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Bond strength, biaxial flexural strength and flexural modulus of dentin bonding systems exposed to water

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ABSTRACT

Purpose: This study evaluated the effects of storage time on dentin bond strength, biaxial flexural strength, and flexural modulus of four adhesive systems.
Materials and Methods: The following adhesive systems were tested: Easy Bond, Scotchbond SE, Single Bond Plus, and Scotchbond Multi-Purpose. Sixty human third molars were used for the microtensile bond strength test ($n=15$). The adhesives were applied to flat occlusal dentin surfaces according to the manufacturers' instructions and a Filtek Supreme resin composite block (6 mm high) was incrementally built up. After 24 h, the teeth were prepared for the bond strength test. The specimens were stored for one week, six months, and one year in distilled water. At the end of each storage period, the specimens were tested under tension (0.5 mm/min) until failure occurred. For the biaxial flexural test, resin discs of each adhesive (0.6 mm thick and 6.0 mm in diameter) were prepared in silicon molds ($n=10$). The discs were stored for the same storage periods in distilled water prior to testing in a universal testing machine (1.27 mm/min). Data were analyzed using two-way analysis of variance and Tukey's test ($\alpha=0.05$).
Results: Bond strength values decreased significantly after six months and one year of water storage only for Scotchbond SE (from 48.1 ± 11.0 to 24.5 ± 15.3 MPa after one year). The storage time did not affect the flexural strength or modulus for any adhesive tested.
Conclusion: Water storage for six months or one year can reduce the dentin bond strength of adhesives; however, the results are product-dependent. No changes in flexural strength or modulus of the adhesives tested were observed after storage of any duration.

1. Introduction

The current classification of dentin bonding agents is based on the adhesion strategy and number of clinical application steps. Depending on the bonding strategy, the adhesives can be etch-and-rinse or self-etching systems, and according to clinical application, they are classified as one, two, or three steps. The first step for the application of an etch-and-rinse adhesive involves phosphoric acid etching, rinsing, and moisture control of the conditioned dentin surface. Subsequently, when using a three-step etch-and-rinse adhesive, a priming step is required, followed by the application of a hydrophobic adhesive resin [1].

Simplified two-step etch-and-rinse adhesives combine the primer and adhesive resin components. Given that three- and two-step etch-and-rinse adhesives are developed and produced by the same manufacturers, most of these bonding agents present similar compositions regarding solvents and adhesive monomers. Nevertheless, despite their similar compositions, *in vitro* and *in vivo* bonding effectiveness data from these etch-and-rinse adhesives have shown that ethanol/water-based, three-step etch-and-rinse adhesives are considered the "gold standard" materials in terms of bonding durability [2–6].

Whereas micro-mechanical interlocking associated with hybrid layer formation is the main bonding mechanism for etch-and-rinse adhesive systems, some self-etching adhesives contain acidic monomers, such as 10-MDP, 4-META and MAC-10, which are able to chemically bond to mineralized dental tissues [7–10]. These acidic monomers form an ionic bond with the calcium in hydroxyapatite crystals, providing additional adhesion for some self-etching adhesives [3,11,12]. Simplified adhesives that combine self-etching primers with the hydrophobic adhesive resin in only one application are known as "all-in-one" or one-step self-etching adhesives. Although there is a tendency towards the simplification of adhesive solutions and bonding

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procedures, inadequate performance of early one-step self-etching adhesives reported as a result of some scientific studies has limited the clinical use of this type of adhesives [3,6,11,13].

Adhesives are important to bond resin-based restorative materials to enamel and dentin. Thus, they must possess a high bond strength to tooth structure, a degree of conversion and adequate mechanical properties such as elastic modulus and strength to resist occlusal loading and hydrolytic degradation. Measurements of such mechanical properties as well as dentin bond strength can provide important information about the different types or categories of adhesive systems. The aim of this study was to analyze four adhesive systems, representing different bonding strategies, to assess their mechanical properties and long-term bonding effectiveness when exposed to water. The null hypotheses to be tested were (1) that adhesives with different application modes would not show significant differences in dentin bond strength, biaxial flexural strength, or flexural modulus and (2) that long-term water storage would not affect the bond strength, biaxial flexural strength, or modulus of any adhesive.

