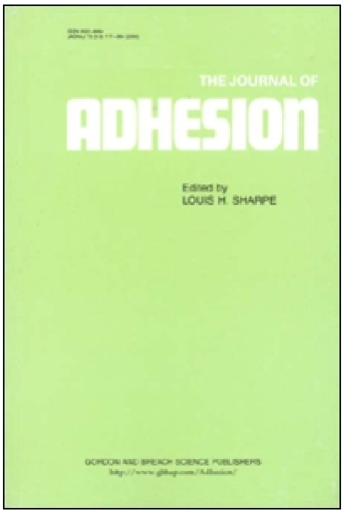

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Water Content in Self-Etching Primers Affects Their Aggressiveness and Strength of Bonding to Ground Enamel

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In this study the influence of water concentration in self-etching (SE) primers on their aggressiveness and bond strength to ground enamel was investigated. Five experimental primers with 0, 5, 10, 20, or 40 wt% of water were formulated, using Clearfil SE Bond® (CSEB) as a commercial reference. Primers were applied to bovine incisors following application of bonding resin and composite restoration. Bond strength was evaluated through shear testing and data were statistically analyzed (5%). The etching aggressiveness was evaluated by SEM. Water concentration significantly affected bond strength and etching aggressiveness. The highest bond strength value (MPa) was found for the primer containing 20% of water (24.7), followed by CSEB (23.8). Groups containing 5% (20.5) and 10% (20.2) of water showed an intermediate performance, while the lowest values were observed for the 0% (17.3) and 40% (16.7) groups. SEM analysis showed increasing aggressiveness for increased water content.

Keywords: Aggressiveness; Bond strength; Etching pattern; Primer; Self-etching adhesives; SEM; Water

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1. INTRODUCTION

Self-etching adhesives (SEA) are increasingly being used in dentistry mainly because of the possibility of eliminating the rinsing step after acid conditioning. SEA reduce the clinical time for application and decrease the risk of making errors during application (technique-sensitivity) [1] compared with etch-and-rinse adhesive systems (ERA). In addition, the self-etching strategy usually exhibits a smaller discrepancy between the depths of demineralization and resin infiltration as found in ERA [2,3], thus reducing post-operative sensitivity and degradation of the bonding interface.

The most popular SEA systems are applied in two steps, whereby the conditioning step of the substrate by the acidic primer is followed by application of a bonding resin [1]. According to some authors [2,4] these two-step systems may possess a bonding ability similar to conventional ERA. Self-etching (SE) systems are basically composed of monomers (mono and bifunctional), initiators, and solvents, and are typically water-based [1]. Water is an essential component to enable the ionization of the acidic monomers for demineralization of the substrates [5]. The bonding strategy also relies on chelation of calcium ions by the ionized monomers, the collagen fibrils being partially solubilized and hybridized [6].

Depending on the concentration, water may be not completely removed from SEA during air-drying procedures [7]. The excess water could compromise the quality of the bonding layer by diluting the monomers, possibly interfering with the polymerization mechanism and the crosslinking density of the formed hybrid layer [8]. Therefore, it is of critical importance to determine the effect of water concentration in SE primers on both the bond strength and etching patterns to dental substrates.

The objective of the present study was to determine the influence of water concentration in experimental SE primers on the aggressiveness and strength of bonding to the ground enamel. The null-hypothesis tested was that the water contained in the experimental SE primer would not affect the etching pattern or the bonding ability with the enamel.

2. MATERIALS AND METHODS

2.1. Reagents

In order to remove impurities, 2-hydroxyethyl methacrylate (HEMA; Aldrich, St. Louis, MO, USA) and methylene chloride (Synth, Diadema, São Paulo, SP, Brazil) were dried under anhydrous sodium sulfate (Nuclear, Diadema, São Paulo, SP, Brazil) for 12 h and filtered before use. 2,6-di-tert-butyl-4-methyl phenol (Aldrich), phosphorus pentoxide (Vetec, Rio de Janeiro, RJ, Brazil), and absolute ethanol (Nuclear) were used as received.

