## Title
Analysis of interfacial structure and bond strength of self-etch adhesives.



## Journal
American Journal of Dentistry, 26(6)

## ISSN
0894-8275

## Authors
Pinzon, Lilliam M
Watanabe, Larry G
Reis, Andre F
et al.

## Publication Date
2013-12-01

Peer reviewed

Published in final edited form as:
*Am J Dent*. 2013 December ; 26(6): 335–340.

# Analysis of interfacial structure and bond strength of self-etch adhesive systems

**Lilliam M Pinzon, DDS, MS, MPH [Assistant Professor]**,
Department of Preventive and Restorative Dental Sciences, University of California, San Francisco, 707 Parnassus Avenue, D-2244. San Francisco, CA 94143-0758

**Larry G Watanabe, BS, MS [Senior Development Engineer]**,
Department of Preventive and Restorative Dental Sciences. University of California, San Francisco

**Andre F Reis, DDS, MS, PhD [Assistant Professor]**,
Department of Operative Dentistry, School of Dentistry, University of Guarulhos, Guarulhos, São Paulo, Brazil

**John M Powers, PhD [Clinical Professor of Oral Biomaterials]**,
Department of Restorative Dentistry and Prosthodontics. University of Texas School of Dentistry at Houston, Houston

**Sally J Marshall, PhD [Professor]**, and
Department of Preventive and Restorative Dental Sciences. University of California, San Francisco

**Grayson W Marshall, DDS, MPH, PhD [Professor]**
Department of Preventive and Restorative Dental Sciences. University of California, San Francisco

## Abstract

**Purpose—**To determine the bond strength, nanoleakage and interfacial morphology of four self-etch adhesives bonded to superficial dentin.

**Methods—**Micro-tensile (MT, n=15) and single plane shear (SP, n=8) bond tests were performed using human dentin polished through 320-grit SiC paper. Clearfil Protect Bond (PB), Clearfil S[3] Bond (S3), Prompt L-Pop (PLP) and G-BOND (GB) were used according to manufacturers' instructions. Composite was applied as cylinders with a thickness of 4 mm with a 1-mm diameter and stored in water at 37° C for 24 hours. Specimens were debonded with a testing machine at a cross-head speed of 1 mm/min. Means and standard deviations of bond strength were calculated. Data were analyzed using ANOVA. Fisher's PLSD intervals were calculated at the 0.05 level of significance. Failure modes were determined at 100X. The hybrid layer was revealed by treatment with 5N HCl/5% NaOCl or fractured perpendicular to the interface and sputter coated with gold. Specimens were viewed at 1000X, 2500X, and 5000X in a field emission SEM at 15 kV. Teeth (n=2) sectioned into 0.9-mm thick slabs were immersed in ammoniacal silver nitrate solution for 24 hours, rinsed and immersed in photo-developing solution for 8h. Specimens were sectioned (90-nm thick) and observed under TEM.

Telephone: (415) 476 2048, Fax: (415) 476 0858, Lilliam.Pinzon@ucsf.edu.

DISCLOSURE STATEMENT: Each author has no conflict of interest.

[j]Philips Electron Optics Inc., Hillsboro

**Results—**Means ranged from 25.0 to 73.1 MPa for MT and from 15.5 to 56.4 MPa for SP. MT values were greater than SP, but were highly correlated ($R^2$ = 0.99, p= 0.003) and provided the same order for the systems studied. Fisher's PLSD intervals ($p<0.05$) for bond strength techniques and adhesives results were 1.7 and 2.3 MPa, respectively. Failures sites were mixed. TEM showed that hybrid layers were ~0.5 μm for PB, GB and S3 and ~5 μm for PLP. SEM showed morphologic differences among adhesives. Silver nitrate deposits were observed within interfaces for all adhesive systems.

**Clinical significance—**Simplification of application procedures appears to induce loss of adhesion capabilities. In this in vitro study we evaluated the bond strength, the micro and nano morphology of four self-etch adhesive systems. In summary, the microtensile bond strengths ranged from 25 to 73 MPa. None of the adhesive systems tested was able to totally prevent nanoleakage, but there were differences among systems. No relationship was observed between thickness of the hybrid layer and bond strength.

## Introduction

Adhesion of resin composite to tooth structure has been a challenge in the history of adhesive resin systems. In order to bond two bodies or surfaces, it is necessary to have both surfaces as close as possible. Furthermore, the adhesive should include characteristics such as flowability, low viscosity, and substrate wettability.

Dentin is composed of 30% organic (collagen fibers), 50% inorganic material (mineralized apatite crystals) and 20% water.[1] These percentages of inorganic and organic material and water content vary proximity to the pulp. The dentinal tubules contain more dentinal fluid and become larger and occupy a higher area fraction as they approach the pulp,[2] Due to this heterogeneity, dentin can be described as a dynamic substrate for bonding.[3] Moisture differences can affect the compatibility between adhesive systems and tooth surfaces.[4]

The hybrid layer was initially described by Nakabayashi as "the structure formed in hard dental tissues by demineralization of the surface and subsurface, followed by infiltration of monomers and subsequent polymerization."[5] The main goal of the self-etching adhesive systems is to infiltrate resin monomer through the smear layer as well as to demineralize and infiltrate the underlying dentin to form a hybrid layer simultaneously.[6] Self-etching adhesive systems are water based and mixed with acidic monomers, such as carboxylic acid and phosphate ester and HEMA and phosphate ester.[7]

With the self-etching adhesive systems, the smear layer becomes part of the bonding substrate since the acidic primer is not rinsed from the surface and the demineralized smear layer may be incorporated into the hybrid layer.[8] The primers in these systems are acidic enough to demineralize the smear layer and the top layer of the underlying dentin surface. As they etch, they also infiltrate the exposed collagen with hydrophilic monomers, which then co-polymerize with the subsequently placed adhesive resin. The concept of an acidic primer is attractive, because in theory this system simultaneously infiltrates the collagen fibers as it decalcifies the inorganic component to the same depth in dentin. This technique should minimize exposed and demineralized collagen since demineralized dentin should be encapsulated by the resin primer and/or adhesive.[9] Self-etch adhesive systems in a longitudinal clinical study showed decreased post-operative sensitivity of resin-based restorations.[10]

It was anticipated that the four self-etching adhesives investigated this study would produce different bond strength, nano-leakeage capabilities and micro-structure results because of their difference in chemical composition and acidity. But they all have the same indications in restorative cases. Dental material manufacturers have developed more simplified self-

etching adhesive systems that contain all their components in a single bottle or in two bottles. These adhesive systems became beneficial by limiting the influence of poor handling of the material by the practitioner[11] but also become a negative factor by restricting the efficiency of the bonding process.[12]

There are innumerable testing techniques used in dental research to assess the bond strength of various materials used in the different dental fields. Authors have reported that shear bond strength is the most common technique used to test bond strength properties of adhesive systems,[13, 14, 15] although other authors have questioned the validity of the technique.[16] However, they affirm that the interface is critical and have suggested that it is perhaps more reliable to use a tensile strength technique.[16]

The microtensile test method[17] offers versatility that cannot be achieved by conventional methods. It is more labor intensive than conventional testing, but attempts to reduce non-uniform stress distribution at the adhesive interface.[18] It also holds potential for providing insight into the strength of adhesion of restorative materials to small clinically relevant sites and substrates.[19] While this technique offers advantages, a distinct disadvantage is technique sensitivity during the preparation phase, caused by alteration in the dimension of the specimens or in the load distribution.[19, 20, 21]

The quality of the adhesion between the dental substrates and the restorative materials is a very important problem to be solved in dentistry. The clinical success of dental restorations can be predicted using different technologies and techniques to measure the mechanical properties and the interface structure quality and characteristics in general. In this study we measured the mechanical property “bond strength” using two different techniques (microtensile and single plane shear bond tests) to evaluate any correlation between the results with the two different techniques. We also assessed the interfacial structure under scanning electron microscopy and transmission electron microscopy in order to characterize the morphological characteristics of the different adhesive/dentin interfaces and better understand the bond strength results.

**The null hypotheses of this study were:**

1. There are no significant differences in bond strength among the four self-etching bonding agents.
2. There are no significant differences in bond strengths between the microtensile and single plane lap shear bond strength testing methods.
3. There are no significant differences in dentin/adhesive microstructure and leakage among the four self-etching bonding agents.

## Methods

Manufacturers of the materials, batch numbers, and products used in this study are listed in Table 1.