2. Materials and methods

2.1. Microtensile bond strength

Sixty caries-free recently extracted human third molars stored in 0.1% thymol solution at 4 °C were used for analysis. The teeth were obtained under a protocol approved by the review board of the Piracicaba Dental School (#146/2010). Occlusal enamel and roots were removed using a diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication to expose a middle-depth dentin surface parallel to the occlusal surface. Flat dentinal surfaces were wet abraded with 600-grit silicon carbide paper (3M of Brazil, Sumaré, SP, Brazil) for 10 s to create a standardized smear layer. The surfaces were randomly divided into four groups according to the different adhesive systems ($n=15$).

Four commercially available dentin adhesive systems (Table 1) were tested: a one-step self-etching (Easy Bond), two-step self-etching (Scotchbond SE), two-step etch-and-rinse (Single Bond Plus), and three-step etch-and-rinse (Scotchbond Multi-Purpose) adhesive (3M ESPE, St. Paul, MN, USA).

The adhesive systems were applied according to the manufacturers' instructions. Following application, a resin composite block (6 mm high) was incrementally built up in three layers with Filtek Supreme (3M ESPE, St. Paul, MN, USA, lot number: N118032) onto the bonded dentin surfaces. Each incremental layer was light cured for 20 s (irradiance of 620 mW/cm^2, XL 3000, 3M ESPE, St. Paul, MN, USA), monitored by radiometer (Demetron Optilux Radiometer, Kerr Corp. Orange, CA, USA). The teeth were then stored in distilled water at 37 °C for 24 h.

The bonded teeth were prepared for microtensile testing using the "non-trimming" technique [14]. Each tooth was vertically and serially sectioned into 0.9-mm thick slices using the same diamond saw under water lubrication. Each slice was then further sectioned to produce twelve bonded specimens of approximately 0.9 mm^2. Four bonded samples were stored in distilled water for one week, four for six months, and another four specimens for one year. In the groups that were stored in water for six months and one year, the water was changed monthly.

At the end of each storage period, the bonded specimens were fixed to the grips of a microtensile testing device using cyanoacrylate glue (Super Bonder Gel, Henkel/Loctite, Diadema, SP, Brazil) and tested under tension at a cross-head speed of 0.5 mm/min until failure in a universal testing machine (Ez-Test, Shimadzu, Kyoto, Japan). After fracture, the specimen was removed from the testing apparatus and the cross-sectional area at the site of fracture was measured with a digital caliper (Starrett Ind. Com. Ltda., Itu, SP, Brazil) to calculate the tensile bond strength. A single failure stress value was then calculated for each tooth by averaging the values of the four bonded slices from that tooth (a total of 720 specimens tested). Bond strength data were analyzed by split-plot two-way analysis of variance (ANOVA) followed by Tukey's test (with a preset alpha of 0.05), considering adhesive and storage time as variables.

Fractured surfaces of the tested specimens were allowed to air-dry overnight at 37 °C, after which they were sputter-coated with gold (MED 010, Balzers, Balzer, Liechtenstein) and examined by a single individual using a scanning electron microscope (VP 435, Leo, Cambridge, UK). Failure patterns were classified as (1) cohesive within the composite, (2) cohesive within the adhesive layer, (3) cohesive within the dentin, (4) adhesive along the dentin surface, (5) mixed when simultaneously exhibiting dentin surface, adhesive layer and remnants of composite. Representative areas of the failure patterns were photographed at 90× magnification.

