Microtensile Bond Strength of 4 Dentin Adhesives to Primary Dentin

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ABSTRACT

Purpose: This study's purpose was to evaluate the aging effect on the bond strength of 4 adhesive systems on primary dentin 24 hours and 6 months after bonding.
Methods: The crowns of extracted, caries-free primary molars were grounded and flat surfaces were prepared and distributed into 4 groups (N=7-9). The adhesives Scotchbond Multi-purpose (SMP), Single Bond (SB), Clearfil SE Bond (CSB), and Adper Prompt L-Pop (APL) were applied with the composite resin Filtek Z-250. Specimens were stored in distilled water (37°C/24h), the crowns were sectioned, and 0.8-mm^2 resin-dentin sticks were obtained and divided into 2 groups: (1) 24h; and (2) 6m. Each group's specimens were tested under tensile at 0.5 mm/minute until they fractured. The fractured sticks were examined by scanning electron microscopy. The data were analyzed by 2-way repeated measures: analysis of variance; Tukey's test (P<.05); and Kruskal-Wallis test (P<.05).
Results: The mTBS (MPa) means values were: SMP=33.28 (2.05), SB=23.27 (4.78), APL=20.64 (10.66), and CSB=18.94 (11.94) for the 24-hour group; and SMP=30.59 (6.29), SB=22.39 (5.9), CSB=21.50 (10.67), and APL=17.19 (6.88) for the 6-month group. The most frequent fracture type found was cohesive failure of the adhesive and mixed, with no statistically significant difference found between the groups.
Conclusions: Resin bond strength on primary dentin was not influenced by aging for the adhesive systems tested.
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KEYWORDS: PRIMARY TEETH, DENTIN, AGING, MICROTENSILE BOND STRENGTH, ADHESIVE SYSTEMS

Adhesive systems are often used in dentistry, allowing for very conservative preparations. Bonding to dentin is critical due its structural complexity.[1,2] It is based on the hybridization's mechanism, where there is a micromechanic bond between adhesive monomers and collagen fibrils from the demineralized dentin, forming the hybrid layer.[3]

Bond strength is an important indication of the adhesive's effectiveness,[4] since the hybrid layer must support polymerization shrinkage as well as occlusal forces[5] to avoid gap formation which favors leakage, bacterial penetration, recurrent caries, and postoperative sensitiveness.[6]

Bond aging by water storage helps estimate a resin filling's durability, since the adhesive interface's components can be degraded by the hydrolysis of collagen fibrils over time. This subpolymerized polymer is, therefore, more prone to the plasticizing effects of water over time.[1,7]

It has been observed that simplification of the adhesive systems does not necessarily render a better bond,[4,7-11] but the simplification of the adhesives procedures by reducing the number of steps and time of

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application may reduce handling possible defects.[4] For pediatric dentistry, this is particularly significant, because faster bond procedures are preferred due to less chair time.[12,13]

The objective of this study was to evaluate the effects of 6-month water storage on primary dentin bond strength for different adhesive systems by means of microtensile tests.

METHODS

Sixty-one extracted, caries-free human primary molars were used. Teeth were stored in a 0.9% saline solution with 0.1% thymol (pH=7) at room temperature. This study was approved by the Ethical Committee for Research on Human Beings of the Federal University of Santa Catarina (protocol no. 205/07). Teeth were used within 6 months after extraction.

The teeth roots were grounded to approximately 2 mm below the dentin enamel junction with the use of a wet no. 200 silicone (**SiC**) grit carbide paper in a polishing machine (Panambra Struers DP-10, Panambra, São Paulo, Brazil). Next, the pulp tissue of each tooth was removed and the pulp chamber was filled with Single Bond adhesive system (3M ESPE, St. Paul, Minn, USA) and Z250 composite resin (3M ESPE) which was impregnated with Rhodamine B in propylene glycol. This procedure was performed to reinforce the tooth structure during sectioning. A new 3-mm layer of composite resin was applied over the filled pulp chamber to simulate part of the root and facilitate the following procedures.

The occlusal enamel of each deciduous molar specimen was grinded with a no. 200 grit SiC paper until a flat dentin area was completely exposed. The exposed dentin surfaces were further wet polished with nos. 400 and 600 grit SiC papers in 4 different directions, 10 seconds each, to standardize the smear layer. The longitudinal surfaces formed were composed of primary and sound dentin once all teeth were caries-free.

BONDING PROCEDURES

After cleaning with distilled water, specimens were divided into 4 groups (N=7-9 teeth) for each adhesive system: (1) Scotchbond Multi-Purpose (3M ESPE); (2) Single Bond (3M ESPE); (3) Clearfil SE Bond (Kuraray Medical, Tokyo, Japan); and (4) Adper Prompt L-Pop (3M ESPE); (Table 1).

The adhesives were applied according to the manufacturer's instructions, and curing was performed via an LED light unit set at 400 mW/cm² (Radii, SDI, Bays Water, Australia).

