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In vivo and *in vitro* Permeability of One-step Self-etch Adhesives

ABSTRACT

Adhesive dentistry should effectively restore the peripheral seal of dentin after enamel removal. We hypothesize that non-rinsing, simplified, one-step self-etch adhesives are effective for minimizing dentin permeability after tooth preparation procedures. Crown preparations in vital human teeth were sealed with Adper Prompt, Xeno III, iBond, or One-Up Bond F. Epoxy resin replicas were produced from polyvinyl siloxane impressions for SEM examination. Dentin surfaces from extracted human teeth were bonded with these adhesives and connected to a fluid-transport model for permeability measurements and TEM examination. Dentinal fluid droplets were observed from adhesive surfaces in resin replicas of *in vivo* specimens. *In vitro* fluid conductance of dentin bonded with one-step self-etch adhesives was either similar to or greater than that of smear-layer-covered dentin. TEM revealed water trees within the adhesives that facilitate water movement across the polymerized, highly permeable adhesives. Both *in vitro* and *in vivo* results did not support the proposed hypothesis.

KEY WORDS: *In vivo,* resin replica, permeability, one-step self-etch, water tree.

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A supplemental appendix to this article is published electronically only at http://www.dentalresearch.org.

INTRODUCTION

An important goal in conservative dentistry is to restore the peripheral seal of dentin that originally exists prior to the removal of enamel (Pashley *et al.*, 2002). For crown preparations of vital teeth that involve a considerable sacrifice of sound tooth structures, the use of provisional cements may permit more microleakage of bacteria and their products than the final restorations (Baldissara *et al.*, 1998). For preservation of the health of the pulpodentinal complex, an alternative approach is for the exposed dentin to be sealed with resin-based adhesives prior to the taking of impressions (Pashley *et al.* 1992; Lam and Wilson, 1999; Jayasooriya *et al.*, 2003).

Being non-rinsing, the milder versions of self-etch adhesives preserve smear plugs and prevent the dilution of resin monomers with dentinal fluid (Perdigão, 2002). For the more aggressive self-etch adhesives that completely dissolve smear plugs, coagulation of plasma proteins by primer components may contribute to a reduction in dentin permeability during the processes of simultaneous etching and priming (Nikaido *et al.*, 1995). Although the complete absence of leakage is not a realistic expectation with the use of these adhesives (Tay *et al.*, 2002a), the recent introduction of one-step self-etch adhesives represents a further reduction in working steps that eliminates some of the technique sensitivity and practitioner variability that are associated with the use of total-etch adhesives (Finger and Balkenhol, 1999; Peschke *et al.*, 2000).

Since dentin adhesives are effective in reducing cervical hypersensitivity (Prati *et al.*, 2001), it is prudent to determine if one-step self-etch adhesives can be used for sealing vital teeth following crown preparations. Thus, the objective of this study was to test the hypothesis that one-step self-etch adhesives are effective in reducing dentin permeability under *in vivo* and *in vitro* conditions.

MATERIALS & METHODS

For the *in vivo* part of the study, 24 vital posterior teeth (maxillary and mandibular premolars and molars from 17 subjects) that required crown preparations for fixed prosthodontics were selected. The age of the subjects ranged from 23 to 42 yrs. Informed consent of the subjects was obtained under an *in vivo* protocol reviewed and approved by an ethics committee from the University of Bologna.

For the *in vitro* part of the study, 35 recently extracted human third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the institutional review board from the Medical College of Georgia. These teeth were stored in a 1% chloramine T solution at 4°C and used within 1 mo following extraction. We prepared each tooth by first removing the occlusal enamel using a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under copious water-cooling. We used 180-grit silicon carbide (SiC) paper to create a smear layer on the exposed dentin surface.