2.2. Synthesis of Methacryloyloxyethyl Dihydrogenphosphate (MEP)/Bis(methacryloyloxyethyl) Hydrogen Phosphate (Bis-MEP)

To a 100-mL round bottom vessel at 0°C with 50 mL of cold methylene chloride, phosphorus pentoxide (6 mmol) was added and the slurry stirred vigorously while 36 mmol of HEMA was slowly added over 1 h using an addition funnel. The reaction was left to run for 5 h at room temperature. After filtering the product, 6 mg of 2,6-di-tert-butyl-4-methyl phenol was added and the methylene chloride was removed under distillation using a rotary evaporator. The product of the reaction was an equimolar mixture of the monomers methacryloyloxyethyl dihydrogenphosphate (MEP) and bis(methacryloyloxyethyl) hydrogen phosphate (Bis-MEP), as previously described by Lima *et al.* [9]. The concentrated product was characterized using Fourier transform infrared spectroscopy (FTIR – IR Solution, Shimadzu, Tokyo, Japan) and ^{1}H nuclear magnetic resonance (^{1}H NMR), and was used for formulating the experimental SE primers.

2.3. Formulation of the Experimental Self-Etching Primers

Five experimental water-based SE primers with different solvent concentrations were tested. The materials were obtained by the intensive mixture of components described in Table 1, which also shows the

TABLE 1 Composition (wt%) and pH of the Six Primers Tested

Composition	P_0	P_5	P_{10}	P_{20}	P_{40}	CSEB
HEMA and MEP/Bis-MEP	60	60	60	60	60	HEMA, MDP, dimethacrylate, photoinitiator, Water
Ethanol	40	35	30	20	0	
Water	0	5	10	20	40	
Total (%)	100	100	100	100	100	100
pH	*	1.5	1.5	1.5	1.5	2.0

HEMA: 2-hydroxylethyl methacrylate; MEP: methacryloyloxyethyl dihydrogen phosphate; Bis-MEP: bis (methacryloyloxyethyl) hydrogen phosphate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate.

*In anhydrous systems, pH cannot be determined.

composition and pH of all materials. SEA Clearfil SE Bond® (Kuraray, Osaka, Japan) was used as a commercial reference material. This system was chosen as it is a well-known, widely used self-etching adhesive.

2.4. Preparation of Specimens for Shear Bond Strength Testing

Fresh bovine incisors were obtained, cleaned, and stored in 0.5% chloramine-T solution for 7 days. The teeth were embedded in epoxy resin and the buccal faces were wet-ground with 180-, 220-, 400-, and 600-grit SiC abrasive papers in order to create a standardized flat surface in enamel. The specimens were randomly separated into six groups according to the primer evaluated. After thorough rinsing, water was removed with a piece of absorbent paper, leaving the surface visibly dry. The prepared enamel surfaces were vigorously etched with primer for 30 s and gently air-dried for 10 s. One coat of an experimental adhesive resin (AD-50), composed of crosslinking methacrylates, hydrophilic methacrylate, photoinitiators, and stabilizers was then applied [9].

2.5. Bond Strength Evaluation and Failure Analysis

For bond strength evaluation, the experimental setup shown in Fig. 1 was carried out [10]. Customized 0.5-mm thick elastomer molds, each with three cylinder-shaped orifices (1.2 mm in diameter), were placed on the teeth surfaces, allowing delimitation of the bonding area. Light-activation of the adhesive agent was carried out for 20 s with the light guide tip placed directly onto the elastomer mold using a light emitting diode curing unit (Radii; SDI, Bayswater, Victoria, Australia). Irradiance was measured with a digital power meter (Ophir Optronics, Danvers, MA, USA) and was found to be $\sim 1400\,\mathrm{mW/cm^2}$. The orifices were filled with a resin composite (Charisma; Heraeus Kulzer, Hanau, Germany); a polyester strip was placed onto the filled molds and the composite was light-activated for 20 s.

