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Water Concentration in Self-etching Primers Affects their Aggressiveness and Bonding Efficacy to Dentin

ABSTRACT

Water is required to ionize acid resin monomers for demineralization of tooth substrates. We tested the null hypothesis that altering the water concentration in two-step self-etching primers has no effect on their aggressiveness and bonding efficacy to dentin. Five experimental self-etching primers were prepared with resin-water-ethanol volume ratios of 9-0-1, 8-1-1, 7-2-1, 5-4-1, and 3-6-1. They were applied to smear-layer-covered dentin, followed by a bonding resin and composite build-ups for microtensile bond testing and TEM examination of tracer penetration. Increasing water concentration from 0-60 vol% improved acidic monomer ionization that was manifested as increasing hybrid layer thickness. However, significantly higher bond strength was observed in the 7-2-1 group, with minimal nanoleakage in the corresponding hybrid layer. When self-etching primers are formulated, a balance must be achieved to provide sufficient water for adequate ionization of the acidic monomers, without lowering the resin concentration too much, to optimize their bonding efficacy to dentin.

**KEY WORDS:** acidic monomer, water content, dentin, microtensile bond strength, nanoleakage.

INTRODUCTION

The creation of stable resin-dentin bonds relies on the formation of hybrid layers that consist of completely or partially demineralized dentin and well-infiltrated, well-polymerized resins (Van Meerbeek *et al.*, 2003). Unlike total-etch adhesives, self-etch adhesives are non-rinsed and should not exhibit discrepancies between the depths of demineralization and resin infiltration (Nakabayashi and Saimi, 1996), since the functions of etching and priming are simultaneously performed by the acidic monomers (Chigira *et al.*, 1994; Watanabe *et al.*, 1994). However, water may not be completely removed from these adhesives, due to the entrapment of free water in adhesive solvent (Pashley *et al.*, 1998) or dentinal tubules (Chersoni *et al.*, 2004), and the retention of bound water *via* hydrogen bonding with the acidic and hydrophilic monomers (Klotz *et al.*, 1996; Tay *et al.*, 2003). These water-filled voids may be visualized as different patterns of nanoleakage with the use of silver nitrate tracers (Tay *et al.*, 2002; Li *et al.*, 2003; Hashimoto *et al.*, 2004).

Bonding to tooth structure *via* the self-etching approach is achieved by increasing the concentrations of acidic methacrylate monomers (Hayakawa *et al.*, 1998). These monomers contain one or more acidic functional groups and have comparatively low, first-acid dissociation constants (pKa_1). Water is required to dissociate these weak acids into ionized forms for permeation of the smear layer and demineralization of the underlying intact tooth substrates (Tay and Pashley, 2001; Pradelle-Plasse *et al.*, 2004). A hydrophilic monomer, 2-hydroxyethyl methacrylate (HEMA), is often included as a transitional polymerizable solvent, since many of the acidic monomers are only mildly water-soluble (Chigira *et al.*, 1989). Although the aggressiveness of self-etch adhesives is largely determined by the concentrations and the pKa values of these acidic monomers (Nishiyama *et al.*, 2004b), differences in water concentration may also contribute to partial or total dissociation of the acid functionalities, creating a variable extent of fractional ionization of these monomers (Anseth *et al.*, 1996).