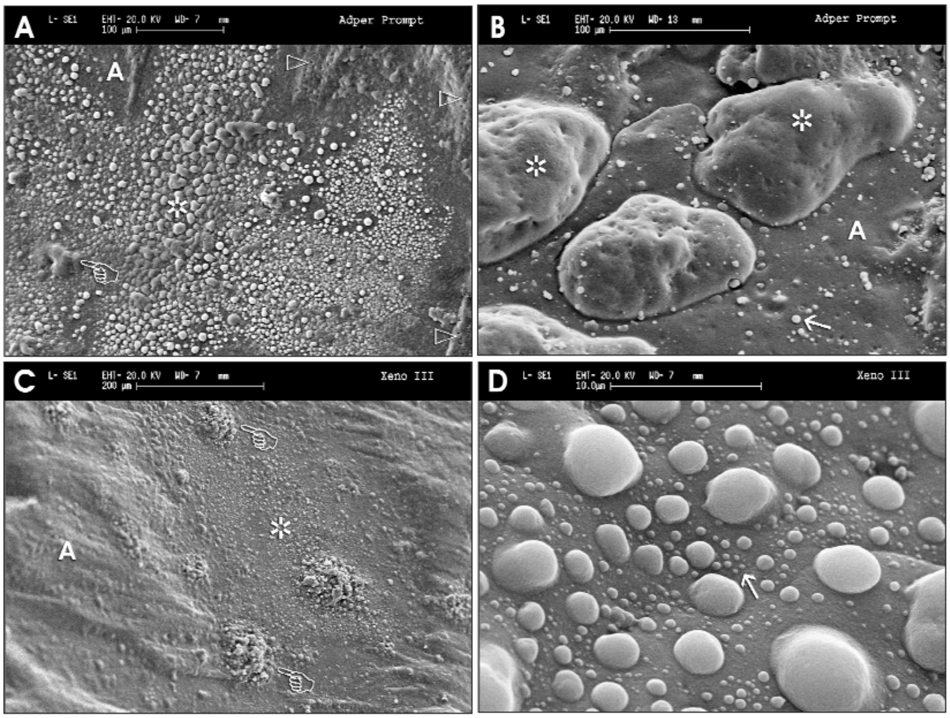

Figure 1. SEM micrographs of epoxy resin replicas of crown preparations of vital human teeth after being bonded with one-step self-etch adhesives Adper Prompt **(A-B)** and Xeno III **(C-D).** **(A)** A low-magnification view of the surface of the adhesive-coated dentin after being sealed with 2 coats of Adper Prompt according to the manufacturer's instructions. The bulk of the dentin surface is covered with adhesive (A), and there are only small areas in which exposed dentinal tubules are observed (open arrowheads). Transudation of dentinal fluid is not evident from the exposed dentinal tubules. However, in areas coated with the adhesive, swelling of the adhesive can be observed (pointer), with transudation of dentinal fluid droplets from the adhesive surface. **(B)** Pooling of multiple droplets resulted in the appearance of large water bundles (asterisks) over the adhesive surface. Small discrete dentinal fluid droplets can also be found (arrow). **(C)** A low-magnification view of an epoxy resin replica of the crown preparation of a vital tooth sealed with Xeno III. The dentin surface is completely coated with adhesive (A), and no exposed dentinal tubules are observed. In isolated regions of the crown preparation that probably correspond with areas of deep dentin, swelling of the adhesive layer can be observed (pointers), together with transudation of dentinal fluid over the surface of the adhesive. **(D)** A high-magnification view of Fig. 1C showing the presence of dentinal fluid droplets over the adhesive surface. No exposed dentinal tubules can be seen. A large number of small, submicron fluid droplets (arrow) can be seen among the larger droplets.

Experimental Design

Four one-step self-etch adhesives were examined. They included 3 two-component systems (Adper Prompt, 3M ESPE, St. Paul, MN, USA; Xeno III, Dentsply DeTrey, Konstanz, Germany; One-Up Bond F, Tokuyama Corp., Tokyo, Japan) and 1 single-component system (iBond, Heraeus Kulzer, Hanau, Germany). They were used according to the manufacturers' instructions. A two-step self-etch adhesive (UniFil Bond, GC Corp., Tokyo, Japan) was used as the control. The chemical compositions of these adhesives are shown in Appendix 1.

Scanning Electron Microscopy (SEM)

For the *in vivo* part of the study, 4 teeth were randomly assigned to each adhesive group. Following crown preparations under local analgesia (mepivacaine hydrochloride 2% with adrenaline 1/100,000), they were sealed with the respective adhesive. The oxygen inhibition layer was gently removed with a cotton pledget soaked in 50% ethanol. Since it has been shown that impression-taking does not affect the integrity of the bonded adhesive (Nahon *et al.*, 2001), a low-viscosity polyvinyl siloxane impression material (Affinis LightBody; Colténe AG, Altstätten, Switzerland) with an intra-oral setting time of 3.5 min was used for taking impressions of these crown preparations. After the research impressions were taken, working impressions were then produced for the construction of the fixed prostheses. Research impressions were also prepared for the remaining 4 crown preparations, in which the smear-layer-covered dentin was not bonded with any adhesive. Epoxy resin replicas were produced from these impressions, according to the protocol reported by Itthagarun and Tay (2000). They were sputter-coated with gold/palladium and examined with a SEM (Cambridge Stereoscan 360, Cambridge, United Kingdom) operating at 20 kV.