After 24 h storage in distilled water at 37°C, a thin steel wire (0.2 mm in diameter) was looped around each cylinder and aligned with the bonding interface; a shear bond strength test was then conducted using a mechanical testing machine (DL500; EMIC, São José dos Pinhais, PR, Brazil), at a crosshead speed of 1 mm/min until failure. For each group, 10 teeth were tested, and the average value of the three resin cylinders was recorded as the bond strength for each specimen. Bond strength values were calculated in MPa and analyzed by

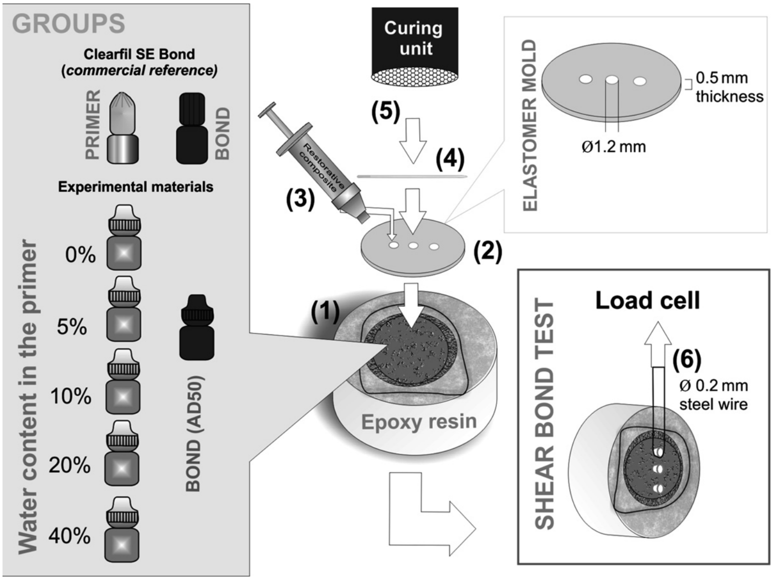

FIGURE 1 Experimental setup used for the bond strength analysis. (1) The primers were applied to the flattened bovine enamel. (2) Elastomer molds with cylinder-shaped orifices were placed on the surfaces and (3) filled with resin composite. (4) A polyester strip was placed onto the filled molds and (5) the composite was light-activated for 20 s. After 24 h, a thin steel wire was looped around each cylinder and aligned with the bonding interface; the shear bond strength test was then conducted using a mechanical testing machine.

the Kruskal-Wallis one-way analysis of variance on ranks and Student-Newman-Keuls' as a *post hoc* test ($P < 0.05$). Fractured specimens were examined by light microscopy under magnifications of 100 and 500×. The predominant mode of failure was classified as follows: adhesive failure, cohesive failure within bonding resin, cohesive failure within enamel or mixed failure.

2.6. Enamel Conditioning Pattern: Morphological Analysis Using SEM

Six enamel fragments (2 × 2, 1 mm in thickness) of bovine incisors were obtained for each SE primer. Additional specimens to which the SE primers were not applied were used as a control (smear layer). The specimens were embedded in epoxy resin (Buehler, Lake Bluff, IL, USA) and dry stored in lightproof containers at 37°C. After 24 h, the surfaces were wet-polished with 600-, 1200-, and 2000-grit SiC papers and with 3-, 1-, and 0.5-µm diamond polishing compound (Metadi II; Buehler). Debris was removed between each polishing step by ultrasonic cleansing for 5 min using distilled water. After washing with distilled water, the enamel fragments were dried with absorbent paper and the experimental SE systems were applied, following the same procedures described for bond strength testing. The samples were ultrasonically cleaned using distilled water and dried at 37°C for 2 h. Thereafter, the specimens were coated with gold and examined under SEM (JSM-5600LV; Jeol Inc., Tokyo, Japan) at 15 kV, in order to analyze the etching morphology provided by the different primers, focusing on its integrity, homogeneity, and continuity along the surface.