The effect of water concentration on the aggressiveness and bonding efficacy of self-etching primers to dentin has not been thoroughly investigated, since commercially available systems differ in their resin monomer compositions, adhesive solvents, and water contents. Thus, it is difficult to study the contribution of a single factor without resorting to a modeling approach. In this study, we created experimental self-etching primers using one acidic resin monomer, one hydrophilic resin monomer, a fixed amount of ethanol, and variable amounts of water for simultaneous etching and priming of dentin. The null hypothesis tested was that altering the water concentration in two-step self-etching primers has no effect on their aggressiveness and bonding efficacy to dentin.
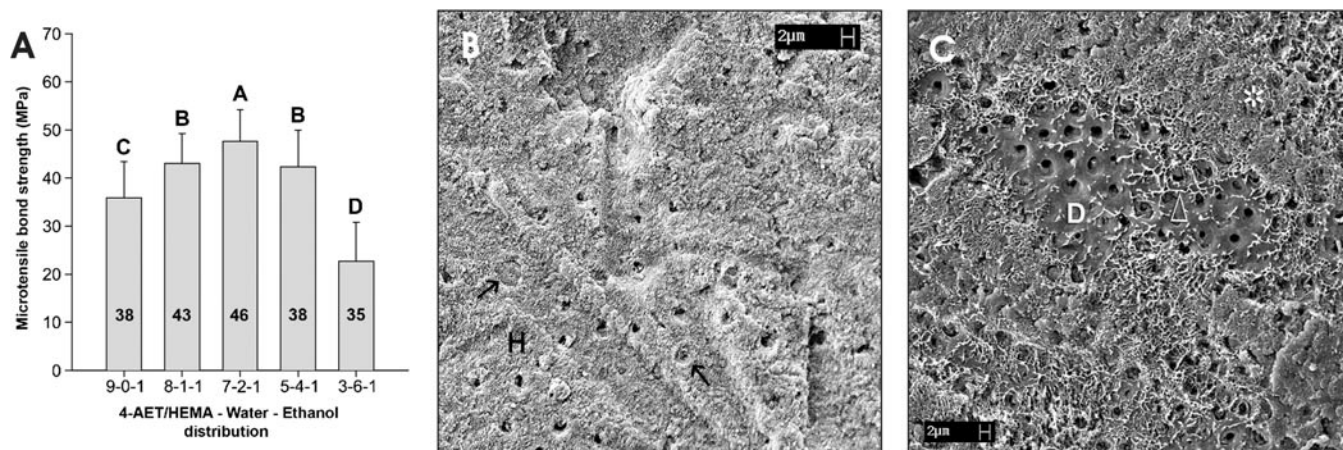
Figure 1. Microtensile bond strengths and representive SEM micrographs from the 5 experimental groups. **(A)** Microtensile bond strengths of the 5 two-step, experimental 4-AET/HEMA self-etching primer solutions: Group 9-0-1 (35.9 ± 7.5 MPa), Group 8-1-1 (43.1 ± 6.1 MPa), Group 7-2-1 (47.6 ± 6.6 MPa), Group 5-4-1 (42.3 ± 7.6 MPa), and Group 3-6-1 (22.6 ± 8.1 MPa). The number inside each bar represents the number of specimens tested for that group. There were no premature failures in any of the groups. Groups with the same upper-case letters are not statistically significant (P > 0.05). **(B)** SEM micrograph of an adhesive failure from the group with the highest mean bond strength (Group 7-2-1), showing the surface of the fractured hybrid layer that appeared comparatively solid. Hybridized smear plugs (arrows) can be seen within some dentinal tubules. **(C)** SEM micrograph of an adhesive failure from the group with the lowest mean bond strength (Group 3-6-1). The surface of the hybrid layer was porous (asterisk), and some incompletely infiltrated collagen fibrils (open arrowhead) can be identified along the fractured interface. The condition of the hybrid layer reflects the extensive nanoleakage observed in the TEM (Fig. 4D). D: non-hybridized dentin.

MATERIALS & METHODS

Twenty-five non-carious human third molars were collected after the patients' informed consent was obtained under a protocol reviewed and approved by the Human Assurance Committee of the Medical Collage of Georgia. The molars were stored in 0.5% chloramine T at 4°C, and were used within 1 mo following extraction. Flat dentin surfaces were created perpendicular to the tooth's longitudinal axis by means of a slow-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA), for the removal of occlusal enamel and superficial dentin. Each surface was ground with 180-grit silicon carbide paper under running water for 30 sec just before bonding occurred.