Fluid Conductance Measurements

We used an *in vitro* fluid-transport model to measure the fluid conductance through adhesives, following the protocol for hydraulic conductance evaluation reported by Pashley and Depew (1986). The roots were removed from each tooth at 3 mm below the cemento-enamel junction, by means of the Isomet saw. We gently removed pulpal tissue with a small spoon excavator so as not to touch the predentin. The dentin surface was further abraded until a remaining dentin thickness of 1 mm was achieved from at least one region of the ground surface to the highest pulp horn, as measured with a pair of Iwonson calipers. The crown segment was cemented to a piece of Plexiglass by means of C&B Metabond (Sun Medical, Shiga, Japan). The Plexiglass was penetrated by a piece of 18-gauge stainless steel tubing that ended flush with the top. This tubing permitted the pulp chamber to be filled with water and to be connected to a water-filled syringe for measurement of the fluid movement across the dentin surface under 15 cm of H_2O pressure (Vongsavan *et al.*, 2000).

We measured fluid conductance ($\mu L/min^{-1}$) by following the displacement of an air bubble in a micropipette with a constant barrel (Appendix 2). Five teeth were selected at random for each adhesive. For each tooth, fluid conductance was measured three times (Bouillaguet *et al.*, 2000): (a) after dentin was acid-etched for the determination of maximum baseline conductance, (b) after the creation of smear-layer-covered dentin by abrasion of the same tooth with 180-grit SiC paper, and (c) after the dentin was sealed with the respective one-step self-etch adhesive under perfusion at 15 cm of H_2O pressure. For each dentin surface, fluid flow ($\mu L/min^{-1}$) across the smear-layer-covered dentin and bonded dentin was expressed as a percentage of that of acid-etched dentin, which was assigned a value of 100% flow rate. This allowed each specimen to serve as its own control by expressing each of the 3 procedures as a percent of the maximum value, and circumvented the use of surface area for the calculation of hydraulic conductance. Fluid flow for acid-etched dentin was measured for 10 min, and those of smear-layer-covered dentin and bonded dentin for 20 min, with all values corrected to *per* min. The results were statistically analyzed by two-way analysis of variance [adhesive type and substrate type (*i.e.*, smear-layer-covered dentin *vs*. bonded dentin)] and Tukey's multiple-comparison tests with statistical significance set at $\alpha = 0.05$.

Transmission Electron Microscopy (TEM)

The remaining 10 teeth were used for the second *in vitro* part of this study, with a resin composite used as an "impression material". Two teeth were selected at random for each adhesive. Each crown segment was similarly connected to the fluid-transport assembly and bonded with the respective adhesive under 15 cm of H_2O pressure. A 2-mm-thick layer of microfilled composite (EPIC-TMPT, Parkell Inc., Farmington, NY, USA) was placed over the cured adhesive under water perfusion. The composite was left in the dark for 3.5 min to simulate the intra-oral setting time of the impression material. The tooth, coupled with the light-cured composite, was sectioned longitudinally into 1-mm-thick slabs and immersed in a 50 wt% ammoniacal silver nitrate tracer solution, following the nanoleakage protocol reported by Tay *et al.* (2002c). Following the reduction of the diamine silver ions into metallic silver, undemineralized, epoxy-resin-embedded, 90-nm-thick sections were prepared and examined with the use of a TEM (Philips EM208S, Philips, Eindhoven, The Netherlands) operated at 80 kV.