2.2. Biaxial flexural strength and flexural modulus

Adhesive solutions from each adhesive bottle were dispensed into a mixing well and air-dried for 20 s to allow the organic

Table 1
Composition of adhesive systems used.

Adhesive (classification)	**Composition** (% by weight)	**Lot number**
Adper Easy Bond (one-step self-etching)	Bisphenol A diglycidyl ether dimethacrylate (15–25%), 2-hydroxyethyl methacrylate (15–25%), ethanol (10–15%), water (10–15%), phosphoric acid-6-methacryloxy-hexyl esters (5–15%), silane treated silica (8–12%), 1,6-hexanediol dimethacrylate (5–10%), copolymer of acrylic and itaconic acid (1–5%), (dimethylamino) ethyl methacrylate (1–5%), camphorquinone (1–3%), 2,4,6-trimethylbenzoyldiphenylphosphine oxide (1–3%)	362007
Adper Scotchbond SE (two-step self-etching)	Liquid a: water (70–80%), 2-hydroxyethyl methacrylate (10–20%) Liquid b: surface treated zirconia (15–25%), triethylene glycol dimethacrylate (15–25%), di-hema phosphates (10–15%), mono hema phosphate (5–10%), methacrylated pyrophosphates (5–10%), tri hema phosphate (< 3%), phosphoric acids-6-methacryloxy-hexyl esters (5–10%), 1,6-hexanediol dimethacrylate (< 4%), diurethane dimethacrylate (1–10%), trimethylolpropane trimethacrylate (5–15%), ethyl 4-dimethyl aminobenzoate (< 2%), dl-camphorquinone (< 2%)	a: 9BU b: 9BW
Adper Single	Etchant: water (55–65%), phosphoric acid (30–40%), synthetic amorphous silica (5–10%).	Etchant: 9NL
Bond Plus (two-step etch-and-rinse)	Adhesive: ethyl alcohol (25–35%), silane treated silica (nanofiller) (10–20%), bisphenol A diglycidyl ether dimethacrylate (10–20%), 2-hydroxyethyl methacrylate (5–15%), glycerol 1,3-dimethacrylate (5–10%), copolymer of acrylic and itaconic acids (5–10%), water (< 5%), diurethane dimethacrylate (1–5%)	Adhesive: 9WP
Adper Scotchbond Multi-Purpose (three-step etch-and-rinse)	Etchant: Water (55–65%), phosphoric acid (30–40%), synthetic amorphous silica (5–10%) Primer: Water (40–50%), 2-hydroxyethyl methacrylated (35–45%), copolymer of acrylic and itaconic acids (10–20%) Adhesive: Bisphenol A diglycidyl ether dimethacrylate (60–70%), 2-hydroxyethyl methacrylate (30–40%)	Etchant: 9NL Primer: 9CE Adhesive: 9RM

solvents to evaporate. The adhesive solutions were then placed in elastomeric impression material molds (Aquasil Ultra LV, Dentsply Calk), positioned on a microscope slide covered with a Mylar sheet. After slight overfilling, a second Mylar sheet was placed on the upper surface, upon which another microscope slide was positioned and maintained under a slight hand pressure to ensure that the fluid perfectly filled the mold. In this manner, a total of 120 disc-shaped specimens (0.6 mm in thickness and 6.0 mm in diameter) were fabricated and were then light-cured by 10-s light exposure on both sides with a halogen light-curing unit (light intensity: 620 mW/cm^2, XL 3000, 3M ESPE). The adhesive discs were then stored in distilled water for one week, six months, or one year at 37 °C before mechanical testing was performed. Thus, 30 discs per adhesive system were randomly assigned to one of three groups according to storage time (one week, six months or one year, $n=10$). For groups that were stored in water for six months and one year, the water was changed monthly.

At the end of the elapsed storage periods, each disc was placed in a custom-made testing jig and tested under biaxial flexion using a universal testing machine (Instron 5844, Instron Corp., Canton, MA, USA) at 1.27 mm/min until failure occurred [15]. Flexural strength and flexural modulus were recorded and analyzed using two-way ANOVA followed by Tukey's test ($\alpha=0.05$), with adhesive and storage time as variables.