Preparation of Experimental Self-etching Primers

An anhydrous stock primer solution was first prepared by dissolving 4-acryloxyethyltrimellitic acid (4-AET) powder in HEMA. 4-AET was synthesized according to the method described by Ikemura and Endo (1998). The stock solution consisted of 40 wt% 4-AET, 59 wt% HEMA, and 1 wt% 2,4,6-trimethylbenzoyl-diphenyl phosphine oxide (Aldrich Chemical Company, Milwaukee, WI, USA) as the photoinitiator. The latter was used in lieu of camphorquinone because of its greater activity for photopolymerization of the acidic HEMA solution (Ikemura, unpublished results).

Five experimental two-bottle-type, self-etching primers were subsequently prepared, with the stock solution as component A, and absolute ethanol/water as component B. The resin-ethanol-water volume ratios after the 2 components were mixed were: 9-0-1 (water-free), 8-1-1 (10 vol% water); 7-2-1 (20 vol%), 5-4-1 (40 vol%), and 3-6-1 (60 vol%). Their pH values at ambient temperature (20-25°C), as determined with a digital pH meter (Model 501, Orion Research, Inc., Beverly, MA, USA), were 1.97 ± 0.06 (Group 8-1-1), 1.93 ± 0.06 (Group 7-2-1), 1.90 ± 0.04 (Group 5-4-1), and 1.86 ± 0.12 (Group 3-6-1). Group 9-0-1 could not be measured with the pH meter, since we could not completely dehydrate the electrolyte, and the pH value of 7.00 ± 0.00 was measured with litmus paper. The experimental self-etching primers were used immediately after the 2 components were mixed, to avoid hydrolysis of the acidic and hydrophilic monomers in the presence of water (Nishiyama *et al.*, 2004a).

Tensile Bond Strength and Fractographic Evaluation

Three teeth were used for each of the 5 experimental groups. Immediately after the 2 components were mixed, each self-etching primer was applied with agitation on the dentin surface for 20 sec. Without being rinsed, the solution was gently air-dried for evaporation of the solvent. The primed dentin was bonded with Fluoro-Bond bonding agent (Shofu Inc., Kyoto, Japan) and light-cured for 20 sec *via* a halogen light-curing unit operated at 600 mW/cm^2. Composite build-ups were constructed with a light-cured resin composite (Beautifil, Shofu Inc.) in 5 one-mm-thick increments.

After being stored in distilled water at 37°C for 24 hrs, the bonded teeth were sectioned occluso-gingivally into 0.9 x 0.9-mm composite-dentin beams, according to the 'non-trimming' technique of the microtensile test. Between 35 and 46 beams were available for bond testing in each group. Specimens were stressed to failure under tension by means of a universal testing machine (Model 4440; Instron, Inc., Canton, MA, USA) at a crosshead speed of 1 mm/min. Bond strength data were statistically analyzed by one-way ANOVA and Tukey's multiple-comparison tests, with statistical significance set at $\alpha = 0.05$.

The failure modes of all specimens were initially examined with a stereomicroscope (Nikon SMZ10, Tokyo, Japan) at 30X magnification and were classified as adhesive, cohesive in dentin/composite, or mixed failures. For each experimental group, each type of failure mode was expressed as a percentage of the total number of specimens in that group. Four representative fractured beams from each group with tensile bond strengths that were close to the mean bond strength of that group were examined with scanning electron microscopy (SEM). In addition, 4 specimens