RESULTS

Resin replicas of *in vivo* crown preparations revealed sporadic regions along the surfaces of the adhesive-coated dentin in which there was swelling of the adhesive. For the one-step self-etch adhesives, transudation of dentinal fluid droplets could be universally identified from the surfaces of all resin replicas examined. Adper Prompt exhibited fairly profuse transudation (Fig. 1A), with coalescence of multiple fluid droplets into large water bundles (Fig. 1B). Fluid transudation appeared in localized areas that were close to pulp horns in Xeno III (Fig. 1C), with the presence of myriad small submicron droplets among the larger droplets (Fig. 1D). The extent of dentinal fluid transudation in iBond and One-Up Bond F was comparable with that from the unbonded smear-layer-covered dentin (Fig. 2A). Fluid transudation was not evident in the two-step self-etch adhesive UniFil Bond (Fig. 2B).

Fluid conductance measurements are summarized in the Table. The presence of a smear layer resulted in a reduction of fluid conductance that was only 12-18% of those recorded for acid-etched dentin. The *in vitro* fluid conductance of dentin bonded with the 4 one-step self-etch adhesives was

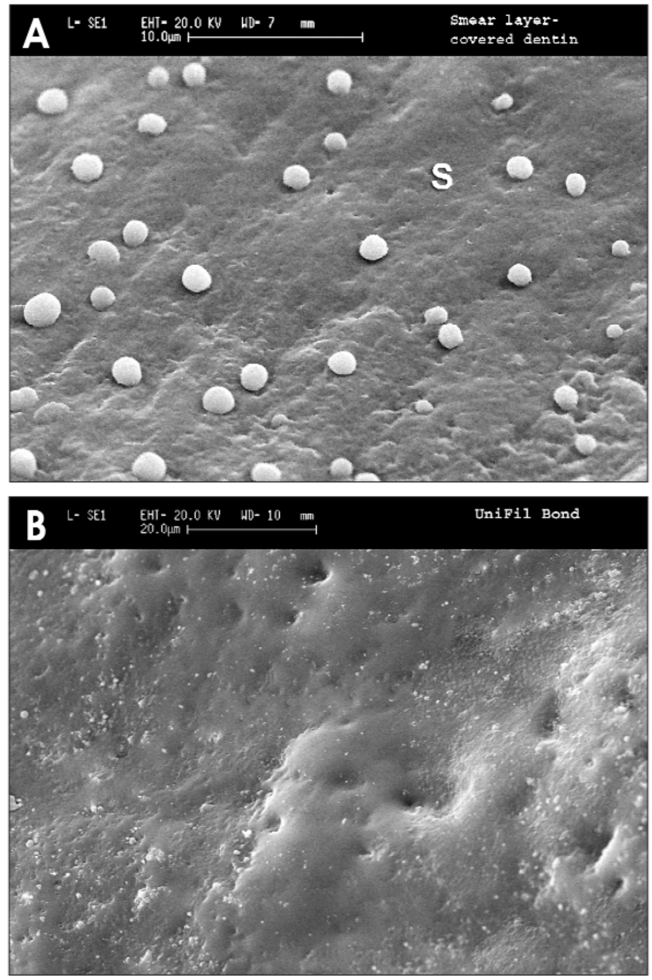

Figure 2. SEM micrographs of epoxy resin replicas of crown preparations of vital human teeth with intact smear layers **(A)** and after being bonded with the control two-step self-etch adhesive UniFil Bond (B). (A) Unbonded smear-layer-covered dentin (S) showing the transudation of sparse, dentinal fluid droplets trapped by the impression material. **(B)** An irregular adhesive surface texture is observed after the oxygen inhibition layer was removed in vital deep dentin bonded with the control two-step self-etch adhesive. No transudation of dentinal fluid droplets can be identified.

Table. Fluid Conductance across Dentin during Different Stages of Application of Self-etch Adhesives

Self-etch Adhesive (N = 5)		% Fluid Flow Induced by 15 cm H_2O of Hydrostatic Pressure	
		Smear-layer-covered Dentin	Bonded Dentin
One-step	Adper Prompt	$17.3 \pm 4.5^{A,1*}$	$28.3 \pm 4.4^{A,2}$
	Xeno III	$12.4 \pm 6.2^{A,1}$	$24.2 \pm 2.9^{AB,2}$
	iBond	$15.1 \pm 5.6^{A,1}$	$18.7 \pm 3.3^{B,1}$
	One-Up Bond F	$14.0 \pm 3.1^{A,1}$	$14.9 \pm 5.0^{B,1}$
Two-step (control)	UniFil Bond (control)	$18.2 \pm 5.0^{A,1}$	$2.1 \pm 2.1^{C,2}$

* Values are means ± standard deviation. Results of *post hoc* multiple-comparison tests are indicated by the superscripts. For each column, groups labeled with the same letter superscripts are not significantly different (P > 0.05). The differences between smear-layer-covered dentin and bonded dentin for each adhesive are indicated by the row results. For each row, groups labeled with the same numeric superscripts are not significantly different (P > 0.05).