3. RESULTS

3.1. Monomer Synthesis

The synthesis of the monoester (MEP) and diester (Bis-MEP) phosphate monomers was accomplished with success (Figs. 2 and 3) with a yield of ~100%. No polymerization was detected during the reaction time or during the purification procedure.

3.2. Bond Strength Evaluation and Failure Analysis

Results for shear bond strength are shown in Fig. 4. Median values are reported as the bond strength data did not achieve homoscedasticity

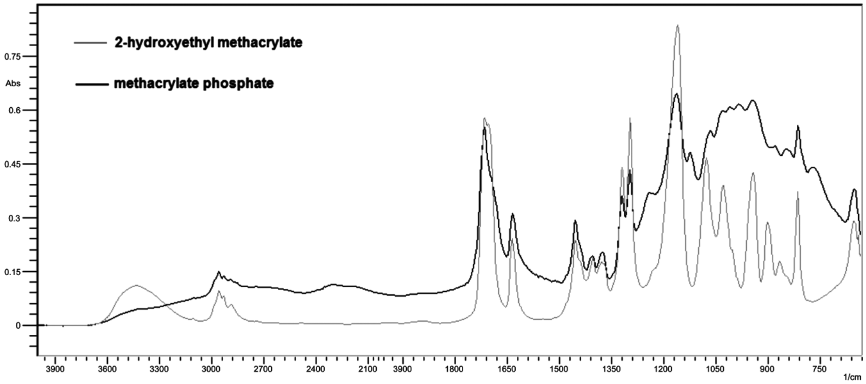

FIGURE 2 FTIR spectra of the starting reagent (2-hydroxyethyl methacrylate) and of the product, an equimolar mixture of the monomers methacryloyloxyethyl dihydrogen phosphate (MEP) and bis(methacryloyloxyethyl hydrogen phosphate (Bis-MEP).

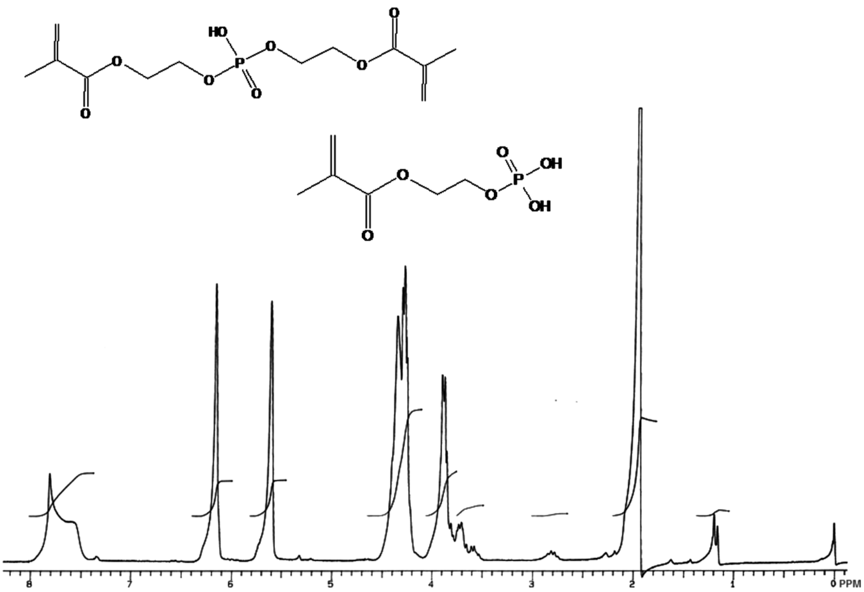

FIGURE 3 ^{1}H NMR spectra of purified MEP and bis-MEP in an equimolar mixture (200 MHz, $CDCl_3$).