### Bonding Procedures (Bond Strength)

It is important to note that both testing methods utilized similar bond areas but different geometries (round for shear vs. square for micro-tensile).

The microtensile bond strength test (MT) part of this study was a randomized, semi-clustered, in-vitro design study testing the bond strength of four self-etch adhesive systems bonded on dentin substrates. Three randomly selected teeth were prepared for MT specimens for each of the 4 adhesive systems. The teeth were ground parallel to the occlusal

surface with 60-grit SiC paper (Carbimet Paper Disc)[a] on a polishing machine (Ecomet 6)[a] to expose superficial dentin and then finished with 320-grit SiC paper (Carbimet Paper Disc).[a] The specimens were randomly bonded following the manufacturers' instructions for the four adhesives with their respective composites [CLEARFIL PROTECT BOND/ CLEARFIL APX (PB), CLEARFIL $S^3$ BOND/CLEARFIL APX,[b] (S3), G-BOND/GC Gradia (GB)[c] and Adper Prompt L Pop Plus/Filtek Supreme,[d] (PLP)].

The instructions for adhesive systems are listed below.

1. Clearfil Protect Bond (PB). The bonding surface was rinsed and blotted dry with a moist cotton pellet. Two drops of Primer were dispensed into a mixing dish and applied to the bonding surface with a brush. The Primer was left in place for 20 seconds and then air dried with a mild oil-free air stream to evaporate the solvent. The bond was then dispensed into a mixing well. It was applied to the bonding surface and blown with a mild air stream to make a uniform film. It was light cured for 10 seconds. A 4-mm thick restoration was prepared with Clearfil APX with light curing of two, 2-mm increments.

2. Clearfil $S^3$ (S3). . The bonding surface was rinsed and blotted dry with a moist cotton pellet.. Two drops of the adhesive were dispensed in a mixing dish. $S^3$ was applied, left on the surface for 20 seconds, and then blown with high pressure air for 5 seconds. It was light cured for 10 seconds. A 4-mm restoration was prepared with Clearfil APX with light curing of two, 2-mm increments.

3. Adper Prompt L Pop Plus (PLP). The bonding surface was rinsed and blotted dry with a moist cotton pellet. Two drops of the adhesive were dispensed in a mixing dish. Two coats he adhesive were applied to the entire surface, rubbing in the solution with moderate finger pressure for 15 sec, a gentle stream of air to thoroughly dry the adhesive to a thin film, and was finally light cure for ten seconds A 4-mm restoration was prepared with Filtek Supreme with light curing of two, 2-mm increments.

4. G-Bond (GB). The bonding surface was rinsed and blotted dry with a moist cotton pellet. Two drops of the adhesive were dispensed in a mixing dish. Two coats he adhesive were applied to the entire surface, left undistributed for 10 seconds, dry thoroughly for 5 seconds under maximun air pressure, and was finally light cure for ten seconds A 4-mm restoration was prepared with GC Gradia with light curing of two, 2-mm increments.

Composites were applied in a thickness of ~ 4 mm. Photopolymerization was accomplished with a halogen light-curing unit (Optilux 501)[e]. The light output was verified with a curing radiometer[f] to be at a level of greater than 770 mW $cm^{-2}$ throughout the study. The bonded specimens were stored in Hank's balanced salt solution for 24 hours at 37 °C in an incubator. After 24 hours, each specimen was mounted in an epoxy resin[g] block and sectioned perpendicular to the bonded surface in 1-mm thick slabs. These 1-mm thick slabs were again remounted in epoxy blocks and sectioned to form sticks perpendicular to the bonding interfaces, resulting in 1-$mm^2$ bonding areas. 15 adhesive/dentin sticks per group were randomly selected for MT testing.

[a]Buehler, Lake Forest, IL, USA
[b]Kuraray America, New York, NY
[c]GC America, Alsip, IL, USA
[d]3M ESPE, St. Paul, MN, USA
[e]SDS/Kerr Demetron, Orange, CA, USA
[f]Demetron Research Corp., Danbury, CT, USA
[g]Cole-Parmer Instruments Co, Vernon Hills, IL, USA

For the single plane shear test[13,14,15], 8 randomly selected freshly extracted third molars were selected and bonded with each adhesive following the procedures of Watanabe et al.[14]

The specimens were debonded with a universal testing machine (Instron)[h] at a cross-head speed of 1 mm/min. Bond strengths were reported in MPa. Failure modes of the debonded specimens were determined at 100X (optical microscopy).

Bond strength data were analyzed using ANOVA. Fisher's PLSD intervals were calculated at the 0.05 level of significance.

## Scanning and Transmission Electron Microscopy Analyses

Four additional teeth were bonded with each one of the adhesives following the manufacturer's instructions. After 24 hours the specimens were then sectioned perpendicular to the adhesive interface with a water cooled slow-speed diamond saw (Isomet)[a] to produce four slices. Two out of the four slabs obtained from each tooth were assigned for scanning electron microscopy (SEM) and the other two were prepared for transmission electron microscopy (TEM). For SEM analyses one slice was acid-etched with 5N HCl for 30 sec followed by 5% NaOCl for 30 minutes and rinsed thoroughly with distilled water to reveal the hybrid layer (HL). The other slice was fractured perpendicular to the interface. Each slice was then dehydrated in successive concentrations of ethanol (33%, 67% and 85%) for 30 minutes at each concentration and absolute ethanol for 60 min. The specimens were left overnight to dry and were then mounted on 12-mm aluminum stubs and sputter coated with approximately 20 nm of gold-palladium alloy. The specimens were viewed at 3 magnifications (1000X, 2500X, and 5000X) and various tilt angles in a XL30 ESEM-FEG 515 field emission microscope at 10 kV[j]. Analyses of the interface microstructure (hybrid layer, resin tag quality, and compactness of the different layers) were based on at least 20 images taken along the length of the dentin-adhesive interface.

## Leakage Evaluation under Transmission Electron Microscopy

The two slabs assigned for TEM analysis were vertically, serially sectioned in the bucco-lingual direction into 0.9 mm-thick slabs using a diamond impregnated saw (Isomet)[a] under water lubrication. Bonded slabs were coated with two layers of nail varnish applied up to within 1 mm of the bonded interfaces. To rehydrate specimens, they were immersed in distilled water for 10 min prior to immersion in the tracer solution for 24 hours. Ammoniacal silver nitrate was prepared according to the protocol previously described by Tay et al.[9] Tooth slabs were placed in the ammoniacal silver nitrate in total darkness for 24 hours, rinsed thoroughly in distilled water, and immersed in photo developing solution for 8 h under a fluorescent light to reduce silver ions into metallic silver grains within voids along the bonded interface.

Undemineralized, epoxy resin-embedded, ultrathin sections were prepared for TEM. One strip approximately 6 mm wide was sectioned from each slab perpendicular to the flat dentin surface using a diamond saw under copious water supply. Specimens were fixed in Karnovsky's solution, post-fixed in osmium tetroxide, dehydrated in ascending ethanol series (30 to100%), and embedded in epoxy resin. Care was taken to ensure proper orientation of the resin–dentin interface. Representative 90-nm thick ultrathin sections were prepared with an ultramicrotome (MT-2C, RMC)[l] using a diamond knife and collected on

[h]Instron Corp., Canton, MA, USA
[i]Enterprise, Norcross, GA, USA
[l]MT-2C, RMC, Miami, Florida

100-mesh, formvar-coated copper grids. Without additional staining, they were observed in a transmission electron microscope (Zeiss EM 900)[k] operated at 50kV.

## Results

Means and ranges in MPa for the bond strength results of the four self-etch adhesive systems as well for the individual techniques are shown (Fig. 1). The means and standard deviations for each adhesive were: PB with MT = 73.1 (5.5) and PB with SP =56.4 (2.3); S3 tested with MT = 39.0 (3.4) and S3 tested with SP = 29.7 (3.5); GB tested with MT = 39.6 (4.9) and GB tested with SP = 30.4 (2.8); and PLP tested with MT = 25.0 (3.0) and PLP tested with SP = 15.5 (2.3). PB showed the highest bond strength compared with the other 3 bonding agents and PLP showed the lowest bond strengths. Furthermore, there was a constant pattern of lower results with single plane shear test compared with micro-tensile test for all the bonding agents, but data from both tests were highly correlated ($R^2 = 0.99$, $p= 0.003$) and provided the same order for the systems studied. Failure sites were mainly adhesive.