Resin composite buildups (Filtek Z250, 3M ESPE, St. Paul, Minn, USA) were constructed on the bonded surfaces in 1.5-mm increments; each was light cured for 20 seconds with the same light unit. All the bonding

Table 1. Adhesives Systems Used: Composition, Application Mode, and Batch No.

Adhesive system	Composition*	pH	Steps†	Batch no.
Scotchbond MP	Primer: Aqueous solution of HEMA, polyalkenoic acid copolymer. Bond: Bis-GMA, 2-HEMA, photoinitiator component.	0.6 (acid)‡	a, b, d, e, f, i	Primer: 7BJ Bond: 7PX
Single Bond	Water, ethanol, HEMA, Bis-GMA, dimethacrylates, photo initiator systems, methacrylate functional copolymer polyacrylic, polyitaconic and polyalkenoic acid.	0.6 (acid)‡	a, c, d, g, h, i	7LY
Clearfil SE Bond	Primer: MDP, HEMA, hydrophilic dimethacrylate, di-camphorquinone, N,N-p-toluidine diethanol, water. Bond: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, di-camphorquinone, N,N-p-toluidine diethanol, colloidal silanated silica	1.9 (primer)§	j, f, h, i	Primer: 00760A Bond: 01094A
Adper Prompt LP	Liquid 1 (red blister): Methacrylated phosphoric esters, Bis-GMA, initiators based on camphorquinone, stabilizers. Liquid 2 (yellow blister): Water, HEMA, polyalkenoic acid e stabilizers	0.4 (primer)§	k, l, m, n, o, i	311556

** HEMA=2-hydroxyethyl methacrylate; Bis-GMA=bisphenyl-glycidyl methacrylate; 10- MDP=10-methacryloyloxydecyl-dihydrogen-phosphate.*

† Application mode: a=acid etch-phosphoric acid 35% (15 s); b=rinsing (15 s); c=rinsing (10 s); d= removing wetness with absorbent paper without over drying the dentin; e=primer application and drying (5 s); f=adhesive; g=2 coats of adhesive; h=drying the adhesive (2-5 s); i=light cure (10 s); j=primer (20 s) and air dry; k=press the red reservoir and bend it to the yellow reservoir; l=press the yellow reservoir towards the green one; m=twist the brush to mix the liquid; n=grit the adhesive (15 s); o=apply the adhesive with brush without gritting (3 s).

† According to the manufacturer.

§ Van Meerbeek et al.

procedures were carried out by a single operator at room temperature.

Sections of 0.9-mm thickness each were made in a longitudinal direction (perpendicular to the adhesive interface) with a 0.3-mm diamond disc (Buehler, Lake Bluff, Ill, USA) in an Isomet 1000 machine (Isomet 1000, Buehler) under water refrigeration at 250 rpm. Initially, those sections were cut in a mesiodistal direction to obtain the specimens' slices. A sticky wax was applied to keep the slices together. Next, another buccolingual sectioning was performed to provide sticks with an 0.8-mm² area (average=15 sticks/tooth).

The bonded sticks were then subdivided into 2 groups, which were assigned to be tested immediately and after 6 months storage in 37°C distilled water containing 0.4% sodium azide.

RESIN–DENTIN MICROTENSILE BOND STRENGTH (MTBS)

The cross-sectional area of each stick was measured individually with a digital caliper (model no. 727, Starett, Itu, São Paulo, Brazil). The sticks were attached to a modified device for microtensile testing with a cyanoacrilate resin gel (Super Bonder, Henkel Loctite Adesivos Ltd, Itapevi, São Paulo, Brazil) and subjected to a tensile force in an universal testing machine (model no. 4444, Instron Corp, Canton, Mass, USA) at a crosshead speed of 0.5 mm/minute.

Bond strength values were calculated in MPa, where the applied force (N) was divided by the stick cross-sectional section's area (mm²). The values attributed to specimens that failed prematurely during preparation were arbitrary and corresponded to approximately half of the minimum bond strength measured in this study (1.34 MPa).[14]

For dentin depth's evaluation after fracture, the dentin of each stick was measured with a digital caliper (model no. 727, Starett). The measurement was made from the adhesive interface to the pulp's chamber roof demarcated by the dye.

The failure modes were evaluated at 65X and 1,000X magnification on a scanning electron microscope (SEM; Philips XL-30, Philips Electric Corp, Eindhoven, The Netherlands) and classified as: cohesive (failure exclusive within dentin or resin composite or adhesive); adhesive (failure at resin/dentin interface); or mixed (failure at the resin/dentin interface that included cohesive failure of the neighboring substrates).[15]

DATA TREATMENT

The average bond strength of each tooth was calculated for each experimental group (time x adhesive), and data were compared using a 2-way repeated measurement analysis of variance (ANOVA) and Turkey's multiple comparisons test ($P<.05$). The microtensile test results were calculated considering the tooth as an experimental unit, to avoid internal variables of each tooth dentin interference.[16-18] Averages of dentin thickness values were individually calculated for each tooth and submitted to ANOVA and Tukey's test ($P<.05$). Failure patterns were individually identified for each dentin resin stick and analyzed via the Kruskal-Wallis test ($P<.05$).