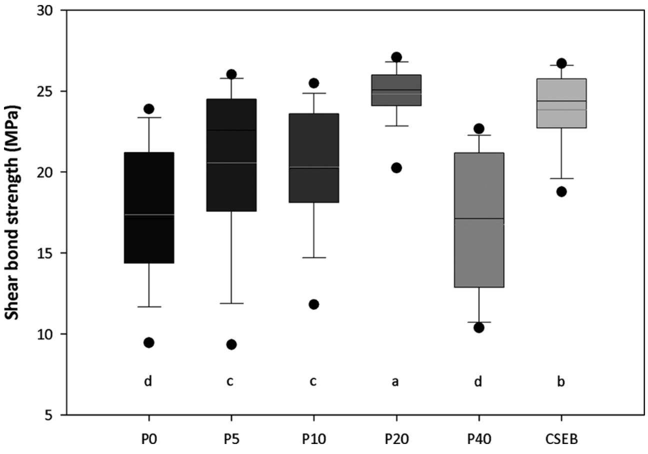

FIGURE 4 Shear bond strength median values of experimental primers ($P_0 - P_{40}$) and commercial reference group (CSEB). Different letters represent statistically significant differences ($P < 0.001$).

(constant variance); thus, a non-parametric statistical analysis was carried out. The water concentration in the primer was a significant factor for bond strength of the experimental SE primers ($P < 0.001$). The primer P_{20} presented a significantly higher bond strength than the other primers, as demonstrated by the *post-hoc* test. The distribution of failure modes is shown in Fig. 5. Analysis of the surfaces fractured during the shear test showed a predominance of adhesive and mixed failures for all materials. Premature failures did not occur in this study.

3.3. Etching Pattern

The morphological changes in enamel surfaces treated with the SE primers are shown in Figs. 6 and 7. Enamel surfaces treated with P_5 and P_{10} appeared very similar, without clear morphologic differences (Figs. 6A and B, respectively). The etching pattern was not aggressive, the surfaces were poorly demineralized, and scratches resulting from the polishing procedures were evident, although slight surface porosities were still observed. On the other hand, the surfaces treated with P_{20} and P_{40} primers showed an extensive loss of enamel crystallites and in comparison with P_5 and P_{10}, a removal of crystals was detected within and around the prisms. Nonetheless, these two groups showed considerable morphological differences. For P_{40}, the demineralization

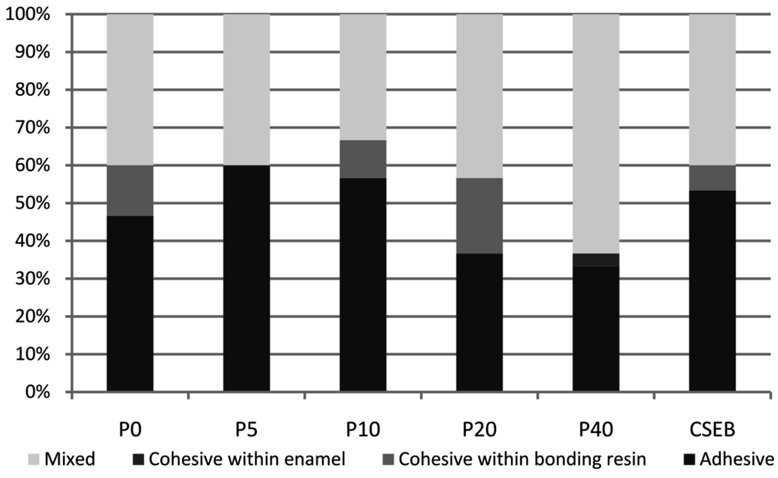

FIGURE 5 Failure mode distribution for the different groups. A predominance of adhesive and mixed failures was detected for all materials.