### Scanning Electron Microscopy Analysis

The fractured specimens evaluated by SEM in the PB group (Fig. 2) showed some resin tags, no visible hybrid layer, and an adhesive layer about 10 μm thick. The GB group (Fig. 3) and S3 group (Fig. 4) showed some resin tags and no visible hybrid layer. The PLP group (Fig. 5) showed some resin tags, and a hybrid layer of about 5 μm. Differences were observed by SEM among the adhesive systems.

### Transmission Electron Microscopy Analysis

Observation of undemineralized, unstained sections for all the adhesives showed some leakage. In fact, the silver deposit patterns were different among the bonding agents.

An undemineralized, unstained TEM micrograph of a resin-dentin interface produced by the two-step self-etching adhesive PB (Fig. 6) showed a thin hybrid layer of ≤ 0.5 μm. After immersion in ammoniacal silver nitrate, very small silver deposits were observed on top of the hybrid layer (HL). The single-step self-etching adhesive S3 (Fig. 7) showed a thin hybrid layer of ≤ 0.5 μm. A few silver deposits were observed within the hybrid and adhesive layers. Smear plugs (SP) were visible. For GB (Fig. 8), a thin hybrid layer of ≤ 0.5 μm was observed. Silver deposits for GB were observed not only within the hybrid layer (HL), but also within the mineralized dentin beneath the hybrid layer. Smear plugs were present (SP). PLP (Fig. 9) presented a hybrid layer of ≤ 5 μm and only partially demineralized dentin.

In summary, PB, GB, and S3 had thin hybrid layers of ≤ 0.5 μm. On the other hand, PLP showed thick hybrid layers of approximately 5 μm. There was no apparent relationship between hybrid layer thickness and bond strength.

## Discussion

Based on the results of this study, all the null hypotheses were rejected due to significant differences in bond strength among the four bonding agents and between the two different bond strength testing techniques.

Durability is one of the most challenging problems of adhesive-dentin bonds.[22] Degradation of adhesive–dentin bonds is caused partially by the acid-etching step in the adhesion procedure that exposes and activates endogenous dentin matrix metalloproteinases (MMPs).

[k]Zeiss, Munich, Germany

Once MMPs are activated during the bonding procedure they can degrade the collagen fibrils, causing failure of the adhesive-dentin bonds.[23, 24, 25, 26] Another factor that affects the resin-dentin bonds is the water-sorption-induced hydrolysis of the hydrophilic resin components present in these adhesives.[27, 28]

PB was the adhesive system with the highest bond strength values and appeared to have the lowest apparent silver deposition among the adhesive systems evaluated in this study. These results might be caused by the chemical composition, compatibility and chemical interaction with the dentin substrate that might be more beneficial for this adhesive system than for the other three adhesive systems. PB contains MDPB monomer, which has an antibacterial cavity cleansing effect[26] and could inhibit MMPs from degrading the collagen.[29] Furthermore, MDPB reportedly creates strong chemical bonds to calcium and polymerized adhesive resin and is water resistant.[26] For these reasons it may allow long-term suppression of MMPs and create hydrolytic stability in the adhesion interface leading to long-term reliability.[28, 29] The adhesive for PB is solvent-free and reatively hydrophobic like SE bond that seals dentin better than solvated adhesives.[30]

On the other hand, PLP had the lowest bond strengths, thickest hybrid layer and highest apparent silver deposition compared to the other 3 adhesive systems evaluated in this study. The acidic monomer in the primer might be too acidic, since it has a pH = 1.1[31], the lowest of the systems studied. This might lead to deeper demineralization that extends beyond the hybrid layer and into the contiguous dentin, producing a weaker substrate after the polymerization of the adhesive system, thus resulting in lower bond strengths. Previous studies showed that PLP has an adhesive/dentin interface morphology similar to the conventional total-etch adhesive systems,[31, 32, 33] probably because of its low pH. The PLP hybrid layer was ~5 μm, which was substantially larger than the other three adhesive systems evaluated in this study (PB, S3 and GB). A previous study[31] reported that the demineralization process caused by PLP was due to the methacrylated phosphoric esters (co-solvent) contained in that adhesive system, with water (solvent) enabling ionization of the acidic monomers and the demineralization of the dentin substrates.

Shear and tensile bond strength tests are commonly used to evaluate the integrity of the adhesion of dental adhesive products to tooth substrates. In fact, the bonding testing methods are highly dependent upon the specimen geometry. The theory behind the microtensile technique is to apply a tensile load through a dentin-adhesive-restorative material beam to debond it at the interface in order to measure the adhesive bond strength. In the shear technique, shear forces are applied through the bond interface to debond the materials (dentin-adhesive-restorative material) as an indication of the adhesive bond strength. The single-plane method of shear-bond strength testing used here has been shown to contain elements of tensile stress.[34] On the other hand, there are differences between single plane method and micro-shear method. In micro-shear studies polyethylene tubes are used as molds, which are then filled with a resin composite. After water storage, the specimens are subjected to test using a scalpel blade to remove these tubes[35, 36, 37] The pressure exerted on the blade in order to cut and remove the polyethylene tubes is transferred to the resin cylinder and consequently can break the specimens. In this study, the shear technique produced lower bond strength results than the microtensile test. However, Micro Tensile and Single Plane Shear techniques results were highly correlated ($R^2$= 0.99, p= 0.003) and provided the same order of bond strength for the systems studied.

## Conclusions

- There were significant differences in bond strengths between techniques and among adhesives. MT values were greater than SP, but the results of the two

techniques were highly correlated ($R^2$= 0.99, p= 0.003) and provided the same order of bond strength for the systems studied.

- SEM analyses showed morphologic differences among adhesives. PLP had a hybrid layer of approximately 5 μm and S3, PB and GB, only partially demineralized dentin, and formed hybrid layers of ≤ 0.5 μm.
- No adhesive system was able to totally prevent leakage, but there were marked differences among systems. Furthermore, PB showed the lowest leakage and highest bond strength and PLP showed the highest leakage and the lowest bond strength.

## Acknowledgments

The authors wish to acknowledge NIH/NIDCRT32 DE07204 (PI: Dr. G. W. Marshall), NIH/NIDCRK23 DE20091-02 (PI: Dr. Pinzon), IADR/NIH/NIDCR Travel Award, Kuraray America, and 3M ESPE for supporting this study.

## References

1. Marshall GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate structure and properties related to bonding. J Dent. 1997; 25:441–458. [PubMed: 9604576]
2. Pashley DH. Dentin: a dynamic substrate – a review. Scanning Microsc. 1989; 3:161–174. discussion 174-6. [PubMed: 2662395]
3. Pashley DH, Sano H, Ciucchi B, Yoshiyama M, Carvalho RM. Adhesion of dentin bonding agents: A review. Dent Mater. 1995; 11:117–125. [PubMed: 8621032]
4. Pereira PNR, Okuda M, Sano H, Yoshikawa T, Burrow MF, Tagami J. Effect of intrinsic wetness and regional difference on dentin bond strength. Dent Mater. 1999; 15:46–53. [PubMed: 10483395]
5. Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res. 1982; 16:265–273. [PubMed: 7085687]
6. Nakabayashi N. Dentinal bonding mechanisms. Quintessence. 1991; 22:73–74.
7. Hayakawa T, Kikutake K, Nemoto K. Influence of self-etching primer treatment on the adhesion of resin composite to polished dentin and enamel. Dent Mater. 1998; 14:99–105. [PubMed: 10023198]
8. Eick JD, Gwinnet AJ, Pashley DH, Robinson SJ. Current concepts or adhesion to dentin. Crit Rev Oral Bio Med. 1997; 8:306–335. [PubMed: 9260046]
9. Gordan VV, Vargas MA, Cobb DS. Evaluation of adhesive systems using acidic primers. Am J Dent. 1997; 10:219–223. [PubMed: 9522695]
10. Gordan VV, Mjör IA. Short- and long-term clinical evaluation of post-operative sensitivity of a new resin-based restorative material and self-etching primer. Oper Dent. 2002; 27(6):543–548. [PubMed: 12413217]
11. Ciucchi B, Bouillaguet S, Holz J, et al. The battle of the bonds 1995. Revue Mensuelle Suisse d'Odontostomatologie. 1997; 107:32–36.
12. Van Meerbeek B, Yoshida Y, Snauwaert J, et al. Hybridization effectiveness of a two-step versus three step smear layer removing adhesive material examined correlatively by TEM and AFM. J Adhes Dent. 1999; 1:7–23. [PubMed: 11725687]
13. Lin E, Hurley E, Perry R. Shear bond strength of three fifth generation adhesive systems. J Dent Res. 1998; 77:132. (Abstract 212).
14. Watanabe LG, Marshall GW, Bedford DT, Marshall SJ. Influence of etching on shear bond strength of dentin adhesives. J Dent Res. 1998; 77:205. (Abstract 797).
15. Sunico MC, Shinkai K, Medina III VO, Shirono M, Tanaka N, Katoh Y. Effect of surface conditioning and restorative material on the shear bond strength and resin14 dentin interface of new one-bottle nanofilled adhesive. Dent Mater. 2002; 18:535–542. [PubMed: 12191667]
16. Della Bona A, van Noort R. Shear vs. tensile bond strength of resin composite bonded to ceramic. J Dent Res. 1995; 74:1591–1596. [PubMed: 7560421]

17. Sano H, Kanemura N, Burrow MF, Inai N, Yamada T, Tagami J. Effect of operator variability on dentin adhesion: students vs. dentist. Dent Mater J. 1988; 17:211–216.