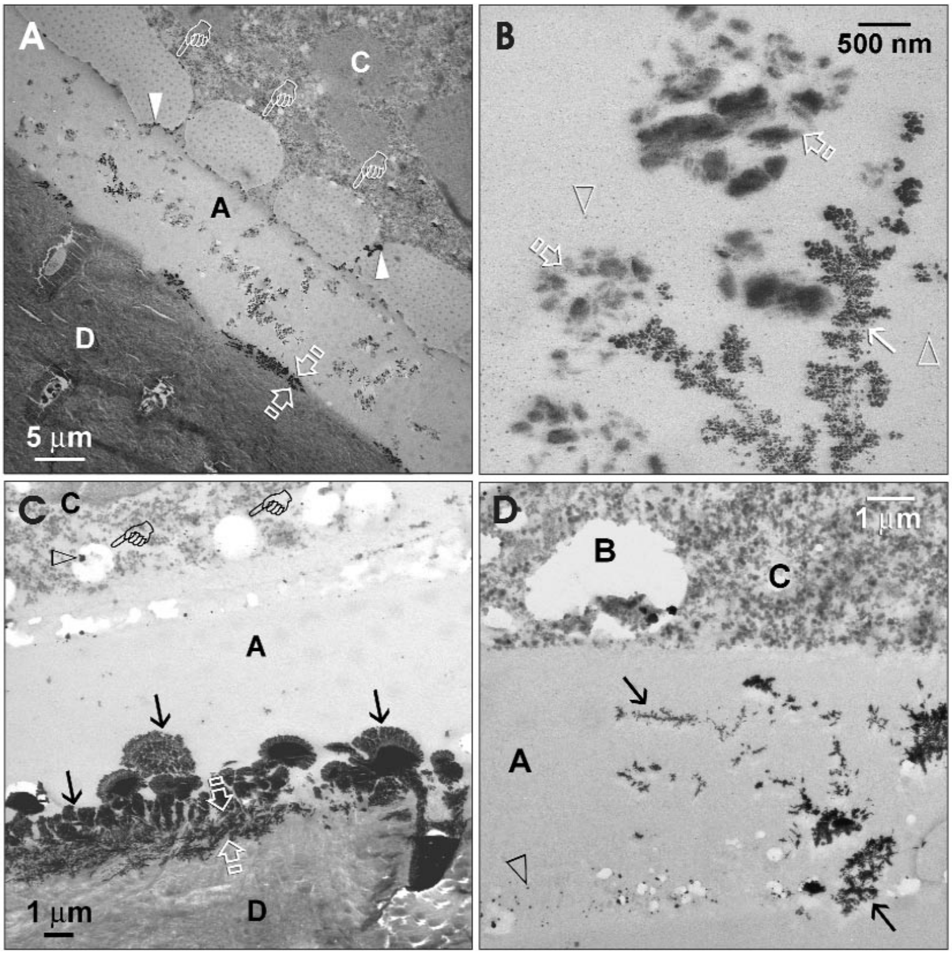

Figure 3. TEM micrographs of unstained, undemineralized resin-dentin interfaces bonded *in vitro* with One-Up Bond F **(A-C)** and iBond **(D)** under a hydrostatic pressure of 15 cm H_2O and further coupled to a resin composite under the same pressure in the dark for 3.5 min before light-activation (to simulate the intra-oral setting time of the impression material). **(A)** Entrapment of water blisters (pointers) between the adhesive (A) and composite (C) in One-Up Bond F. Silver remnants can be seen along the periphery of some blisters (solid arrowheads), but the majority of the blisters are filled with epoxy resin. A 1-µm-thick, partially demineralized hybrid layer can be seen along the adhesive-dentin interface. D, intertubular dentin. **(B)** A high-magnification view of the adhesive layer in One-Up Bond F, showing the existence of a water tree (arrow) among the basic glass filler clusters (open arrows). Very fine, isolated silver grains (open arrowhead) are dispersed throughout the entire adhesive layer. **(C)** The resin-dentin interface in iBond showing the presence of water blisters (pointers) between the adhesive (A) and the composite (C). Remnant silver deposits (open arrowhead) can be identified with the water blisters. Between open arrows = hybrid layer; arrows = water trees; D = intertubular dentin. **(D)** A high-magnification view of the adhesive-composite interface in iBond, showing the presence of additional water trees (arrows) and isolated silver grains (open arrowhead) within the bulk of the adhesive (A). Water blisters (B) can be found within the microfilled composite (C).