Table 2
Mean microtensile bond strength (SD) in MPa to dentin of the adhesive systems tested after various storage periods.

Adhesive systems	Storage periods		
	One week	Six months	One year
Easy Bond	47. 8 (10.2) Aa	45.1 (11.7) Aab	42.8 (15.2) Aa
Scotchbond SE	48.1 (11.0) Aa	33.8 (8.1) Bb	24.5 (15.3) Bb
Single Bond Plus	52.8 (10.6) Aa	52.2 (5.8) Aa	46.7 (8.8) Aa
Scotchbond Multi-Purpose	50.5 (10.2) Aa	47.7 (14.5) Aa	40.7 (11.3) Aa

Means indicated by the same letters (uppercase – row, lowercase – column) were not significantly different ($P>0.05$).

3. Results

3.1. Microtensile bond strength

Results of two-way ANOVA indicated that both adhesive ($P<0.0001$) and storage time ($P<0.0001$) as well as their interaction ($P=0.0339$) had a significant influence on bond strength. Summarized statistics for the different experimental groups are shown in Table 2. After one week of water storage, the adhesives did not exhibit any significant difference in mean bond strength ($P>0.05$). Following long-term water storage, data indicated that the bond strengths of Easy Bond, Single Bond Plus and Scotchbond Multi-Purpose were not affected by water storage ($P>0.05$), whereas the six-month and one-year specimens of Scothbond SE showed a significantly lower bond strength compared with their controls, which were tested after one week of water storage ($P<0.05$).

Fig. 1 shows the proportional prevalence (%) of the different failure patterns in all experimental groups. Representative images depicting the failure types are presented in Figs. 2–7. All groups

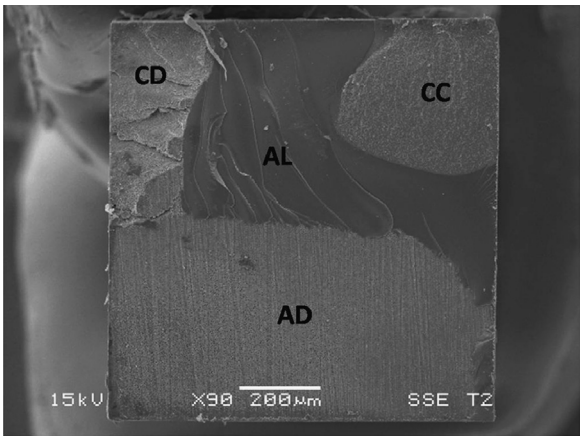

Fig. 2. Mixed Failure showing the dentin surface (AD), the fractured dentin (CD), remnants of composite (CC) and adhesive layer (AL) for Scotchbond SE (storage in water for 6 months) (original magnification 90 ×).