RESULTS

Table 2 shows the overall premature failures for each adhesive system tested. The microtensile mean values for each group are presented in Table 3, as well as the statistical analysis (ANOVA and Tukey's test).

The average dentin thickness was 2.60 mm, ranging from 1.91 mm to 3.42 mm per stick (Table 4). Failure patterns representative of each adhesive system are presented in Table 5. Figures 1 to 6 show representative failures observed in SEM.

DISCUSSION

The evaluation of bond strength by microtensile tests has been widely used in dental research[11,16,19-23] because this leads to a lower occurrence of adhesive failures due to a reduced bond area (<2 mm²). Also, it allows for obtaining many specimens from one tooth and permits the evaluation of bond strength in small specific areas such as sclerotic or affected dentin.[4,20,23,24]

In this study, the microtensile test was performed and an aging factor was introduced to evaluate not only bond efficacy but also bond durability. The specimens were stored for 6 months in distilled water containing 0.4% sodium azide to prevent bacterial grow during storage. Also, they were stored in a stick form to accelerate aging of the adhesion.[7,16,21]

Premature failures were reduced and mainly occurred on the self-etch adhesive groups (Clearfil SE Bond and Adper Prompt L-Pop; Table 2).

Although the components of the adhesive's interface can be degraded over time by hydrolysis, a plasticizing effect on the polymers also may occur,[1,7] which may lower bond strength.[19,21] In this investigation, all adhesives have shown stability regarding bond strength after the storage

Table 2. No. of Premature Failures (%) for Each Adhesive System

Time	Adhesive	Total (N)	Premature failures (N)	%
Baseline	Single Bond	125	3	2
	Scotchbond MP	109	0	0
	Adper Prompt LP	153	9	6
	Clearfil SE Bond	156	15	10
6 mos	Single Bond	94	0	0
	Scotchbond MP	83	0	0
	Adper Prompt LP	96	3	3
	Clearfil SE Bond	95	3	3

* (N) means the number of sticks.