from each group with tensile bond strengths that were either higher or lower than the corresponding mean bond strength of that group were also examined. The dentin sides of the fractured beams were air-dried, sputter-coated with gold/palladium, and examined by SEM (Cambridge Stereoscan 440, Cambridge, United Kingdom) at 10 kV.
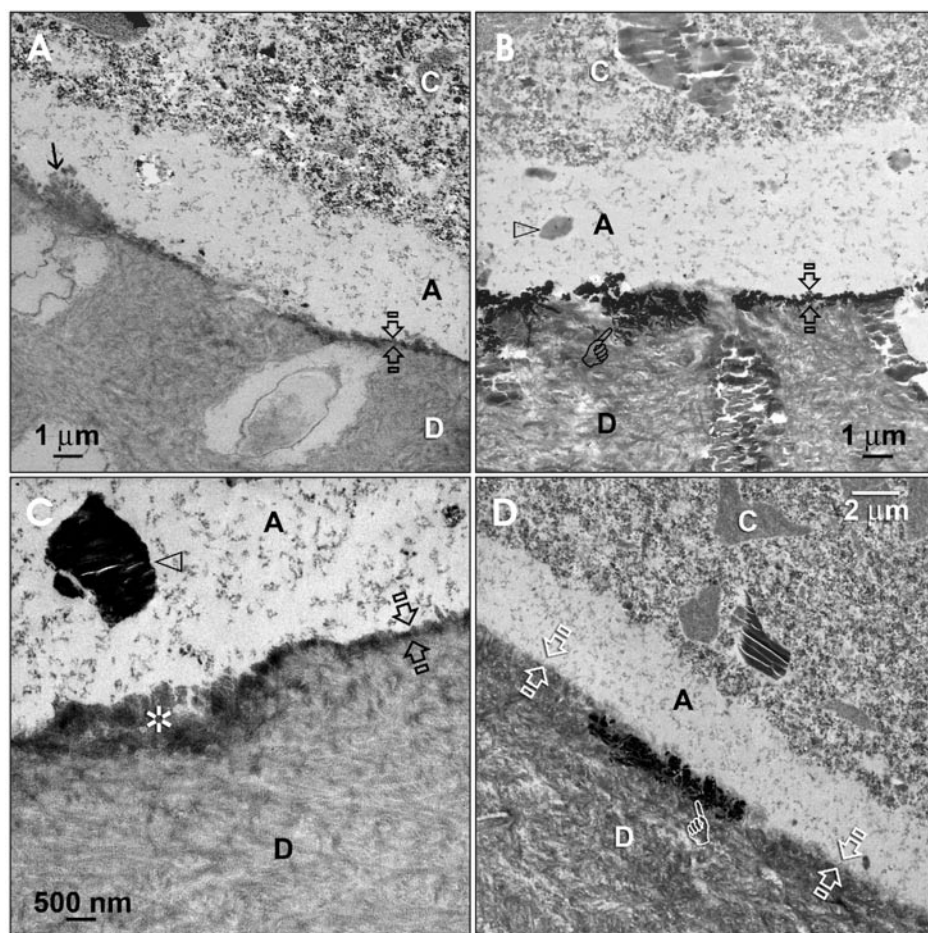
Transmission Electron Microscopy (TEM)

Two teeth from each group were similarly treated with the self-etching primer solution and further bonded with Fluoro-Bond bonding agent. The bonded teeth were coupled to a microfilled resin composite (EPIC-TMPT, Parkell Inc., Farmingdale, NY, USA) to facilitate ultramicrotomy. The teeth were sectioned into 0.9-mm serial slabs. The 2 widest slabs from each tooth were used for TEM examination. For evaluation of the aggressiveness of various self-etching primers, 1 slab from each tooth was completely demineralized in ethylene diamine tetraacetic acid. After laboratory processing according to the protocol reported by Tay *et al.* (1999), 90-nm-thick epoxy-resin-embedded sections were prepared and stained with uranyl acetate and lead citrate.