similar to or greater than that of the corresponding smear-layer-covered dentin. Conversely, fluid conductance of dentin bonded with the control two-step self-etch adhesive was significantly less than that of the corresponding smear-layer-covered dentin ($P < 0.001$). Two-way ANOVA revealed a significant difference for the factor "substrate type" when the two types of adhesives were pooled for analysis ($P = 0.015$). When the two types of substrates for fluid conductance evaluation were pooled, a highly significant difference was noted for different adhesives ($P < 0.001$). A significant interaction between "substrate type" and "adhesive type" ($P < 0.001$) was also observed. Results of the multiple-comparison tests are represented in the Table.

For Adper Prompt and Xeno III, separation of sections along the composite-adhesive interfaces occurred during ultramicrotomy, and no intact section could be retrieved. TEM micrographs of One-Up Bond F and iBond bonded under perfusion revealed the presence of water blisters along the composite-adhesive interface (Figs. 3A, 3C), without the loss of integrity between the hybrid layer and the adhesive. Apart from nanoleakage within the hybrid layer, two modes of silver deposition could be identified within the adhesive. Water trees (*i.e.*, silver-filled water channels) extended from the surface of the hybrid layer into the adhesive, and could be observed either adjacent to the basic glass filler clusters (Fig. 3B) or in the unfilled adhesive (Fig. 3D). Fine, isolated silver grains were also present in the adhesive layers (Figs. 3B, 3D). Water blisters were not observed along the adhesive-composite interface in the control two-step self-etch adhesive.

DISCUSSION

The smear layer and smear plugs account for 86% of the total resistance to fluid movement in deep dentin (Pashley *et al.*, 1978). Both the *in vivo* and *in vitro* results of this study showed that when bonded under dentin perfusion, none of the one-step self-etch adhesives examined was any more effective at sealing dentin than the original smear layer (Gillam *et al.*, 1997). Thus, the hypothesis that one-step self-etch adhesives are effective in reducing dentin permeability must be rejected.

The dentinal fluid droplets that were observed *in vivo* along the surface of adhesive-bonded dentin were not artifacts produced by moisture condensation during impression-taking, since they were absent when vital dentin was bonded with the control two-step self-etch adhesive. Transudation of dentinal fluid was found to be non-uniform and localized to specific regions, reflecting the variation in permeability from different regions of a crown preparation (Richardson *et al.*, 1991). Moreover, these droplets were absent from epoxy resin replicas of dehydrated dentin bonded *in vitro* with one-step self-etch adhesives (Chersoni, unpublished results), or from the adhesive-composite interfaces when dentin was replaced with processed composite as a bonding substrate (Tay *et al.*, 2003). TEM results further showed that the permeability associated with these adhesives is not caused by a loss of integrity between the adhesive and dentin, but by the presence of water channels (*i.e.*, water trees) that probably expedite such water movement *via* capillary fluid flow (Tay and Pashley, 2003). Furthermore, the isolated silver grains that were detected throughout the adhesive layer may provide an additional diffusion mechanism for the movement of ions and small molecules across an amorphous polymer matrix based on the free volume theory—*via* a process known as jump diffusion or ion hopping (Dürr *et al.*, 2002). This study confirms the *in vitro* model, previously proposed by Tay *et al.* (2002b), that one-step self-etch adhesive behaves as a permeable membrane after polymerization.

For the two less-permeable adhesives, iBond and One-Up Bond F, *in vitro* fluid conductance was comparable with that of smear-layer-covered dentin. This may be due to their less aggressive etching effects, that preserve rather than dissolve smear plugs. It is pertinent to note that transudation of dentinal fluid was also observed *in vivo* for iBond, since this adhesive contains Gluma desensitizer, which is supposed to coagulate plasma proteins (Schüpbach *et al.*, 1997) and form partitions within the dentinal tubules to reduce the dentinal fluid flow (Bergenholtz *et al.*, 1993). The inclusion of cubical/spherical glass fillers in One-Up Bond F or fumed silica fillers in Xeno III did not completely block the paths of water migration through the adhesive, as predicted by the "tortuous path theory" of Nielsen (1967).