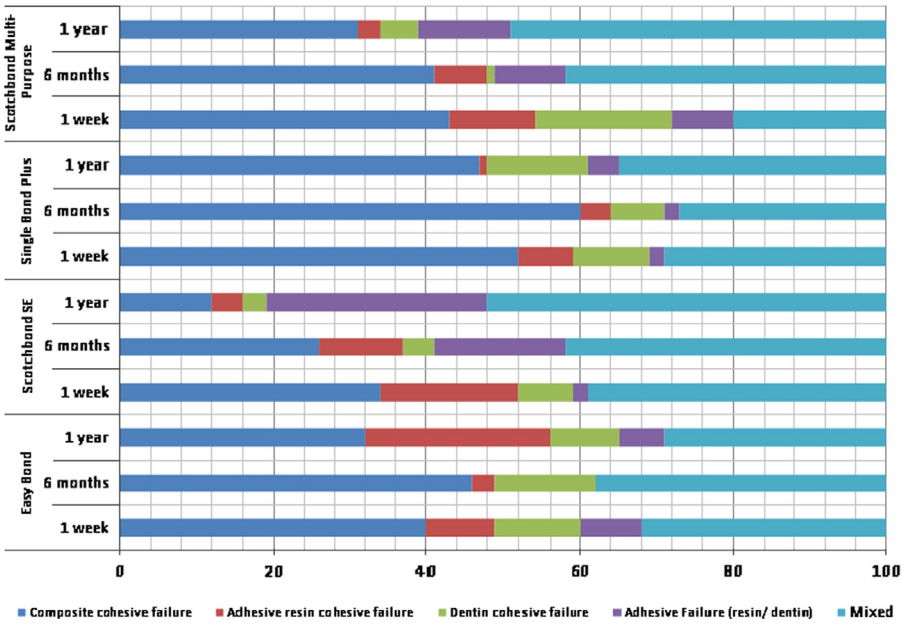

Fig. 1. Distribution of failure modes among experimental groups.

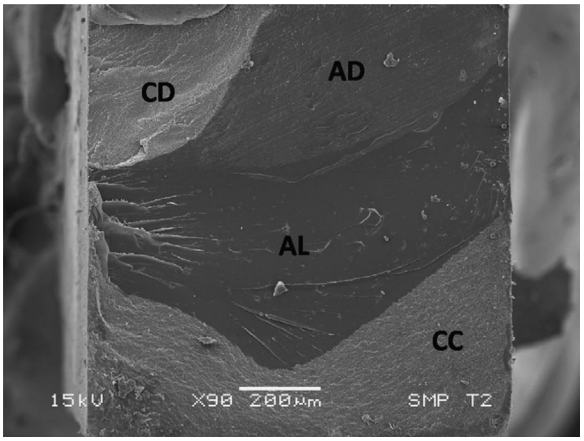

Fig. 3. Mixed Failure showing the dentin surface (AD), the fractured dentin (CD), remnants of composite (CC) and adhesive layer (AL) for Scotchbond Multi-Purpose (storage in water for 6 months) (original magnification 90 ×).

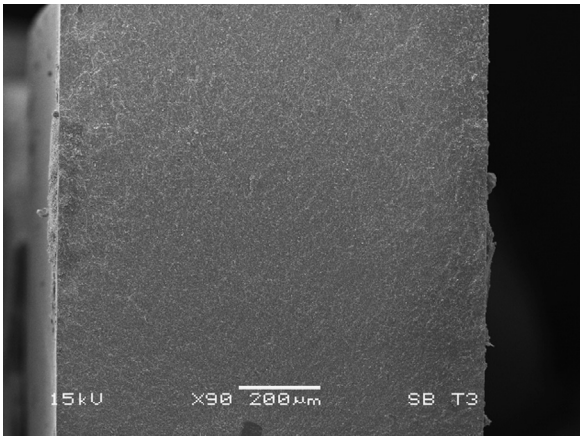

Fig. 4. Cohesive failure within the composite resin for the Single Bond Plus adhesive (storage in water for one year) (original magnification 90 ×).

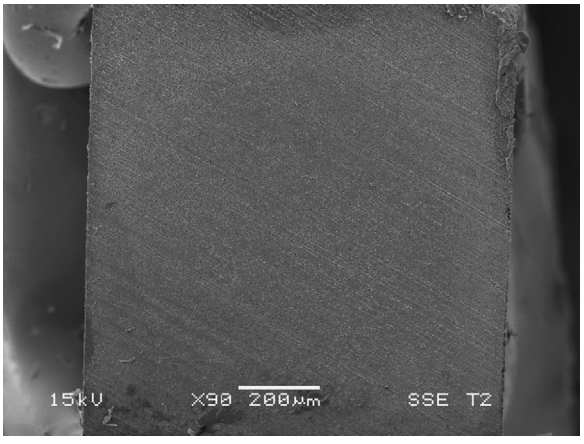

Fig. 5. Adhesive failure along the dentin surface for the Scotchbond SE adhesive (storage in water for 6 months) (original magnification 90 ×).

showed a high incidence of mixed fractures (Figs. 2 and 3) and cohesive failure within the composite resin (Fig. 4). Lower incidences of other failure modes were observed (Figs. 5–7).