For examining the extent of resin infiltration with resin-dentin interfaces formed by the self-etching primers, we immersed the second slab from each tooth in a 50 wt% ammoniacal silver nitrate solution for 24 hrs, according to the silver-impregnation protocol reported by Tay *et al.* (2002). After reduction of the diamine silver ions, the silver-impregnated slabs were processed for TEM examination without further demineralization and examined unstained. Both types of sections were examined by TEM (CM100, Philips, Eindhoven, The Netherlands) at 80 kV.
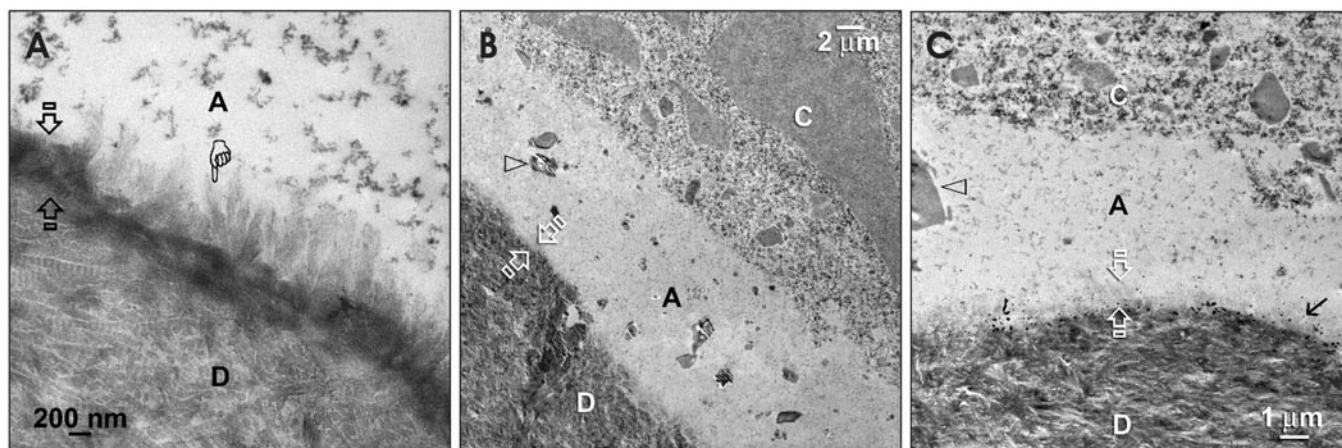

Figure 2. TEM micrographs from (A-B) Group 9-0-1 (anhydrous), and (C-D) Group 8-1-1 (10 vol% water). C, composite; A, filled adhesive; D, dentin. Open arrowhead: F-PRG fillers. **(A)** Stained, demineralized section from Group 9-0-1 showing a 200-nm-thick, electron-dense hybrid layer (between open arrows). Some of the smear layer remnants (arrows) were not completely dissolved. **(B)** The corresponding unstained, undemineralized section, showing that the 200-nm-thick, partially demineralized zone (between open arrows) is heavily filled with silver deposits (nanoleakage). In addition, reticular silver deposits are sporadically present along the interfibrillar spaces (pointer) of the dentin beneath the hybrid layer. Such a feature could not be identified from the stained demineralized sections. **(C)** Stained, demineralized section from Group 8-1-1, showing a similar 200-nm-thick hybrid layer (between open arrows) with thicker regions containing smear layer remnants (asterisk). **(D)** The corresponding unstained undemineralized section, showing that the partially demineralized zone (between open arrows) is generally devoid of nanoleakage. Similar to Group 9-0-1, sporadic regions of silver-filled interfibrillar spaces (pointer) can be observed in the underlying intact, mineralized dentin.

RESULTS

Significant differences were observed for the tensile bond strengths of the 5 experimental self-etching primers (Fig. 1A). Group 7-2-1 specimens exhibited the highest strength (47.6 ± 6.6 MPa) (P<0.05). This was followed by Groups 8-1-1 (43.1 ± 6.1 MPa) and 5-4-1 (42.3 ± 7.6 MPa), which were not significantly different from each other, but were significantly higher than Group 9-0-1 (35.9 ± 7.5 MPa). Group 3-6-1 exhibited the lowest bond strength (22.6 ± 8.1 MPa). Between 8 and 15% mixed and cohesive failures were observed in all groups, with the exception of Group 3-6-1, in which bond failures were exclusively adhesive.