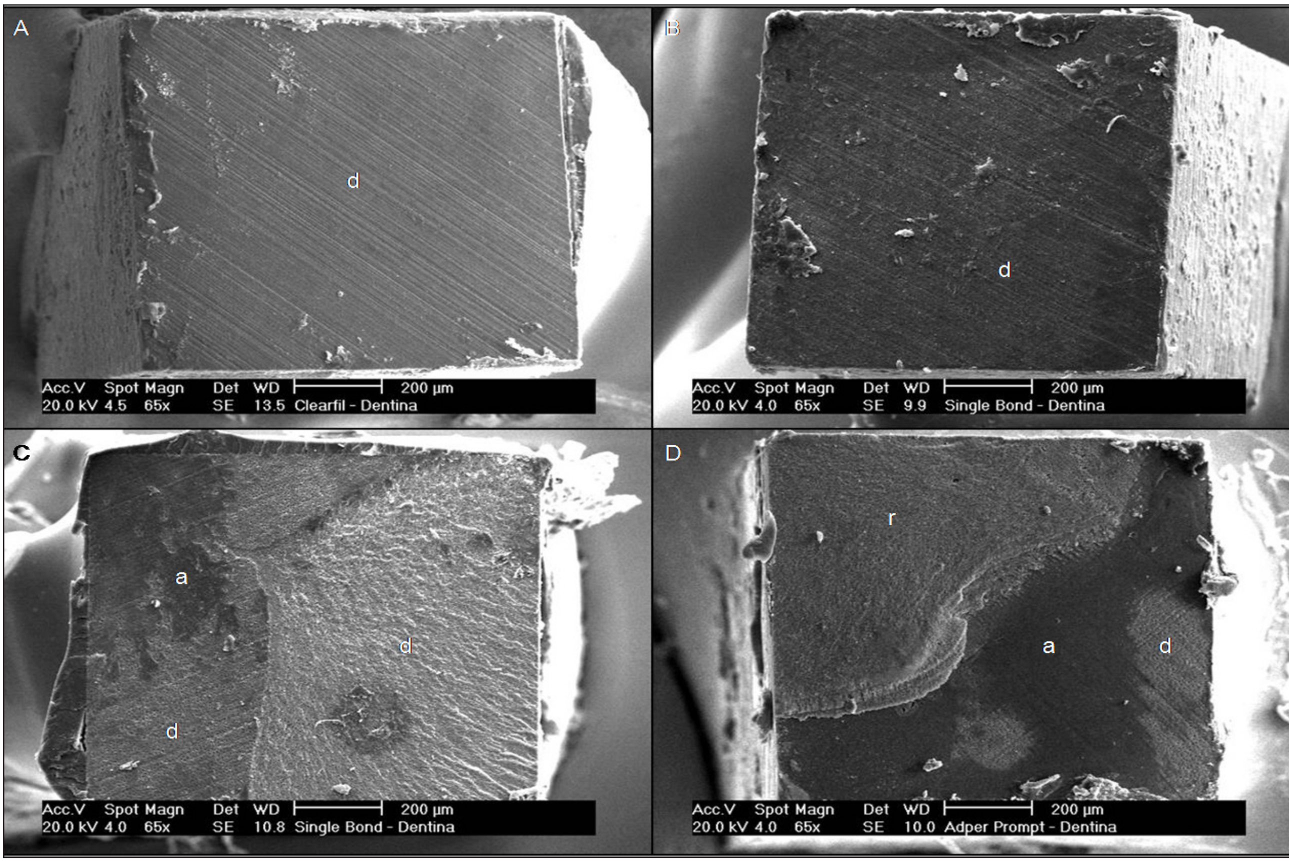

Figure 1. Dentin surface (65X) after microtensile test, baseline groups, (A–B) apparent adhesive failure, and (C–D) mixed failure (d=dentin; a=adhesive; r=resin).

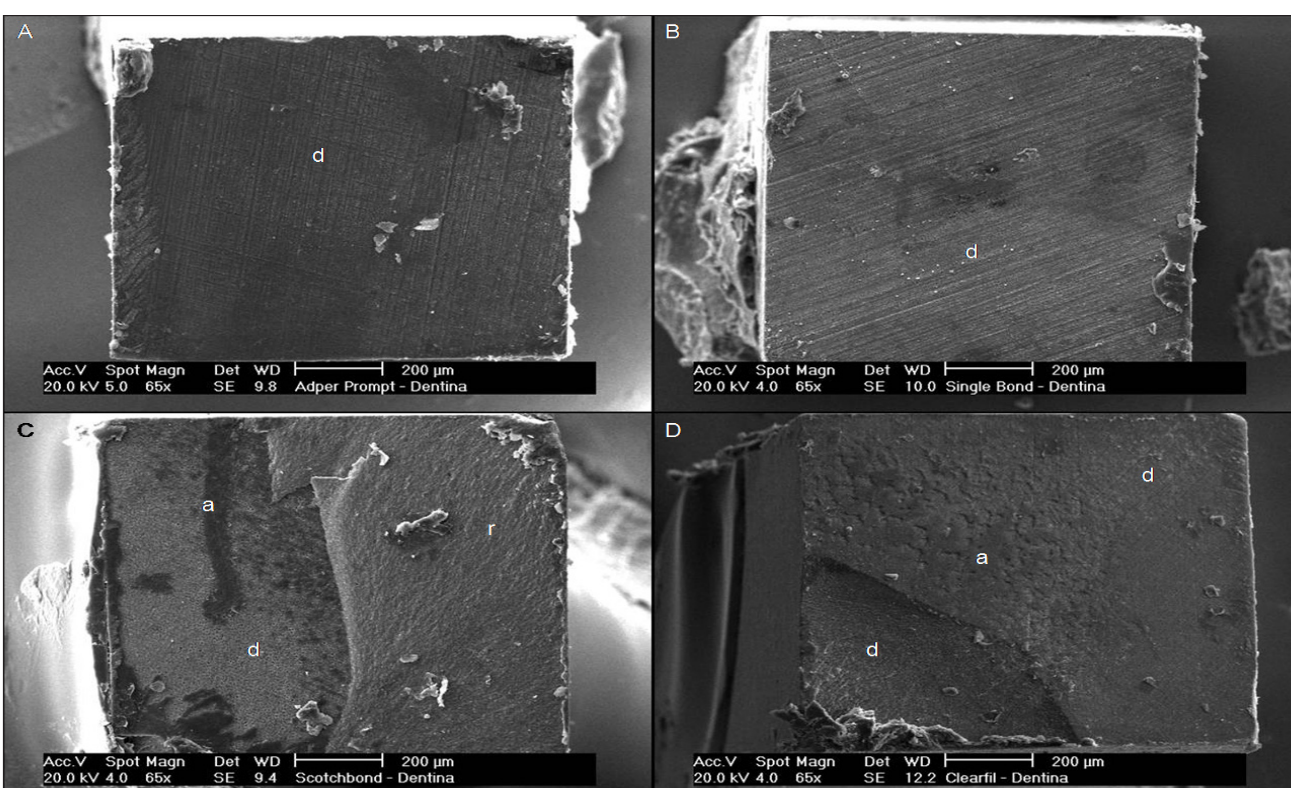

Figure 2. Dentin surface (65X) after microtensile test, 6-month groups, (A–B) apparent adhesive failure, and (C–D) mixed failure (d=dentin; a=adhesive; r=resin).