3.2. Biaxial flexural strength and flexural modulus

Biaxial flexural strength and modulus values are presented in Tables 3 and 4, respectively. Statistically significant differences

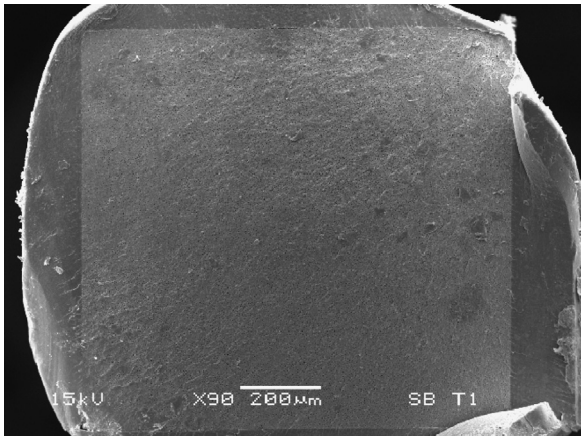

Fig. 6. Cohesive failure within the dentin for the Single Bond Plus adhesive (storage in water for one week) (original magnification 90 ×).

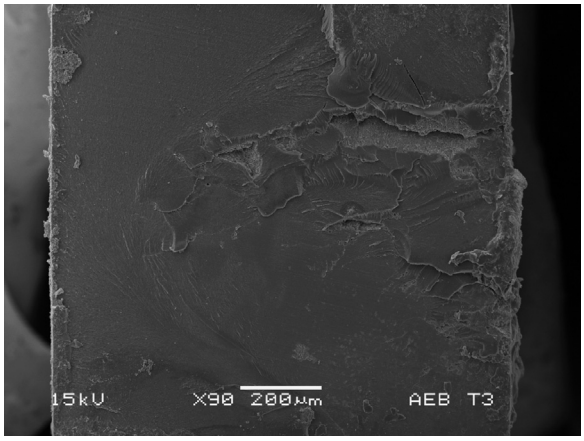

Fig. 7. Cohesive failure within the adhesive layer for the Easy Bond adhesive (storage in water for one year) (original magnification 90 ×).

Table 3
Mean biaxial flexural strength (SD) in MPa of the adhesive systems tested after various storage periods.

Adhesive systems	Storage periods		
	One week	Six months	One year
Easy Bond	73.8 (7.1) Aa	74.4 (11.8) Aa	77.4 (6.2) Aa
Scotchbond SE	67.5 (15.4) Ab	61.4 (21.4) Ab	53.1 (13.5) Ab
Single Bond Plus	58.6 (6.6) Ab	59.1 (16.9) Ab	59.9 (3.2) Ab
Scotchbond Multi-Purpose	85.1 (9.3) Aa	88.3 (29.2) Aa	78.5 (8.1) Aa

Means indicated by the same letters (uppercase – row, lowercase – column) were not significantly different ($P > 0.05$).

Table 4
Mean biaxial flexural modulus (SD) in GPa of the different adhesive systems tested after various storage periods.