Morphologic differences among the adhesive failures were seen between the higher-bond-strength groups and those with lower bond strengths. In Group 7-2-1 (higher bond strengths), for example, the fractured surface of the hybrid layer remained comparatively solid (Fig. 1B). Conversely, the fractured hybrid layer surface was porous in Group 3-6-1 (lower bond strengths), from which denuded collagen fibrils were detached from the resinous components (Fig. 1C). Specimens with bond strengths higher or lower than those of the corresponding mean bond strength of a particular group depicted either mixed failures or cohesive failures within the weaker dentin (not shown).

Increasing the water concentration from 0-60 vol% resulted in improved acidic monomer ionization, manifested as an

Figure 3. TEM micrographs from the experimental group with the highest bond strength (Group 7-2-1; 20 vol% water). C, composite; A, filled adhesive; D, dentin; open arrowhead, fully pre-reacted glass-ionomer-type fillers (F-PRG) from the Fluoro-Bond bonding resin. **(A)** Stained, demineralized section showing a 500-nm- to 1-μm-thick hybrid layer (between open arrows). The smear layer was completely dissolved, and collagen fibrils (pointer) from the intact dentin exhibit a 'shag carpet' appearance. **(B)** The corresponding unstained, undemineralized section after tracer penetration, showing the overall absence of nanoleakage from the resin-dentin interface. The partially demineralized zone (between open arrows) corresponded to the hybrid layer depicted in the stained section. **(C)** A high-magnification view of Fig. 3B, showing the presence of minimal nanoleakage that existed in the form of isolated silver grains (arrow) within the partially demineralized zone (between open arrows).

increase in the depth of the hybrid layers created by the self-etching primers. Hybrid layer thickness varied from 0.2 μm in Groups 9-0-1 (Fig. 2A) and 8-1-1 (Fig. 2C), to 0.5-1 μm in Group 7-2-1 (Fig. 3A), to 1.5-2 μm in Groups 5-4-1 (Fig. 4A) and 3-6-1 (Fig. 4C).

Extensive nanoleakage was observed within the 0.2-μm-thick partially demineralized zone in Group 9-0-1 (Fig. 2B). In addition, an unusual reticular pattern of nanoleakage that was sporadically located in the mineralized dentin beneath the hybrid layers could frequently be identified in Group 9-0-1 (Fig. 2B), and occasionally in Group 8-1-1 (Fig. 2D).

Group 7-2-1 exhibited minimal nanoleakage within the partially demineralized zone (Fig. 3B) that was predominantly of the spotted type of nanoleakage (Tay *et al.*, 2002) (Fig. 3C). A further increase in the water content of the self-etching primers was not accompanied by an improvement in the quality of dentin hybridization, with moderate (Group 5-4-1; Fig. 4B) to severe nanoleakage (Group 3-6-1; Fig. 4D) observed within the 1.5- to 2-μm-thick partially demineralized zones.

DISCUSSION

It is surprising that very mild etching of the dentin and appreciable tensile bond strength were observed in the anhydrous Group 9-0-1, since water is necessary to ionize the acidic functional groups of self-etching adhesive monomers. The effective ion that causes demineralization of dentin is not the hydrogen ion (H^+), but the hydronium ion (H_3O^+), the hydrated hydrogen ion. Since such ions cannot be formed in the absence of water, we speculated that the water responsible for the ionization of the acidic monomers could have been derived from the underlying dentin.