18. van Noort R, Howard IC, Cardew G. A critique of bond strength measurements. J Dent. 1989; 15:61–67. [PubMed: 2659632]

19. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay F. The Microtensile bond test: a review. J Adhes Dent. 1999; 1:299–309. [PubMed: 11725659]

20. van Noort R, Brown D, Clarke R, Combe EC, Curtis R, Fletcher AM, Lloyd CH, Marquis P, McCabe JF, Nicholson J, et al. Dental materials: 1991 literature review. J Dent. 1993; 21(1):5–30. [PubMed: 8425984]

21. Sano H, Shono T, Sonoda H. Relationship between surface area for adhesion and tensile bond strength – evaluation of microtensile bond test. Dent Mater. 1994b; 10:236–240. [PubMed: 7664990]

22. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: methods and results. J Dent Res. 2005; 84:118–132. [PubMed: 15668328]

23. Hamouda IM, Al-Khodary AM, El-Shami FM. Degree of conversion and antimicrobial activity of etch-and-rinse versus self-etching adhesives. J Adhes Dent. 2010; 12:33–38. [PubMed: 20155228]

24. Zhang Y, Wang Y. Distinct photopolymerization efficacy on dentin of self-etch adhesives. J Dent Res. 2012; 91:795–799. [PubMed: 22736445]

25. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res. 2004; 83:216–221. [PubMed: 14981122]

26. Zhang S-C, Kern M. The role of host-derived dentinal matrix metalloproteinases in reducing dentin bonding of resin adhesive. Int J Oral Sci. 2009; 1:163–176. [PubMed: 20690420]

27. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials. 2005; 26:6449–6459. [PubMed: 15949841]

28. Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. Dent Mater J. 2009; 28:11–19. [PubMed: 19280964]

29. Imazato S, Kuramoto A, Takahashi Y, Ebisu S, Peters MC. In vitro antibacterial effects of the dentin primer of Clearfil Protect Bond. Dent Mater. 2006; 22:527–532. [PubMed: 16198404]

30. Brackett WW, Tay FR, Looney SW, Ito S, Haisch LD, Pashley DH. The Effect of Subject Age on the Microtensile Bond Strengths of a Resin and a Resin-modified Glass Ionomer Adhesive to Tooth Structure. Oper Dent. 2008; 33:282–286. [PubMed: 18505218]

31. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. Dent Mater. 2001; 17:296–308. [PubMed: 11356206]

32. Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dental Materials. 2001; 17:430–444. [PubMed: 11445211]

33. Sundfeld RH, Valentino TA, de Alexandre RS, Briso AL, Sundefeld ML. Hybrid layer thickness and resin tag length of a self-etching adhesive bonded to sound dentin. J Dent. 2005; 33:675–681. [PubMed: 16139699]

34. Watanabe LG, Lacy DR, Davis DR. Shear bond strength, Single Plane vs Conventional Lap Shear. J Dent Res. 1987; 67:383. Abst. 2159.

35. Ishikawa A, Shimada Y, Foxton RM, Tagami J. Micro-tensile and micro-shear bond strengths of current self-etch adhesives to enamel and dentin. American Journal of Dentistry. 2007; 20(3):161–166. [PubMed: 17672257]

36. Shimada Y, Senawongse P, Harnirattisai C, Burrow MF, Nakaoki Y, Tagami J. Bond strength of two adhesive systems to primary and permanent enamel. Operative Dentistry. 2002; 27(4):403–409. [PubMed: 12120779]

37. de Andrade AM, Moura SK, Reis A, Loguercio AD, Garcia EJ, Grande RHM. Evaluating resin-enamel bonds by microshear and microtensile bond strength tests: effects of composite resin. Journal of Applied Oral Science. 2010; 18(6):591–598. [PubMed: 21308290]

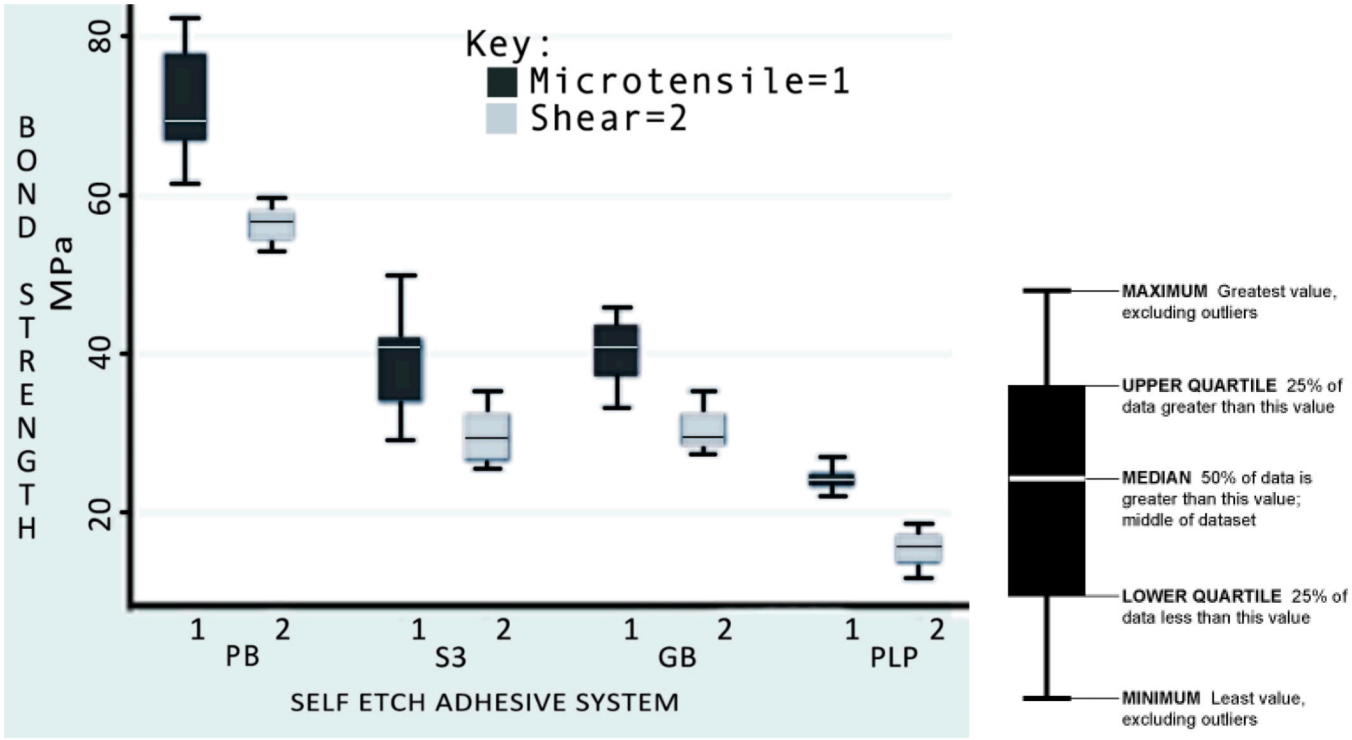


**Figure 1.**
Means and standard deviations of the bond strength for four self-etch systems with the two different techniques. (1 = T; 2 = SP)