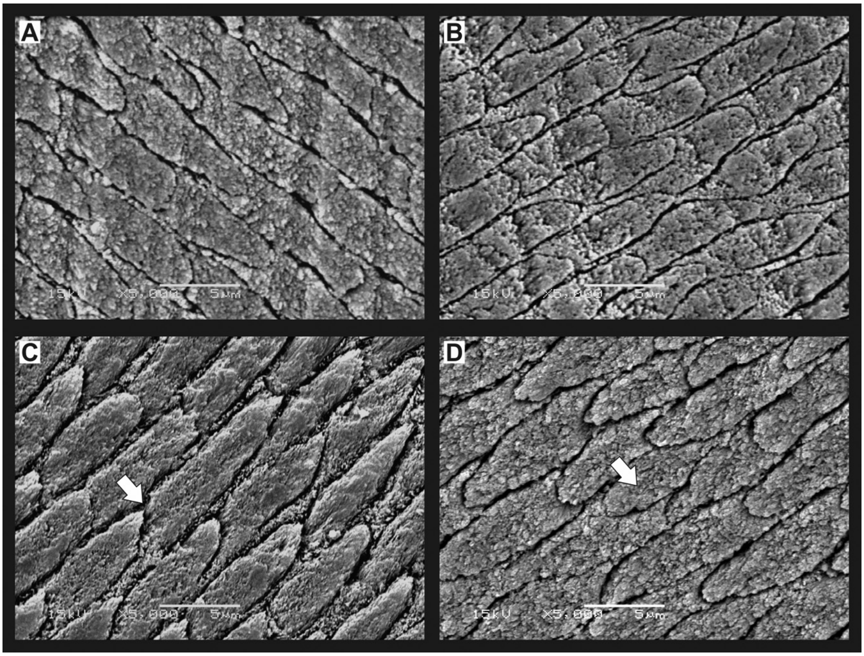

FIGURE 6 SEM analysis showed increased aggressiveness associated with the increase in water content of the experimental SE primers. In (A) 5% of water, and (B) 10% of water, it is possible to observe slight demineralization of the ground enamel. In (C) 20% of water, the demineralization was more accentuated on the interprismatic zone (arrow). The primer with (D) 40% of water showed the most aggressive etching pattern, with higher presence of porousity on the surface of the prisms (arrow) as compared with the other primers. (Scale markers indicate 5 μm.)

was more pronounced, with increased porosity in the center of the enamel prisms, and greater irregularity at the edge of enamel prisms compared with P_{20}.

Figure 7A shows an SEM picture of the enamel smear layer produced, while in Fig. 7B the etching pattern for the P_0 primer is depicted. For this primer, slight demineralization was noted, which was just able to remove the smear layer and the presence of surface scratches was observed, as in the previous micrograph. Figure 7C shows the etching pattern for the primer with the highest bond strength values (P_{20}). In this group, scratches and remnants of apatite crystals loose on the surface could be observed. In Figs. 7C and D it is possible to compare the etching pattern of P_{20} with CSEB: P_{20} caused