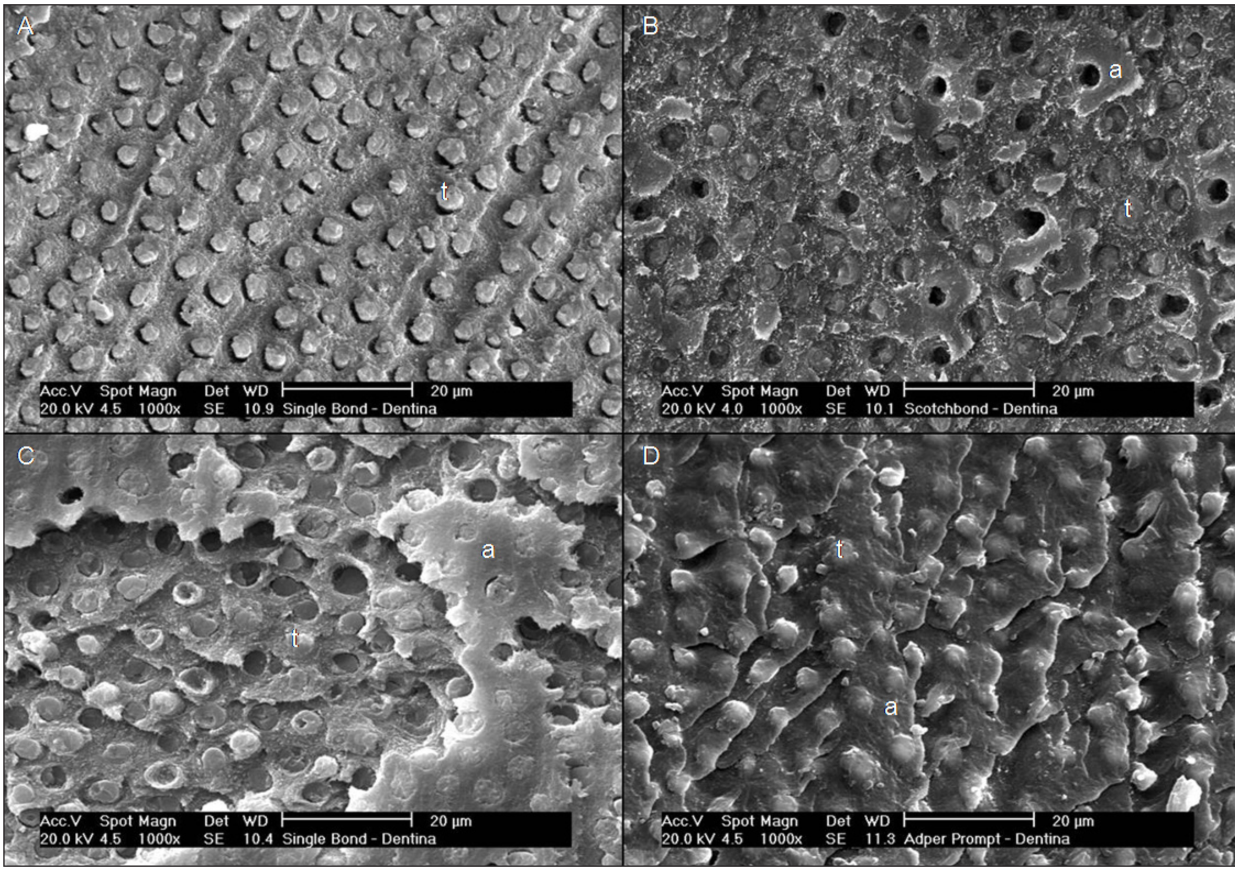

Figure 3. Dentin surface (1,000X) after microtensile test, baseline groups. (A) Dentin tubules with resin tags. (B–D) The tubules are filled and an adhesive layer is placed over the dentin surface. (B–C) Exposed collagen fibers in dentin are observed. (A–D Cohesive ailure of the adhesive (a=adhesive; t=resin tags).