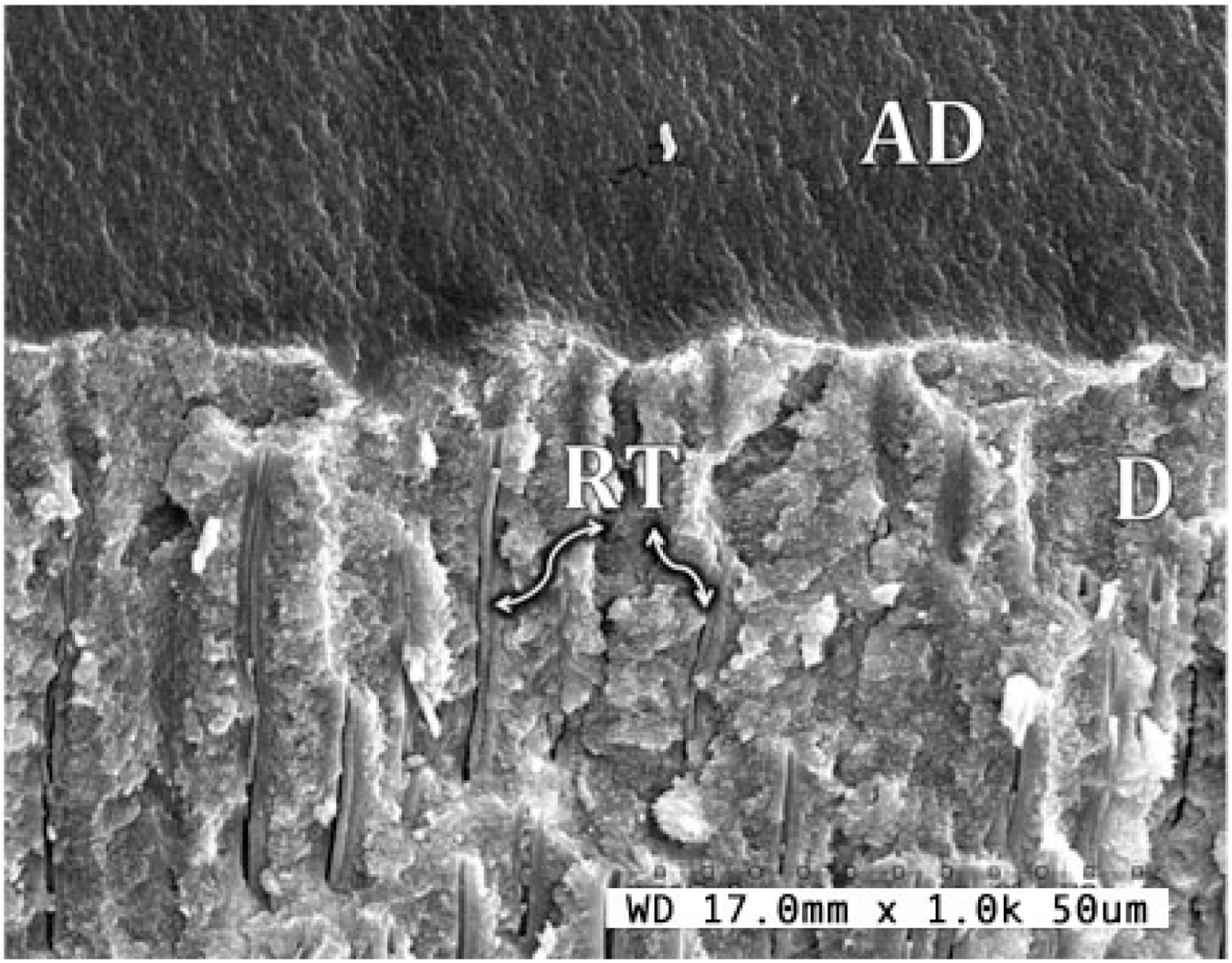


**Figure 2.**
Representative SEM (1000× magnification) of the resin-dentin interface produced by the 2-step self-etching primer system PB. D, dentin; AD, adhesive layer; RT, resin tags.

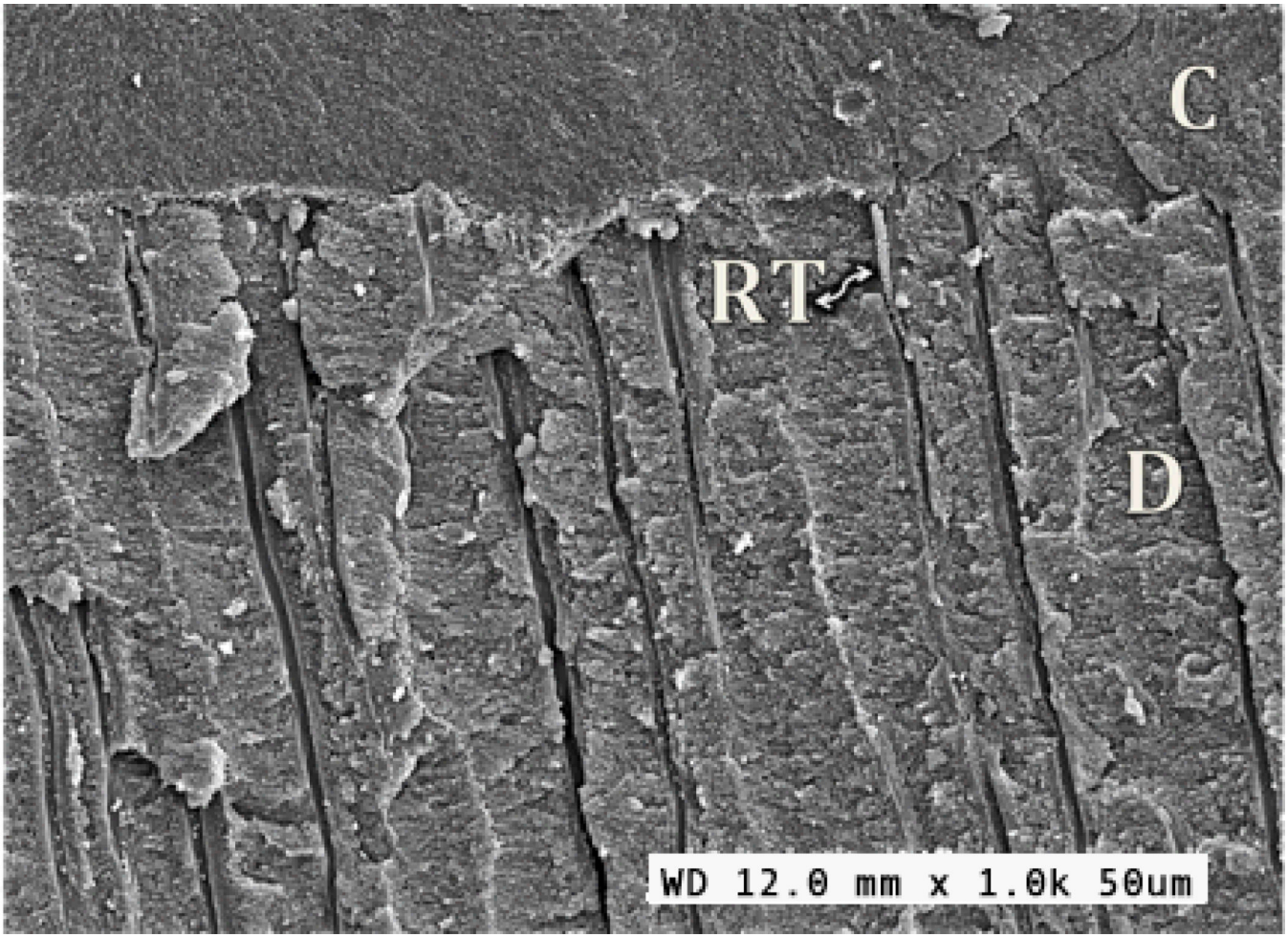


**Figure 3.**
Representative SEM (1000× magnification) of the resin-dentin interface produced by the single-step self-etching adhesive GB. D, dentin; C, composite; RT, resin tags.