Despite the thinness of the hybrid layers (*ca.* 200 nm) in Groups 9-0-1 and 8-1-1, extensive nanoleakage could be identified within the zones of partially demineralized dentin. Conversely, minimal nanoleakage was observed in Group 7-2-1, with a thicker hybrid layer (0.5-1 μm). Water molecules
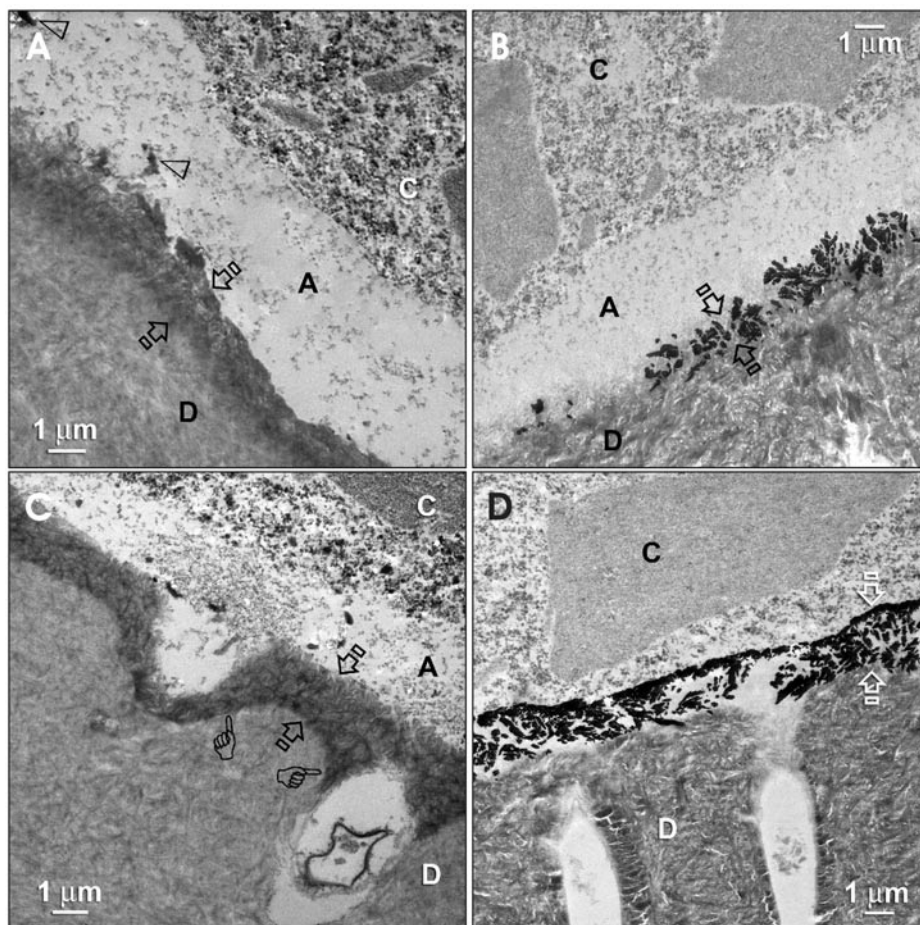
form hydration clusters around functional groups on collagen peptides and proteoglycans, thereby preventing the interpeptide or peptide-proteoglycan hydrogen bonding that can collapse interfibrillar spaces in the demineralized matrix (Suzuki and Nakai, 1994; Nakaoki *et al.*, 2000; Pashley *et al.*, 2000, 2001). Thus, apart from its role in ionization of the acidic resin monomers, it appears that a minimal water concentration is necessary to keep the demineralized matrix in an expanded stage, even for self-etching primers in which resin infiltration occurs simultaneously with etching of the dentin. This could have accounted for the better resin infiltration and the minimal nanoleakage observed in Group 7-2-1.

The appearance of a reticular pattern of silver-infiltrated interfibrillar spaces beneath the hybrid layers in Group 9-0-1 and occasionally in Group 8-1-1 was unusual, and suggested that continuous etching of the dentin beneath the hybrid layer could have occurred, since the initially non-ionized acidic monomers were converted into their ionic states by water that was derived from the underlying dentin, or during the period of specimen storage in water prior to their laboratory processing. This phenomenon did not occur in the other 3 groups, probably because the acidic monomers were more optimally ionized with the water that was present in these mixed primer solutions, enabling the ionized form of the acidic monomers to exert an immediate etching effect on the underlying dentin. Conversely, delayed ionization of the acidic monomers in Groups 9-0-1 and 8-1-1 could have resulted in non-infiltrated spaces beneath the hybrid layers that were subsequently penetrated by the tracer solution. Water is also useful to solubilize the dissolved calcium and phosphate ions, thereby lowering their ion products below the solubility product constants for various forms of calcium phosphates (McDowell *et al.*, 1977; Shellis *et al.*, 1997). When insufficient water is present, re-precipitation of calcium phosphates within the very fine, partially demineralized interfibrillar spaces of the underlying dentin may have prevented resin infiltration into these regions, resulting in the unusual form of nanoleakage beneath the hybrid layers. The

hypotheses of delayed ionization of acidic monomers under the condition of an inadequate supply of water, and its possible effect on etching and resin infiltration of dentin, are speculative and require further testing for their validity to be confirmed.