Clinically, since water movement through the polymerized adhesive layer involves slow diffusion rather than rapid fluid transport through the dentinal tubules (Mjör and Ferrari, 2002), it is unlikely that their capability for reducing post-operative sensitivity will be affected. However, the results indicate that the new simplified adhesives do not seal dentin very well. If water and small ions can move across the adhesives, one wonders how large molecules must be before their diffusion is restricted. The potential detrimental effect of increased adhesive permeability associated with one-step self-etch adhesives can be seen in low-viscosity self-etching resin cements that contain activator components to render them compatible with acidic adhesives. For those resin cements that utilize one-step self-etching adhesive components, fluid transudation through the adhesive may result in emulsion polymerization of the resin cement to form resin globules under the influence of water (Mak *et al.*, 2002). Adhesive permeability accounts for the compromised bond strength observed when such resin cements were used for bonding to dentin (Carvalho *et al.*, 2004). Conversely, bonding of indirect restorations was improved when dentin was first bonded with a two-step self-etch adhesive prior to impression-taking (Jayasooriya *et al.*, 2003).

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APPENDICES

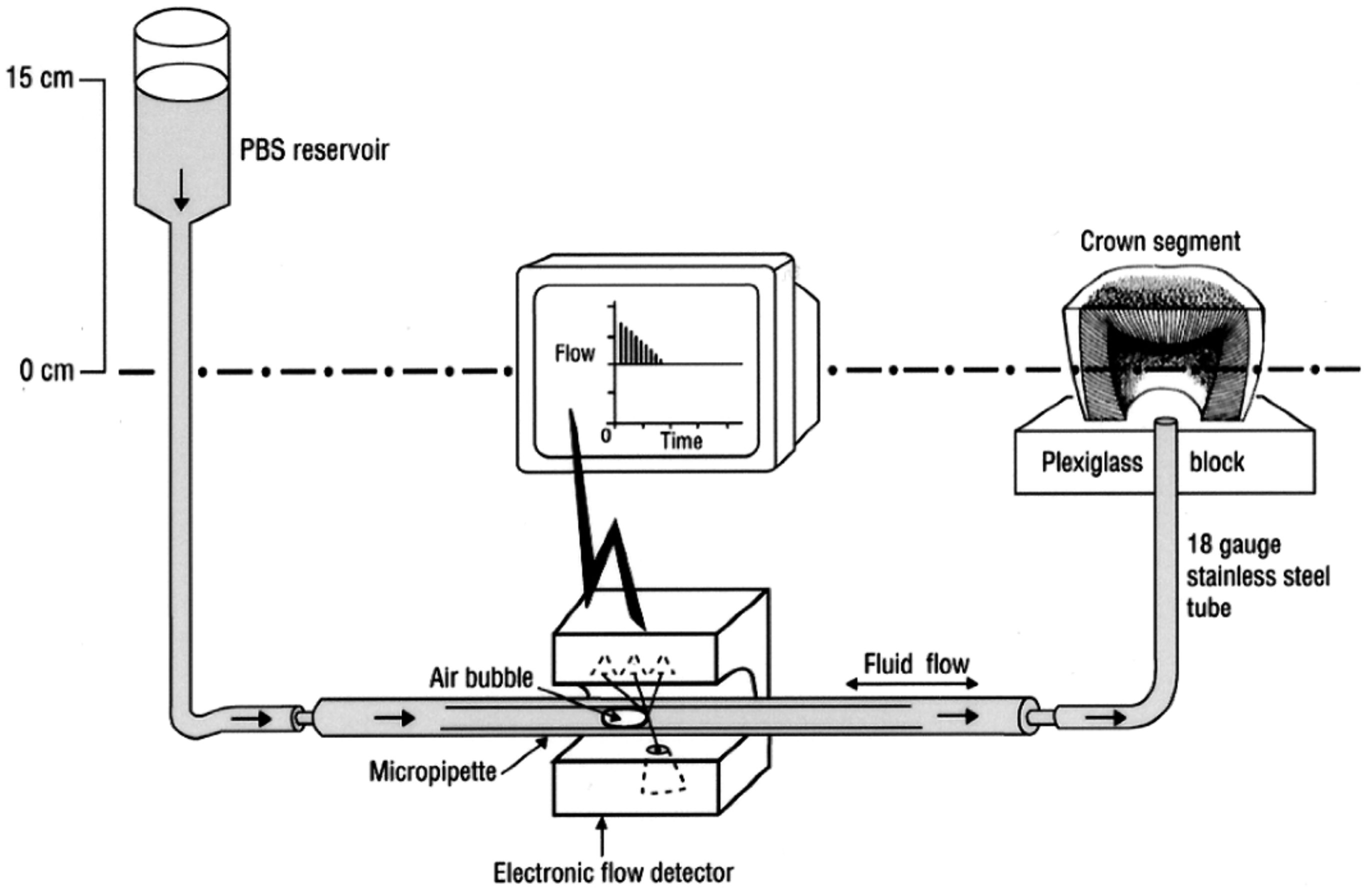
APPENDIX 1

Compositions and Application Protocols of the Self-etch Adhesives Used in This Study

Adhesive	Components	Composition	Application Protocol	Manufacturer
Adper Prompt	Blister A	Methacrylated phosphoric acid esters, photo-initiator (camphorquinone), stabilizer	Mix blisters A and B. Scrub continuously for 15 sec and re-apply until glossy surface appears. Dry thoroughly.	3M ESPE, St. Paul, MN, USA
	Blister B	Water, complexed fluorides, stabilizer	Re-apply a second coat (no waiting time). Dry thoroughly and light-cure.	