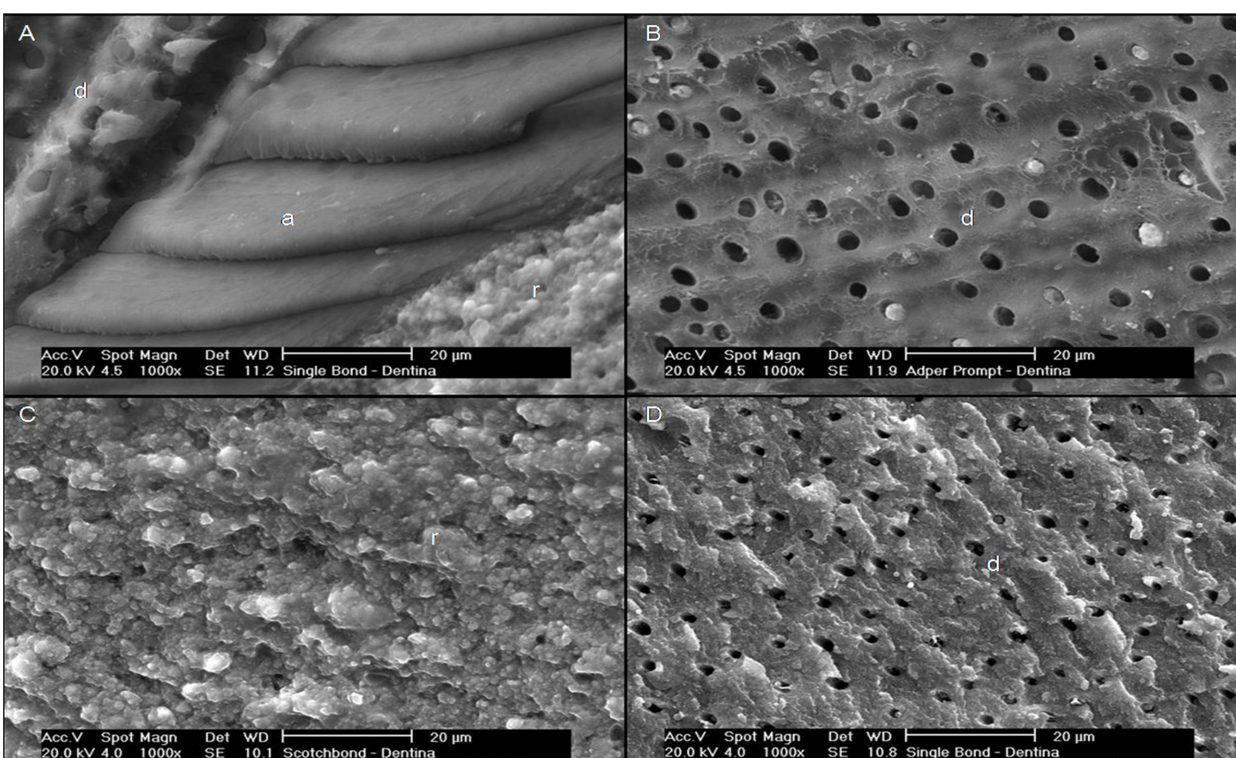

Figure 4. Dentin surface (1,000X) after microtensile test, baseline groups. Mixed, adhesive, cohesive of the resin, and cohesive of the dentin are represented, respectively, by a–d (d=dentin; a=adhesive; r=resin).

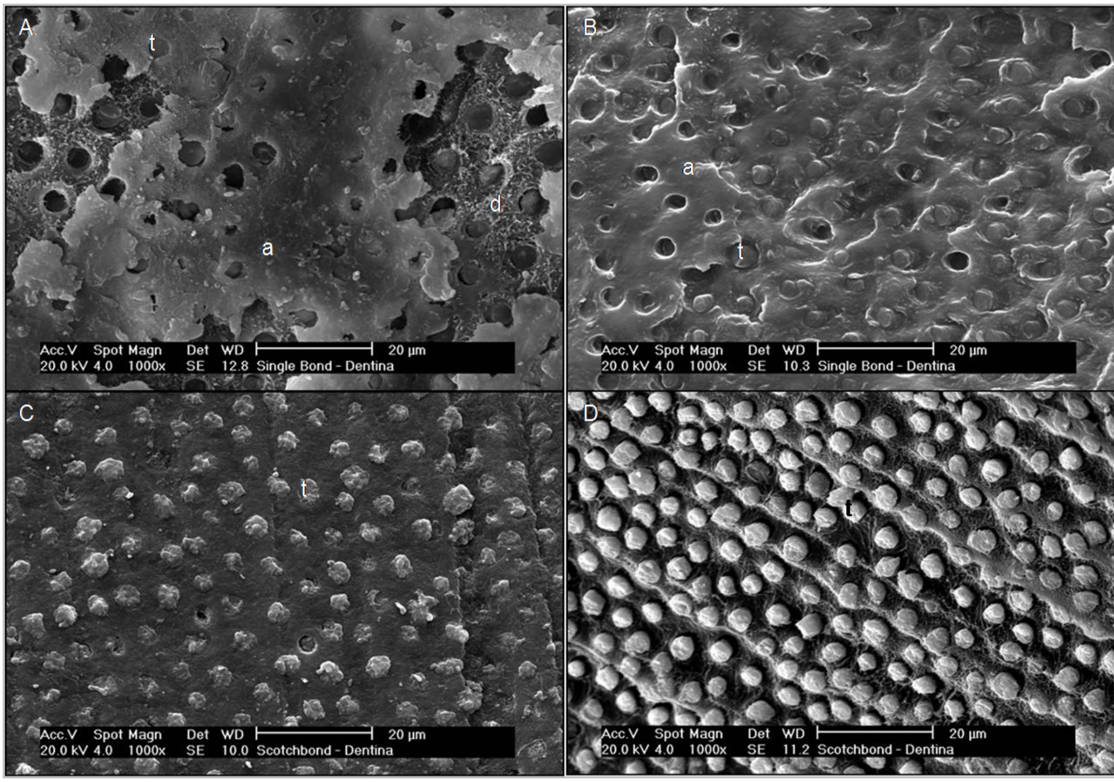

Figure 5. Dentin surface (1,000X) following microtensile test after 6 months. (A–B) Notice the dentin tubules filled with resin tags, adhesive layer over the dentin surface, and exposed collagen fibers. (C–D) Filled dentin tubules. (A–D) Cohesive failure of the adhesive (a=adhesive; t=resin tags).