Adhesive systems	Storage periods		
	One week	Six months	One year
Easy Bond	1.4 (0.3) Ab	1.3 (0.3) Ab	1.6 (0.3) Ab
Scotchbond SE	1.8 (0.2) Aa	1.6 (0.3) Ab	1.8 (0.4) Aab
Single Bond Plus	1.3 (0.2) Ab	1.3 (0.4) Ab	1.6 (0.3) Ab
Scotchbond Multi-Purpose	2.1 (0.2) Aa	2.3 (0.3) Aa	2.2 (0.2) Aa

Means indicated by the same letters (uppercase – row, lowercase – column) were not significantly different ($P > 0.05$).

were found among adhesives with regard to flexural strength ($P < 0.0001$) and modulus ($P < 0.0001$). The flexural strength and modulus of adhesives were not affected by storage time ($P = 0.4051$ and $P = 0.3901$, respectively). In addition, the interaction between adhesive system and storage time did not significantly affect flexural strength ($P = 0.3995$) or modulus ($P = 0.0630$).

The mean flexural strength values for Easy Bond and Scotchbond Multi-Purpose were higher than those observed for Scotchbond SE and Single Bond Plus at all storage times ($P < 0.05$). The biaxial flexural modulus for Scotchbond Multi-Purpose was higher than that of Easy Bond and Single Bond Plus ($P < 0.05$). Nevertheless, no significant difference was detected between the three-step etch-and-rinse adhesive and Scotchbond SE after one week or one year of water storage.

4. Discussion

The first null hypothesis stating that adhesives with different application modes would not show any significant difference in dentin bond strength, biaxial flexural strength, or flexural modulus was rejected since the adhesive systems presented different results mainly regarding flexural strength and modulus. The second hypothesis was also rejected since the two-step self-etching system showed reduced dentin bond strength after water storage for six months and one year.

Until recently, simplified "all-in-one" adhesives presented inadequate clinical and laboratory performance, because they contained a high concentration of hydrophilic monomers. Even after polymerization, they acted as semi-permeable membranes, because they contained a high organic solvent concentration that formed a thin adhesive resin layer, with voids from residual solvent and water. The monomer/water ratio changes during solvent evaporation, which can result in phase separation and blistering [6,11,13].

In this study, the dentin bond strength, biaxial flexure strength and modulus of Easy Bond one-step self-etching adhesive did not decrease significantly after six months or one year of water storage. Previous studies using adhesives belonging to this category reported lack of dentin bond strength stability after long-term water storage [3,11,13]. However the one-step self-etching adhesive tested in this study showed similar performance to the three-step etch-and-rinse adhesive known as the "gold standard" in terms of bonding durability, with the exception of the flexural modulus. The functional monomer of Easy Bond is the phosphoric acid-6-methacryloxy-hexyl ester. It also contains other resin monomers, such as Bis-GMA and dimethacrylates that are important for the higher mean biaxial flexural modulus values compared to those obtained with Scotchbond SE and Single Bond Plus adhesives, but results were not significantly different from those obtained with a three-step adhesive (Scotchbond Multi-Purpose). The acidic functional monomer is responsible for dentin conditioning and interaction with the substrate; however, no chemical reaction of this acidic monomer with dentin has been described. The concentration of ethanol, an organic solvent, is approximately 10–15%. The water content is similar and is important for ionization of the functional monomer [16].

The two-step self-etching system tested (Scotchbond SE) contains the same phosphoric acid-6-methacryloxy-hexyl ester. Nevertheless, other acidic monomers are also present in its formulation, such as methacrylated pyrophosphates and mono-, di-, and tri-hema phosphates. This adhesive cannot be considered as a self-etching primer, because the primer solution is not acidic and contains only an aqueous solution of HEMA monomer. The water (70–80%) present in bottle "A" is responsible for ionization of the acidic monomers contained in bottle "B", which occurs "*in situ*" when the contents of the two bottles are mixed. Other resin monomers are also present in the composition of Scotchbond SE (bottle "B"), including TEGDMA, UDMA, and di- and tri-methacrylates. Such monomers are related to the formation of a more highly cross-linked polymer network [17], which can increase the flexural modulus. When comparing the flexural modulus of this self-etching system to that of Scotchbond Multi-Purpose (the "gold standard"), the results showed no significant difference after one week or one year of water storage. Regarding the dentin bond strength of Scotchbond SE, a significant decrease was observed after storage in water for six months, but no further significant decrease was observed after one year. One possible explanation for this bond strength reduction and lower biaxial flexural strength is the amount of water from bottle "A" and hydrophilic monomer remaining after primer application and air-drying, which may reduce monomeric conversion, worsen mechanical properties and accelerate hydrolytic degradation.