Increasing the water concentration beyond 20 vol% resulted in further increases in the degree of ionization of the 4-AET monomers, lowering of the pH of the self-etching primer solutions, and further increasing their depth of demineralization. However, increasing the water concentration also inadvertently diluted the concentrations of the adhesive monomers, thereby lowering their inward rates of diffusion. The water molecules also competed with the adhesive monomers for binding sites on collagen (Vaidyanathan *et al.*, 2003). The excess water may also prevent optimal polymerization of the adhesive monomers. Using 62% BisGMA/37% HEMA-based bonding agent, Jacobsen and Söderholm (1995) reported the effect of increasing water concentration on the percent conversion of double bonds, bond strength, and phase changes. As water was added to the bonding agent, the percent conversion fell rapidly from 53.5% at zero water, to 22.7% when 20% water was added. Phase changes may also occur as water is added. Spencer and Wang (2002) examined the amount of water necessary to produce phase changes in 70% BisGMA/30% HEMA formulation dissolved in 45% ethanol. This formulation exhibited phase separations at 28 vol% water.

Figure 4. TEM micrographs from (A-B) Group 5-4-1 (40 vol% water), and (C-D) Group 3-6-1 (60 vol% water). C, composite; A, filled adhesive; D, dentin. Open arrowhead: F-PRG fillers. **(A)** Stained, demineralized section from Group 5-4-1. There was an increased aggressiveness of the self-etching primer with increasing water concentration, with the formation of a 1.5- to 2-μm-thick hybrid layer (between open arrows). **(B)** The corresponding unstained undemineralized section revealed a 1.5- to 2-μm-thick partially demineralized zone (between open arrows) in which reticular patterns of nanoleakage could occasionally be seen. There were no silver deposits within the interfibrillar spaces of the underlying dentin. **(C)** Stained, demineralized TEM from Group 3-6-1, showing a similarly thick hybrid layer (between open arrows), but with further extensions to form peritubular cuffs (pointers) around the dentinal tubules. **(D)** The corresponding unstained, undemineralized TEM. The low resin concentration and the increased water content resulted in the extensive deposition of silver within the partially demineralized zone (between open arrows).

Without ethanol solvation, the same 70% BisGMA/30% HEMA underwent phase separations at around 5 vol% water. These factors, individually or in combination, could have contributed to the continuous decline in bond strength, and the increasing severity of the nanoleakage in Groups 5-4-1 and 3-6-1, despite the formation of thicker hybrid layers (*ca.* 1.5-2 μm) by these more aggressive self-etching primers.

As the water concentration decreases, the adhesive monomer concentration increases. This tends to increase the viscosity of the formulation, permitting the creation of thicker adhesive concentrations. In a study that examined the microtensile bond strength of an acetone-based adhesive to phosphoric-acid-etched dentin, the highest bond strengths (64 MPa) were obtained with 37% acetone and 63% comonomers, while the lowest bond strength (38 MPa) was produced by 67% acetone and 33% comonomers (Cho and Dickens, 2004).

Clearly, higher monomer concentrations are preferred.

Within the limits of this study, the null hypothesis—that altering the water concentration in two-step self-etching primers has no effect on their aggressiveness and bonding efficacy to dentin—has to be rejected. When self-etching primers are formulated, compromises must be made to provide sufficient water for adequate ionization of the acidic monomers, but without lowering the resin concentration too much to optimize their bonding efficacy to dentin.

ACKNOWLEDGMENTS

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