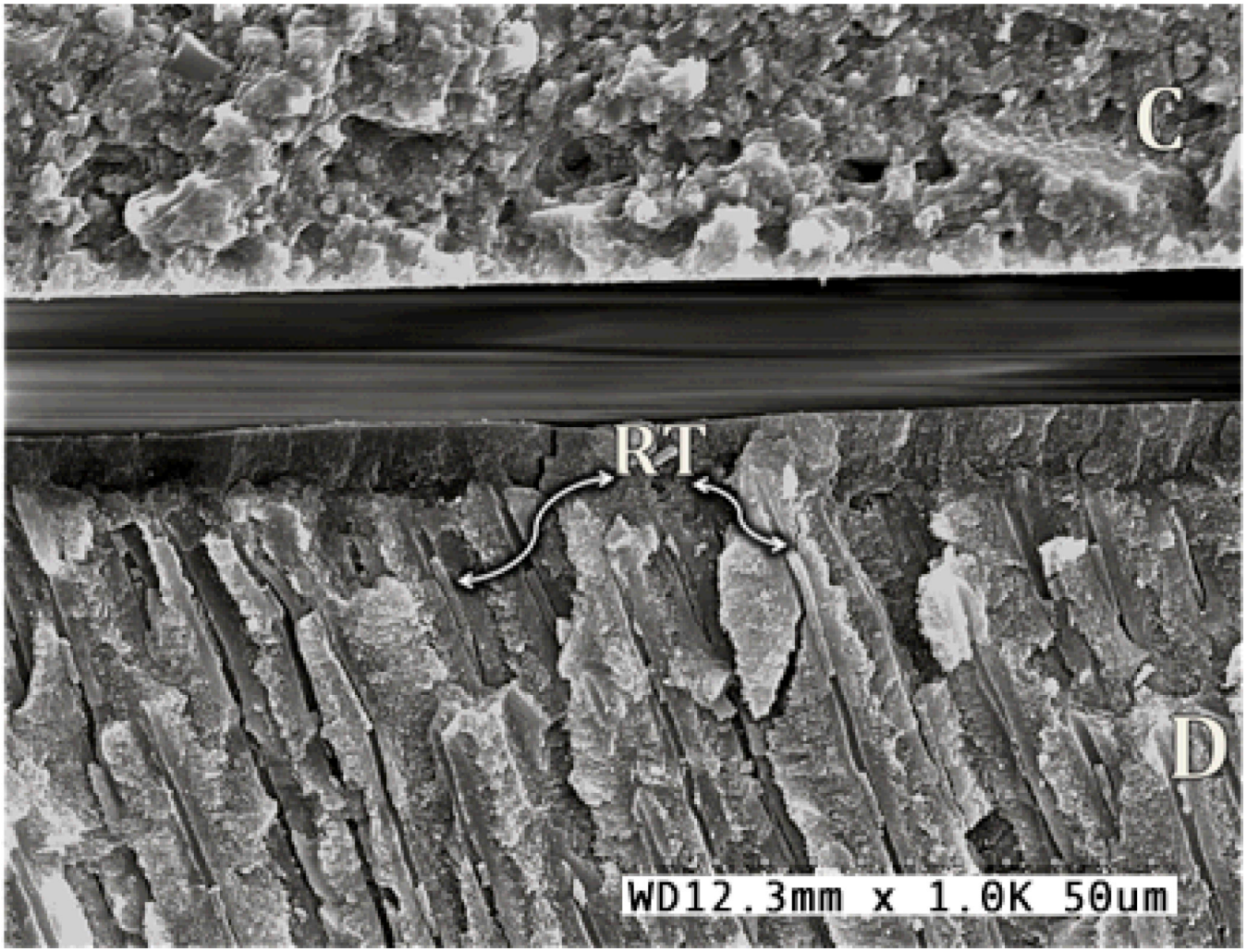


**Figure 4.**
Representative SEM (1000× magnification) of the resin-dentin interface produced by the single-step self-etching adhesive S3. D, dentin; C, composite; RT, resin tags. The gray area between C and D is a gap (artifact)

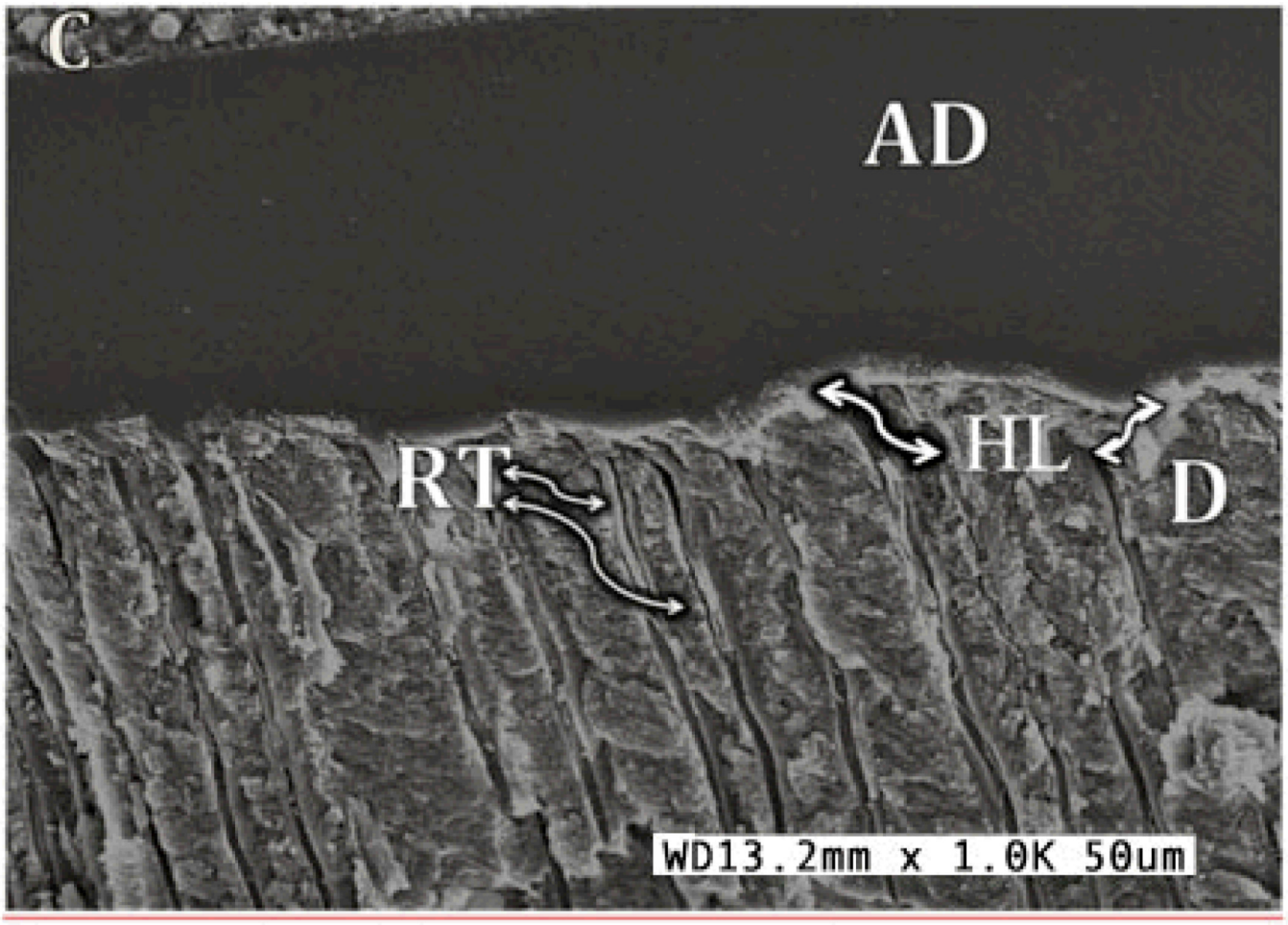


**Figure 5.**
Representative SEM (1000× magnification) of the resin-dentin interface produced by the single-step self-etching adhesive PLP. D, dentin; AD, adhesive layer; C, composite; RT, resin tags, HL: hybrid layer.

