Liebenberg WH (1999) Flowable resin composites as "filled adhesives": Literature review and clinical recommendations *Quintessence International* **30(4)** 249-257.
7. Ferrari M & Tay FR (2003) Technique sensitivity in bonding to vital, acid-etched dentin *Operative Dentistry* **28(1)** 3-8.
8. Gwinnett AJ (1992) Moist versus dry dentin: Its effect on shear bond strength *American Journal of Dentistry* **5(3)** 127-129.
9. Kanca J 3rd (1992) Effect of resin primer solvents and surface wetness on resin composite bond strength to dentin *American Journal of Dentistry* **5(4)** 213-215.
10. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P & Vanherle G (1992) Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems *Journal of Dental Research* **71(8)** 1530-1540.
11. Sano H, Shono T, Takatsu T & Hosoda H (1994) Microporous dentin zone beneath resin-impregnated layer *Operative Dentistry* **19(2)** 59-64.
12. Wang Y & Spencer P (2003) Hybridization efficiency of the adhesive/dentin interface with wet bonding *Journal of Dental Research* **82(2)** 141-145.
13. Kato G & Nakabayashi N (1998) The durability of adhesion to phosphoric acid etched, wet dentin substrates *Dental Materials* **14(5)** 347-352.
14. Hashimoto M, Ohno H, Sano H, Kaga M & Oguchi H (2003) *In vitro* degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy *Biomaterials* **24(21)** 3795-3803.
15. 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.
16. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H & Oguchi H (2000) *In vivo* degradation of resin—dentin bonds in humans over 1 to 3 years *Journal of Dental Research* **79(6)** 1385-1391.
17. Cheong C, King NM, Pashley DH, Ferrari M, Toledano M & Tay FR (2003) Incompatibility of self-etch adhesives with chemical/dual-cured composites: Two-step vs one-step systems *Operative Dentistry* **28(6)** 747-755.

18. Tay FR, Pashley DH & Yoshiyama M (2002) Two modes of nanoleakage expression in single-step adhesives *Journal of Dental Research* **81(7)** 472-476.

19. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C & Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives *Biomaterials* **26(9)** 1035-1042.

20. Tay FR, Pashley DH, Suh BI, Carvalho RM & Itthagarun A (2002) Single-step adhesives are permeable membranes *Journal of Dentistry* **30(7-8)** 371-382.

21. Tay FR & Pashley DH (2003) Water treeing—a potential mechanism for degradation of dentin adhesives *American Journal of Dentistry* **16(1)** 6-12.

22. Tay FR, Pashley DH, García-Godoy F & Yiu CK (2004) Single-step, self-etch adhesives behave as permeable membranes after polymerization Part II Silver tracer penetration evidence *American Journal of Dentistry* **17(5)** 315-322.

23. Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH, Carvalho RM & Lai CN (2004) Single-bottle adhesives behave as permeable membranes after polymerization. I. *In vivo* evidence *Journal of Dentistry* **32(8)** 611-621.

24. 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.

25. De Munck J, Vargas M, Iracki J, Van Landuyt K, Poitevin A, Lambrechts P & Van Meerbeek B (2005) One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin *Operative Dentistry* **30(1)** 39-49.

26. ISO-Standards (2003) ISO/TS 11405:2003 Dental materials—Testing of adhesion to tooth structure Geneve: International Organization for Standardization 2nd edition 2-7.

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

28. Perdigão J, Lambrechts P, Van Meerbeek B, Vanherle G & Lopes AL (1995) Field emission SEM comparison of four post-fixation drying techniques for human dentin *Journal of Biomedical Materials Research* **29(9)** 1111-1120.

29. Carvalho RM, Yoshiyama M, Brewer PD & Pashley DH (1996) Dimensional changes of demineralized human dentine during preparation for scanning electron microscopy *Archives of Oral Biology* **41(4)** 379-386.

30. Goracci C, Sadek FT, Monticelli F, Cardoso PE & Ferrari M (2004) Microtensile bond strength of self-etching adhesives to enamel and dentin *Journal of Adhesive Dentistry* **6(4)** 313-318.

31. Sensi LG, Lopes GC, Monteiro S Jr, Baratieri LN & Vieira LC (2005) Dentin bond strength of self-etching primers/adhesives *Operative Dentistry* **30(1)** 63-68.

32. Itou K, Torii Y, Oyama F, Yoshiyama M & Pashley DH (2003) Effect of drying methods on hybrid layer thickness *American Journal of Dentistry* **16(5)** 335-339.

33. Prati C, Biagini G, Nucci C, Castaldini C & Zucchini C (1990) Effects of chemical pretreatments on dentin bonding *American Journal of Dentistry* **3(5)** 199-206.

34. Breschi L, Gobbi P, Mazzotti G, Falconi M, Ellis TH & Stangel I (2002) High resolution SEM evaluation of dentin etched with maleic and citric acid *Dental Materials* **18(1)** 26-35.

35. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P & Van Meerbeek B (2005) Monomer-solvent phase separation in one-step self-etch adhesives *Journal of Dental Research* **84(2)** 183-188.

36. Arita A, Uno S & Yamada T (2004) Adhesive interface observation of one-step/one component adhesive *Journal of Dental Research* **83(Special Issue B)** Abstract #0065 (http://www.dentalresearch.org)

37. Koshiro K, Ikeda T, Inoue S, Sidhu S & Sano H (2005) Nano interaction zone: A new concept of resin-dentin interface *Journal of Dental Research* **84(Special Issue A)** Abstract #2935 (www.dentalresearch.org)

38. Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Perdigão J, Lambrechts P & Vanherle G (2000) Microscopy investigations. Techniques, results, limitations *American Journal of Dentistry* **13(Special Issue)** 3D-18D.

39. Nakabayashi N & Saimi Y (1996) Bonding to intact dentin *Journal of Dental Research* **75(9)** 1706-1715.

40. Perdigão J, May KN Jr, Wilder AD & 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.

41. Inoue S, Van Meerbeek B, Vargas M, Yoshida Y, Lambrechts P & Vanherle G (2000) Adhesion mechanism of self-etching adhesives. In: Tagami J, Toledano M, Prati C (eds) *Advanced Adhesive Dentistry. Proceedings Book of the Granada 3rd International Kuraray Symposium: December 3-4, 1999* Cirimido: Grafiche Erredue, p 131-148.

42. Turkun SL (2003) Clinical evaluation of a self-etching and a one-bottle adhesive system at two years *Journal of Dentistry* **31(8)** 527-534.