Xeno III	Universal	HEMA*, aerosil R-947 (fumed silica), BHT (stabilizer), ethanol, water	Mix liquids A and B. Apply mixed adhesive and leave undisturbed for 20 sec	Dentsply DeTrey, Konstanz, Germany
	Catalyst	Pyro-EMA-SK, PEM-F, UDMA, BHT, camphorquinone, *p*-dimethyl amine ethyl benzoate (co-initiator)	Spread adhesive gently for 2 sec until no more flow of the adhesive occurs and light-cure for 10 sec	
One-Up Bond F	Liquid A	Water, HEMA, methyl methacrylate, coumarin dye, Methacryloyloxyalkyl acid phosphate, MAC-10	Mix liquids A and B. Apply mixed adhesive within 1.5 min after mixing. Leave the mixed adhesive on dentin for at least 20 sec, briefly air-dry and light-cure for 10 sec.	Tokuyama Corp., Tokyo, Japan
	Liquid B	Multifunctional methacrylic monomer, Fluoroaluminosilicate glass; Photoinitiator (aryl borate catalyst)		
iBond	Single bottle, no-mix system	Acetone, water, glutaraldehyde, 4-META	Apply a minimum of 3 consecutive coats of adhesive with no drying in between. Agitate for 30 sec, gently air-dry, and light-cure for 20 sec.	Heraeus Kulzer, Hanau, Germany
UniFil Bond	Self-etching primer	Water, ethanol, 4-MET, HEMA, UDMA, photoinitiator	Apply Primer, leave undisturbed for 20 sec. Apply Bond, light-cure for 10 sec.	GC Corp., Tokyo, Japan
	Bonding resin	HEMA, UDMA, TEGDMA, silanized fumed silica		

* Abbreviations: 4-META, 4-methacryloxyethyltrimellitic anhydride; 4-MET, 4-methacryloxyethyltrimellitic acid; BHT, 2,6-di-tert-butyl-p-cresol; Bis-GMA, (1-methylethylidene)bis[4,1-phenyleneoxy(2-hydroxy-3,1-propanediyl)] bismethacrylate; HEMA, 2-hydroxylethyl methacrylate; MAC-10, Methacryloxyundecane dicarboxylic acid; PEM-F, Penta-methacryl-oxy-ethyl-cyclo-phosphazen-monofluoride; Pyro-EMA-SK, tetra-methacryl-ethyl-pyrophosphate; and UDMA, urethane dimethacrylate (1,6-dimethacryl-ethyl-oxy-carbonylamino-2,4,4-trimethyl hexane).

APPENDIX 2



A schematic illustrating the set-up for measuring hydraulic conductance through adhesive-bonded dentin.