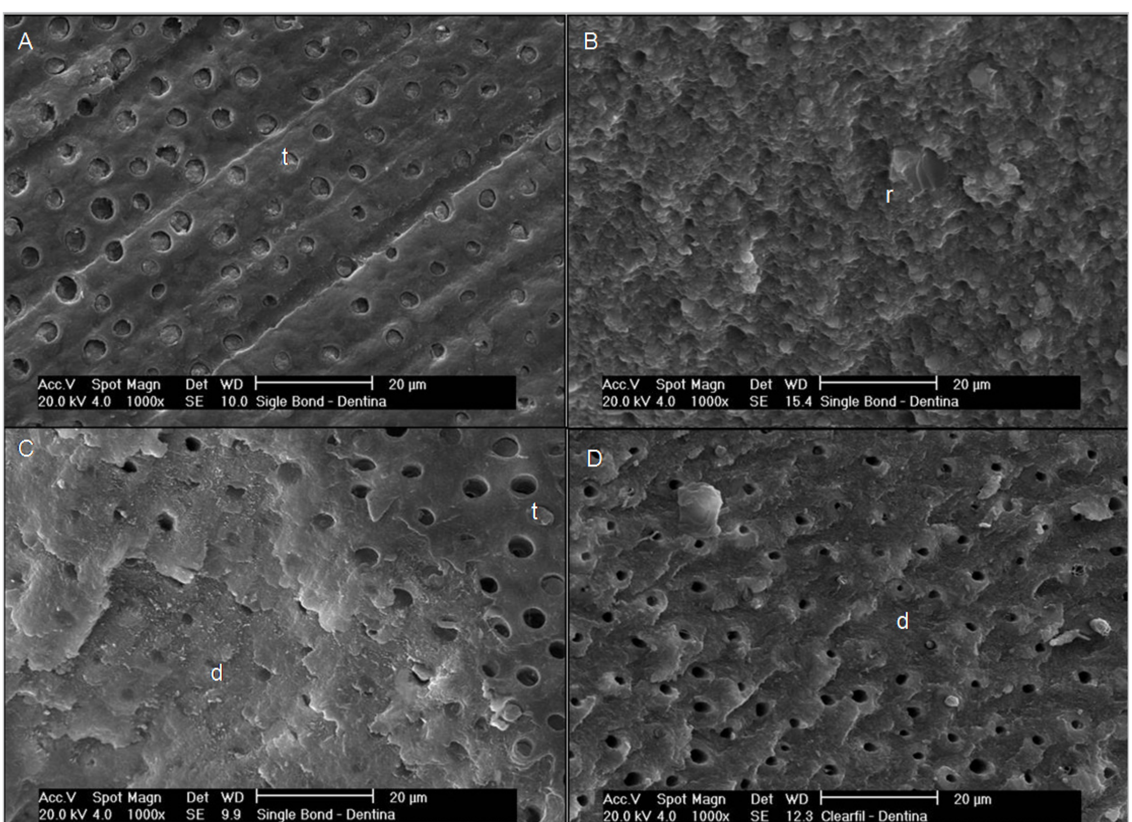

Figure 6. Dentin surface (1,000X) following microtensile test after 6 months. (A) Dentin tubules with resin tags showing cohesive failure of the adhesive. (B–D) Cohesive fracture of the resin, mixed failure, and cohesive failure of the dentin (d=dentin; r=resin; t=resin tags).

period, this could be due to the short period of analysis, which was corroborated by Ernhardt et al.[11] Another factor that may have contributed to the results is the no renewing of the storage substance, since there is some evidence that an acceleration of the aging process may occur when this solution is periodically renewed.[25]

We found differences in bond strengths among the adhesive systems only at the immediate evaluation. Scotchbond Multi-Purpose adhesive has shown significantly better results than Clearfil SE Bond. Although the highest average bond strength was found for Scotchbond Multi-Purpose adhesive in both evaluation times, there was no statistically significant difference between this adhesive and the others, except at the immediate time for the Clearfil SE Bond. This could be due to the variability of some results that had increased standard deviations, mainly for self-etch adhesives (Clearfil SE Bond and Adper Prompt L-Pop; Table 3).