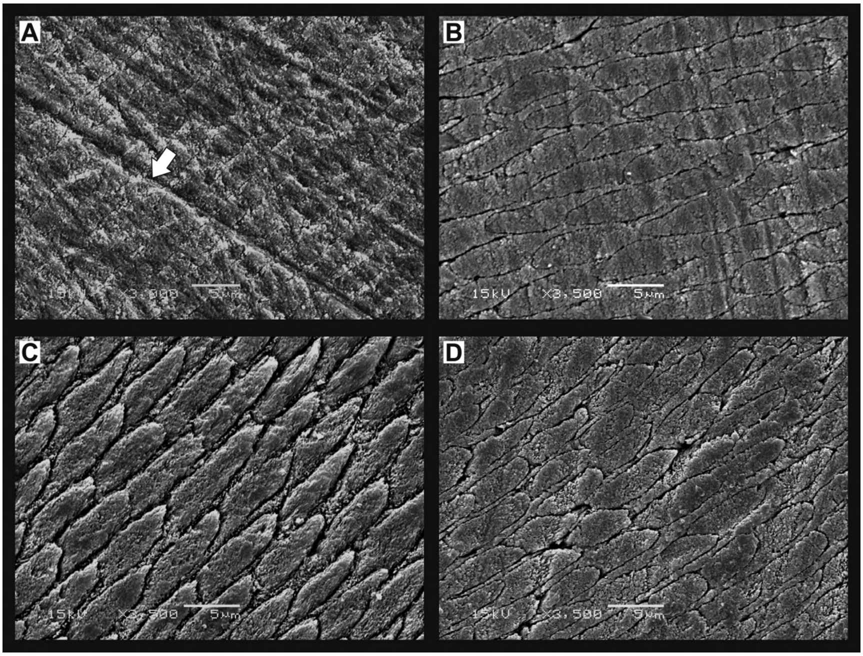

FIGURE 7 SEM pictures of (A) untreated, control surface, (B) anhydrous primer, (C) primer P_{20}, and the (D) commercial reference CSEB. In (A), the presence of smear layer and scratches resulting from the polishing procedures can be observed (arrow). In (B), slight demineralization of the enamel surface and the presence of scratches can be noticed. In (C), no surface scratches are detected; demineralization occurred in the center of the prisms and around them. In (D), slighter demineralization compared with P_{20} and scratches are observed. (Scale markers indicate 5 μm.)

increased demineralization with no surface scratches being observed, while surfaces treated with CSEB still showed scratches.

4. DISCUSSION

Results of the present study provide evidence that water concentration significantly affected the bonding of the experimental SE primer to the enamel. The primer containing 20% water presented the highest bond strength values, followed by the primers with 5 and 10%. Apart from bond strength, the etching aggressiveness was also affected by water concentration. An increase in aggressiveness was observed with increased water content. The acidic monomers contained in the primer present low dissociation constants, water being required for dissociation into ionized forms to allow demineralization. Increasing the water available may increase the rate of ionization of the monomers, enhancing the creation of retention for bonding.

The activity of the SEA might be modified by a neutralization reaction with the mineral component of the tooth (*i.e.*, calcium compounds). Buffered acidic monomers gradually lose their ability to etch dental tissues [11]. Water may solubilize dissolved calcium and phosphate ions, avoiding re-precipitation of calcium phosphates that would neutralize the primer and even prevent resin infiltration into the underlying substrate. However, the lowest bond strength was detected for the primer containing 40% water, in spite of its increased aggressiveness. This indicates there is a limit to the amount of water that might enhance the bond strength of the primer. Although with an increase in the rate of ionization of the monomers, excess water could decrease the strength of bonding due to competition with monomers for infiltration into the substrate. On the other hand, the bonding technique used a piece of absorbent paper to remove water from the enamel surface. It can be speculated that the bond strength of the primer with 40% water would be improved had a more aggressive drying technique being used.

Not surprisingly, the primer with no water added presented significant bond strength values. Previous studies confirm that anhydrous primers may demineralize and bond to dentin [5,9]. Hiraishi *et al.* [5] suggested the water responsible for the ionization of the acidic monomers could have been derived from the underlying dentin. It might be further speculated that the ethanol used as a solvent in the primer might also ionize the monomers [12]. However, water molecules are characterized by a higher tendency to donate hydrogen than other solvent molecules, and could explain the lower bonding performance of the anhydrous primer in comparison with the others primers, except for P_{40}.