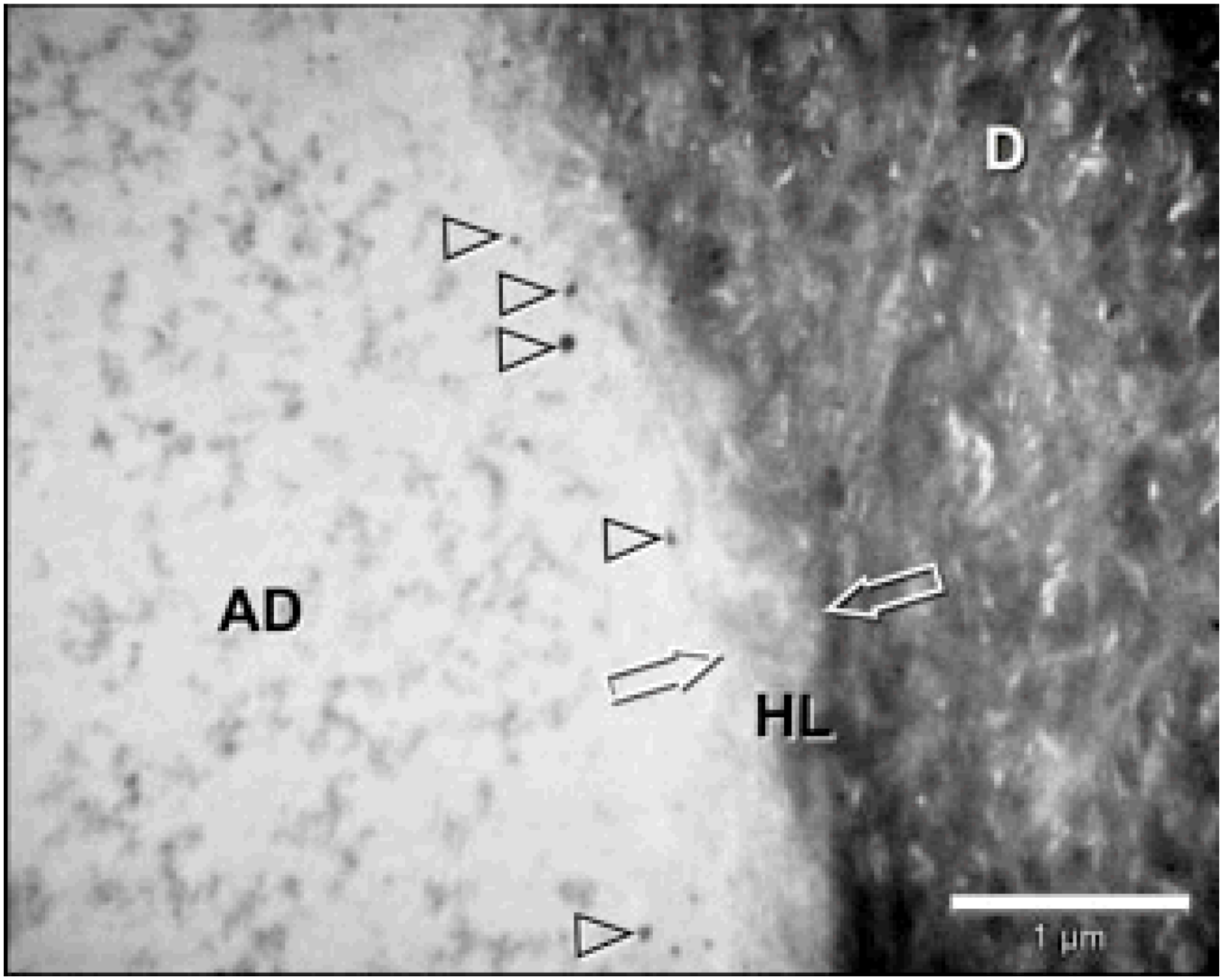


**Figure 6.**
Representative TEM of the resin-dentin interface produced by the 2-step self-etching primer system PB. A thin hybrid layer (HL) of ≤ 0.5 µm was observed (between white arrows). A few silver deposits were observed for this adhesive (arrowheads), which were mainly located on top of the hybrid layer. The gray areas within the adhesive layer are filler particles. D, dentin; AD, adhesive layer; HL, hybrid layer.

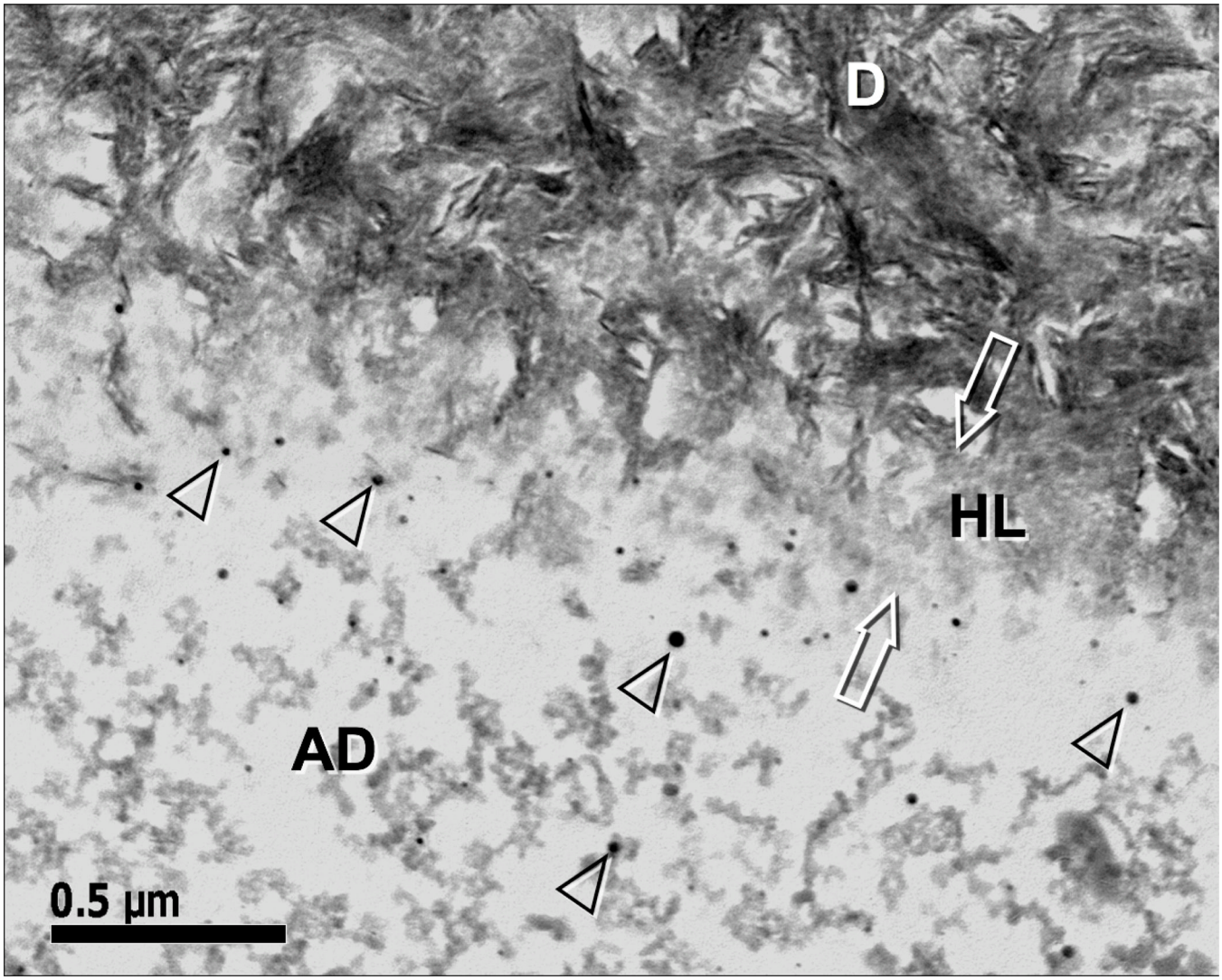


**Figure 7.**
Representative TEM of the resin-dentin interface produced by the single-step self-etching adhesive S3. A thin hybrid layer (HL) of ≤ 0.5 µm was also observed (between white arrows). A few silver deposits were observed (arrowheads), which were mainly located on top of the hybrid layer and within the adhesive layer. The dark spots (arrowheads) are silver deposits, and the gray areas within the adhesive layer are filler particles. D, dentin; AD, adhesive layer; HL, hybrid layer.