The etch-and-rinse systems (Single Bond Plus and Scotchbond Multi-Purpose) showed no difference in dentin bond strength; however, lower biaxial flexural strength and flexural modulus values were observed for Single Bond Plus when compared to Scotchbond Multi-Purpose. The Single Bond Plus adhesive contains up to 20% Bis-GMA hydrophobic monomer, while the "Bonding Resin" bottle of Scotchbond Multi-Purpose contains approximately 60–70%. Such a high concentration of hydrophobic monomers is responsible for the increase in flexural strength and modulus of the adhesive polymer [12,18].

Ito et al. [19] and Hosaka et al. [20] showed that the elastic modulus of the adhesives reduced significantly after short-term water-storage. The water absorption promoted by polymers may cause plasticization, decreasing the mechanical property studied. They also reported that the water absorption level depends on the monomeric composition of each adhesive system. In these studies, the effects of increasing hydrophilicity on water absorption and elastic modulus of commercial and experimental adhesive resins were evaluated. The least hydrophilic resin showed a 15% decrease in elastic modulus, while the most hydrophilic experimental resin showed a 73% reduction in the elastic modulus after 3 days of water storage. Further, they also reported that the commercial resins presented a 19–42% reduction in elastic modulus [19]. The study performed by Hosaka et al. [20] tested five one-step self-etching adhesives and reported that water-storage for 24 h also reduced the modulus of elasticity from 584 to 1073 MPa.

The primary concern related to the etch-and-rinse adhesives is the degradation of collagen fibrils within incompletely resin-infiltrated hybrid layers. Degradation of resin–dentin bonding can result from two factors: (1) the collagenolytic activity of specific proteases such as matrix metalloproteinases and cysteine–cathepsins [21–24] that contribute to the auto-degradation of collagen fibrils and (2) early hydrolytic degradation of the polymer network due to the poor polymerization reaction of hydrophilic monomers [12]. Our results clearly indicate that one year of water storage was not sufficient to cause severe degradation of adhesives or their respective hybrid layers. In addition, the storage in pure water without the addition of enzymes or essential ions that are required to activate host-derived proteases, which accelerate the *in vitro* degradation of resin-bonded interfaces, may require a longer period to affect the *in vitro* integrity of resin-bonded specimens. Thus, additional studies extending the storage time and/or using water supplemented with enzymes and/or other components which activate proteases should be performed in order to enhance the comparison among these categories of dental adhesive systems.

The failure patterns obtained in this study are in agreement with those of previous studies and involve cohesive (composite, dentin or adhesive resin), adhesive (resin–dentin) or mixed fractures [6,25,26]. Single Bond Plus was the least affected by water storage, while the other three adhesives presented more changes. More adhesive and cohesive fractures were observed

within adhesive layer failures over time in the Scotchbond SE and Easy Bond self-etching systems, respectively. Some adhesive failures may be related to degradation of the adhesive dentin and the bonding between adhesive and dentin. Scotchbond Multi-Purpose showed decreasing cohesion within the composite/adhesive layer and an increase in mixed failures over time.

5. Conclusion

The present study indicates that the dentin bond strength of Scotchbond Multi-Purpose, Single Bond Plus and Easy Bond adhesives is unaffected by water storage for one year. Water storage did not reduce the biaxial flexural strength or the flexural modulus of any of the adhesive systems over time. The data regarding the three important properties of the adhesives evaluated in this study will increase knowledge of the clinical behavior of composite restorations made with these bonding agents.

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