The present results indicate that the aggressiveness of the primer had a direct effect on the bonding outcome. Poor bond strength was verified for primers showing less aggressive etching patterns. In spite of that, the failure modes were similar among all groups, with a predominance of adhesive and mixed failures. For conventional ERA systems, increased etching times might favor the occurrence of failure within the dental substrate because of the discrepancy of conditioning and infiltration depths. For SEA, adhesion is obtained through shallow hybridization with residual hydroxyapatite (HAp), usually generating adhesive or mixed failures. The only exception was for P_{40}, which showed aggressive etching but low bond strength. In addition, for P_{40}

alone, cohesive failures within the bonding resin were detected, which might be taken as evidence of the excess water affecting the polymerization of the resin. Water might reduce the degree of conversion [13] and interfere with polymerization. As a result, unpolymerized acidic monomers could continue to etch the dentin, leading to a detrimental impact on the bond [14,15].

Compared with the reference commercial material, most of the experimental SE primers presented similar or increased etching aggressiveness but yielded lower enamel bond strengths, except for P_{20}. This is another piece of evidence that the etching aggressiveness is not the only factor influencing the adhesion of functional monomers to hard dental tissues. The experimental primers presented similar pH (according to Table 1) independent of the water concentration, while CSEB showed higher pH. The effect of pH of SE primers on the bonding to dentin has been investigated [16], and the results indicate that the bond strength was not affected by the acidity of the solution. The acidity of the primer depends on its pKa, which in turn depends on the molar concentration of the acid and the acidity constant, which is a quantitative measure of the strength of an acid in solution [16].

The differences in bond strength between the commercial material and experimental primers may also be related to the distinct functional monomers MDP and MEP/Bis-MEP. When phosphorylated monomers are employed in SEAs, some reactions with the dental substrate are triggered. The mechanism starts with demineralization of the HAp until equilibrium of the reaction is achieved. From this point, chemical adsorption between the acidic monomers and the HAp may occur by means of ionic bonds [17]. A previous study [18] described a reaction mechanism with an ionic binding model of phosphate monomers interacting electrostatically with the Ca^{+2} ions of HAp, where either one or two of the P-OH groups of the monomer dissociated H^+ to form one or two $P\text{-}O^-$ groups during chemical interaction with HAp. Additionally, a covalent binding model of phosphate monomer is described with its condensation with the PO_4^{3-} ions from HAp to form pyrophosphate groups. These reactions may be expected to vary according to the phosphate monomer presented in the primer.

In this study it was found that, within certain limits, increasing water concentration in SE primers might be an efficient way of enhancing their aggressiveness and bonding ability. Generally, by increasing the percentage of an organic solvent in the aqueous medium, the relative permittivity of the medium is lowered [12], decreasing the acid ionization constants. This indicates that other solvent effects such as hydrogen bonding and solvent basicity, as well as proton-solvent interaction and dispersion forces, in addition to the electrostatic effect, exert a profound influence on the ionization processes [12]. However, the present results also indicate that a primer with high water content (P_{40}) may present increased etching aggressiveness but decreased strength of bonding to enamel. As previously mentioned, this finding might be related to the failure mode, as the increased etching may generate areas of stress concentration within the etched enamel, and also related to the difficulty in fully removing the free, unused water in the primer, interfering with the polymerization of the material.

Under clinical conditions, enhanced bonding of SEA to enamel might result in an increase of the durability of the restoration, and even in a preservation of the dental tissues. For example, when the effect of SEAs on the bond strength of orthodontic brackets is evaluated, an improvement in the bonding procedure by minimizing enamel loss and reducing chair time, while still maintaining sufficient bond strengths between brackets and enamel, might occur. In addition, the clinician should be aware that some SEA can leave the enamel surface healthier after debonding as compared with ERA [19].

5. CONCLUSION

The water content in self-etching primers presents a significant influence on the strength of bonding and etching aggressiveness to ground enamel. The higher the water content, the higher the etching aggressiveness. However, the highest bond strength was obtained for the primer with 20% of water, while the primer with 40% of water showed bonding ability comparable with the anhydrous primer.

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