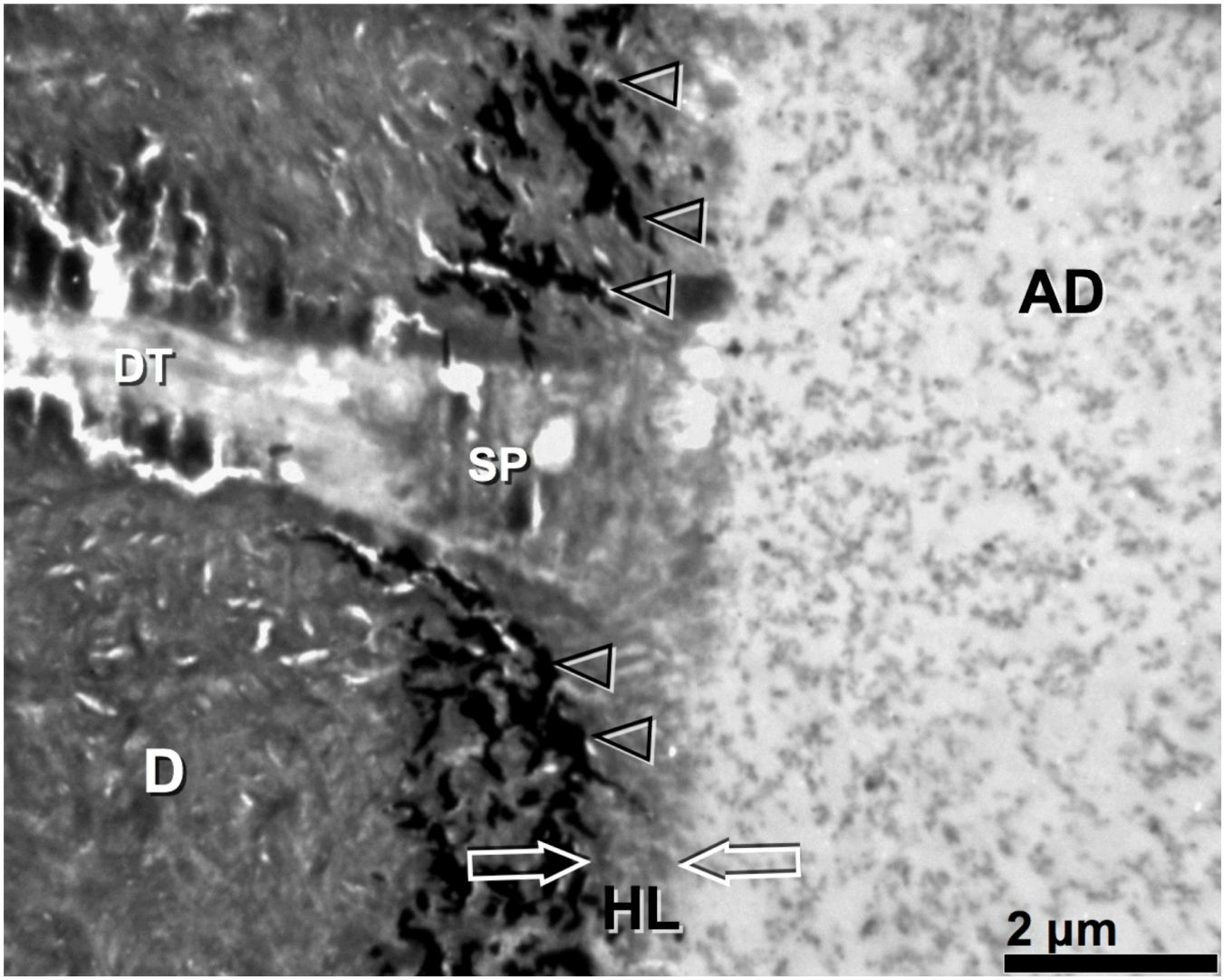


**Figure 8.**
Representative TEM of the resin-dentin interface produced by the single-step self-etching adhesive GB A thin hybrid layer (HL) of ≤ 0.5 µm was observed (between white arrows). More silver deposits were observed for this system, which were mainly located within the hybrid layer (arrowheads). D, dentin; AD, adhesive layer; SP, Smear plug; DT, dentin tubule; HL, hybrid layer.

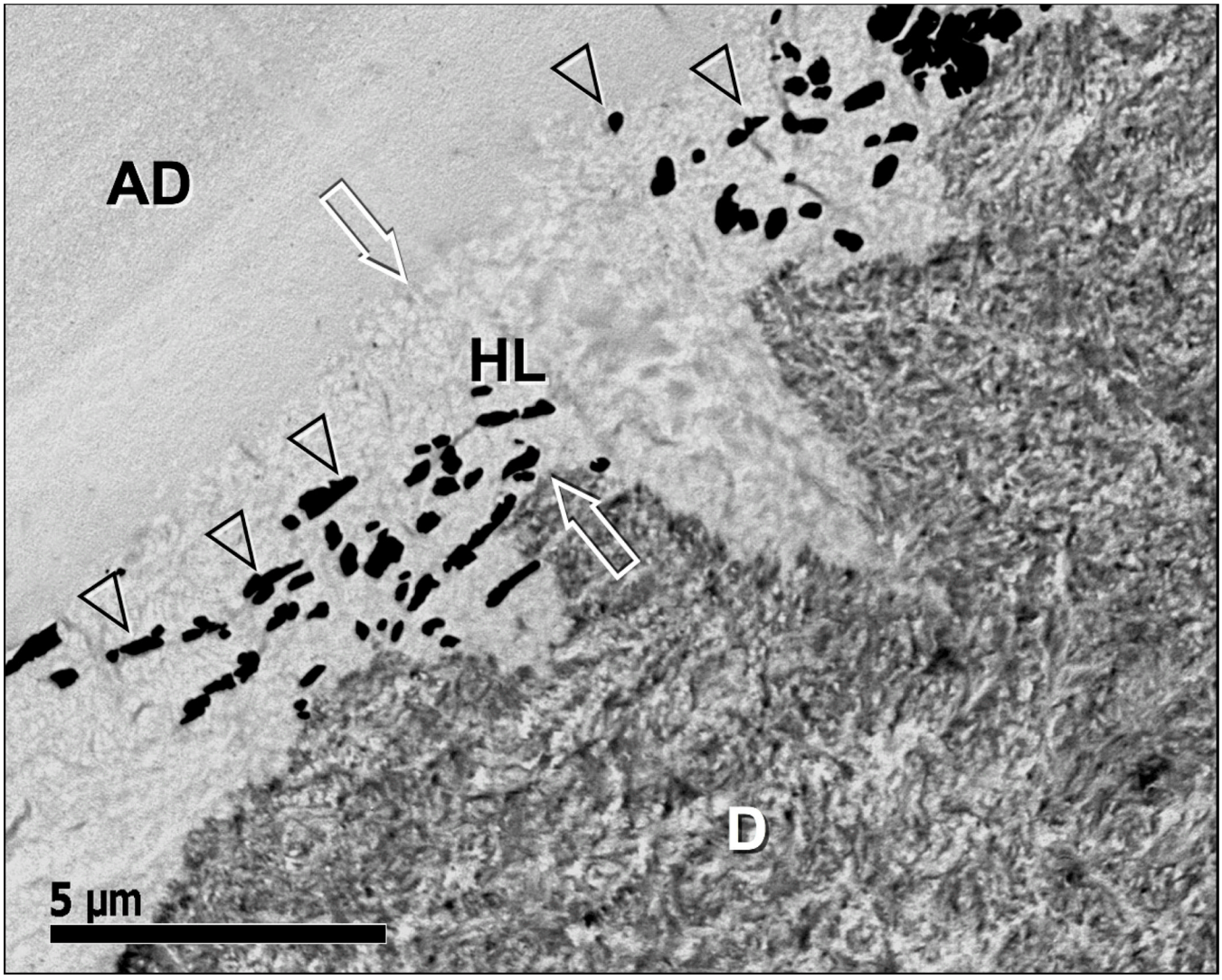


**Figure 9.**
Representative TEM of the resin-dentin interface produced by the single-step self-etching adhesive PLP. A thick hybrid layer (HL) of ≤ 5 µm was observed (between white arrows). A larger amount of silver deposits was observed for this system, which were mainly located within the hybrid layer (arrowheads). D, dentin; AD, adhesive layer; HL, hybrid layer.

**Table 1**

Materials

| Product | Manufacturer | Batch number |
|---|---|---|
| Adper Prompt L Pop (PLP) | 3M ESPE, St. Paul, MN | 5BY |
| Filtek Supreme | | 5FH |
| G-BOND (GB) | GC America, Alsip, IL | 0404161 |
| GC Gradia | | 0501181 |
| CLEARFIL PROTECT BOND (PB) | Kuraray America, New York, NY | 00487A |
| CLEARFIL APX | | 00089C |
| CLEARFIL $S^3$ BOND (S3) | | 00003C |