The variability of results was probably related to the characteristics of self-etching adhesives, which have more water when compared to the total-etch adhesives. They need water to ionize the acidic monomers to produce an effective demineralization of the hard dental tissues.[26] Not all the residual water entrapped into the hybrid and adhesive layer is removed, which is harmful, since polymerization of the adhesive is negatively influenced by the presence of water.[27] As more residual water is entrapped into the hybrid and adhesive layer, the polymer's mechanical properties are lowered, reducing bond strength values.[28]

Conversely, one of the factors that most affects total-etch adhesive systems' manipulation and performance is the primer's solvent. Primers containing water are less susceptible to handling variables than those with acetone or alcohol.[7] According to Table 1, Scotchbond Multi-Purpose uses water as a solvent, while Single Bond has water and ethanol. Therefore, Single Bond's adhesion is more vulnerable to the water content at the dentin surface after acid etch. This difference may explain the 3-step technique's superiority, although there was no statistically significant difference (Table 3).

The SEM evaluation shows that the most frequent fracture type found was cohesive failure of the adhesive and mixed modes, with no statistically significant difference found between the groups; this confirms the results of other studies.[16,29]

For all adhesive systems at both evaluation times, exposed collagen fibers were found at the hybrid layer's base (Figures 3b-c, 5a, 5d). This may indicate a poor infiltration of resin monomers into collapsed demineralized dentin, since a homogeneous hybrid layer in an effective infiltration is observed without the presence of exposed fibrils.[22] Exposure of collagen fibers may lead to a hybrid layer more prone to hydrolytic degradation.[17,30,31]

Determination of the fracture's type as well their method of analysis differs among studies: therefore, interpretation of the results may vary according to the

Table 3. Mean Values (MPa) of Microtensile Bond Strength Tests

Adhesive	Time*			
	Baseline		6 mos	
	N	Mean±(SD)	N	Mean±(SD)
Single Bond	8	23.27±4.78 ABa	7	22.39±5.9 Aa
Scotchbond MP	7	33.28±2.05 Aa	7	30.59±6.29 Aa
Clearfil SE Bond	9	18.94±11.94 Ba	7	21.5±10.67 Aa
Adper Prompt LP	9	20.64±10.66 ABa	7	17.19±6.88 Aa

** Different capital letters in the same column and different lower-case letters in the same line indicates a statistically significant difference using Tukey's test (P<.05).*

Table 4. Mean Values (mm) of Dentin Thickness

Adhesive	Time			
	Baseline		6 mos	
	N	Mean±(SD)	N	Mean±(SD)
Single Bond	8	2.50±0.29 Aa	7	2.45±0.23 Aa
Scotchbond MP	7	2.52±0.38 Aa	7	2.48±0.36 Aa
Clearfil SE Bond	9	2.65±0.55 Aa	7	2.81±0.47 Aa
Adper Prompt LP	9	2.81±0.38 Aa	7	2.52±0.39 Aa

** Capital letters in one column and the same lowercase letters in the same line indicates statistical similarity using Tukey's test (P<.05).*

Table 5. Fracture Patterns after Microtensile Tests

Fracture patterns (%)					
Time	Patterns/ Adhesive	Scotchband MP	Single Bond	Clearfil SE Bond	Adper Prompt LP
	Adhesive	0	0	5	0
	Cohesive/dentin	2	5	0	7
Baseline	Cohesive/resin	11	21	6	8
	Cohesive/adhesive	42	29	43	54
	Mixed	45	45	47	31
	Adhesive	0	0	0	0
	Cohesive/dentin	3	7	4	0
6 mos	Cohesive/resin	12	13	11	5
	Cohesive/adhesive	35	33	50	60
	Mixed	51	47	35	35

** No statistically significant difference observed between groups using Kruskal-Wallis's test (adhesive, P=.55/time, P=.47).*

equipment and magnification. In this study, it was difficult to distinguish adhesive failure from cohesive failure of the adhesive at a lower magnification (65X). Most failures were apparently adhesive-related at lower magnification, which was reinforced by the presence of grooves left by the SiC paper on the dentin surface (Figures 1a-b, 2a-b). When visualized at 1,000X (Figures 3-5), however, a cohesive failure of the adhesive was observed, as seen by others.[32,33] Therefore, failure evaluations after tensile bond strength tests should preferably be done by SEM at a high magnification (approximately 1,000X).[24]

The present study did not evaluate dentin bond strength on permanent teeth. However, it is possible to establish, based on a literature review, a similar behavior (ie, there are morphological and structural differences between primary and permanent teeth; however, there is no difference in the bond strength comparing them).[13,22,34,35]

CONCLUSIONS

Based on this study's results, the following conclusions can be made:

1. The bond strength of dentin adhesives in primary dentin, analyzed by the microtensile tests, was not influenced by 6 months of water storage.
2. The 3-step adhesive (total-etch) technique demonstrated a higher bond strength; however, a statistically significant difference was only found when compared to the self-etch adhesive (2-step technique) at the baseline.

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