Short-term aging and the dentin bond strength of adhesive systems

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The aim of this study was to compare the effects of 2 aging methods on the dentin bond strength of different adhesive systems, including a universal adhesive. Seventy-two third molars were sectioned to create flat midcoronal dentin surfaces, which were randomly assigned to 12 groups (n = 6 each) according to the aging method (conventional aging, defined as 6 months of water storage; accelerated aging by means of a pH-cycling method; or negative control [immediate bond strength]) and adhesive system (Adper Single Bond 2, Clearfil SE Bond, Prime & Bond 2.1, or Scotchbond Universal). Composite resin blocks were constructed on the flattened dentin surfaces after application of the appropriate adhesive, and the specimens were stored in water for 24 hours. Specimens from the control group were immediately sectioned into resin-dentin sticks (0.8 mm^2) and subjected to a microtensile bond strength test. Specimens from the experimental groups were sectioned and tested after undergoing the assigned aging method. Data were analyzed with 2-way analysis of variance and a Tukey test ($\alpha = 0.05$). The study findings showed that neither aging method significantly affected the dentin bond strength ($P = 0.917$). Of the 4 adhesives, Adper Single Bond 2 had the highest bond strength value after aging ($P < 0.001$). Scotchbond Universal adhesive demonstrated statistically significantly higher bond strength values than Clearfil SE Bond and Prime & Bond 2.1, which had statistically similar values. Adhesive failures at the resin-dentin interface or adhesive failures mixed with cohesive failure of the adjacent substrate predominated in all groups. The 2 aging processes did not result in degradation of the adhesive interface or jeopardize the dentin bond strength of any of the adhesives tested.

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The ongoing development of commercially available adhesive systems demands intensive in vivo or in vitro evaluation of their properties. Because in vivo studies are expensive and time-consuming and require patients' compliance, laboratory tests are an effective alternative to assess some adhesive properties.[1] Some of these tests are dynamic and try to replicate alterations in the oral environment (eg, thermal conditions and masticatory loading); other tests are static and evaluate bonding degradation over time by means of water storage or other aging methods.

Most adhesive systems perform well when the immediate bond strength is assessed.[2,3] However, bonding durability seems to be a problem for some materials, and diminished bond strength or increased nanoleakage have been observed after a long period of storage.[4-6] Water storage is the most common aging method used to evaluate the degradation of adhesive properties, as it attempts to mimic saliva in the oral environment.[2] However, degradation of adhesive properties leading to diminished bonding requires at least 6 months of water storage.[7] Some authors have suggested that 1 year of water storage or daily water change is needed.[8]

Some alternatives to water storage are available for inducing degradation of the adhesive interface.[7,9] Some authors advocate the use of aging solutions, such as propionic acid, acetic acid, or ethanol, to accelerate bonding degradation and assess the long-term effectiveness of adhesive systems.[9] However, other researchers initiate degradation by means of caries induction on the mineralized tissues near the adhesive interface, as it is one of the most commonly observed conditions in the oral environment. Several methods are available to reproduce caries in vitro.[10] Regardless of the method, caries induction consists of alternating cycles of demineralization and remineralization. This pH fluctuation is also constantly observed in the oral environment via ingestion of beverages or acid production resulting from plaque accumulation.

Different approaches to assessing aging of adhesive properties are documented in the literature. One method consists of exposing an entire restoration to in vitro aging models and then preparing the specimens for the test, while another method consists of preparing the specimens and then immersing the sectioned specimens in different aging solutions. In the latter scenario, more surfaces are exposed to the aging solution; hence, this approach seems reasonable to accelerate bonding degradation.[11] In an attempt to produce more durable interfaces, some manufacturers have added functional monomers, antibacterial agents, and fluoride to their products. However, controversy exists regarding the beneficial effects of some of these properties, especially for prevention of secondary caries.[12,13]

Few data are available regarding the amount of time needed for water storage—if the water is not changed daily—or whether

Table 1. Adhesive systems and application modes.

Adhesive system	Composition	Type	Mode of application[a]
Adper Single Bond 2 (batch: N508311)	*Etchant:* 37% phosphoric acid *Bond:* Bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate, functional copolymer of polyacrylic and poly(itaconic) acids, 10 wt% of 5 nm-diameter spherical silica particles	Etch-and-rinse	1. Actively apply etchant for 15 s. 2. Rinse for 10 s. 3. Blot excess water. 4. Actively apply 2 consecutive layers of adhesive for 15 s. 5. Gently air dry for 5 s. 6. Light cure for 10 s.
Clearfil SE Bond (batch: primer, 012333A; bond, 01865A)	*Primer:* MDP, HEMA, hydrophobic dimethacrylate, camphorquinone, water, *N,N*-diethanol toluidine *Bond:* MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, camphorquinone, *N,N*-diethanol-*p*-toluidine, colloidal silica	Self-etching	1. Actively apply primer to the tooth surface and leave in place. 2. Dry with an air stream to evaporate the volatile solvents. 3. Actively apply bond to the tooth surface. 4. Create a uniform film using a gentle air stream. 5. Light cure for 10s.
Prime & Bond 2.1 (batch: 922355F)	UDMA, PENTA, resin R5, camphorquinone, EDAB, BHT, bisphenol A, cetylamine fluoride, acetone	Self-etching	1. Actively apply the first coat of adhesive to the entire surface and wait for 20 s. 2. Gently air dry for 5 s. 3. Repeat steps 1 and 2. 4. Light cure for 10 s.
Scotchbond Universal (batch: 509806)	*Etchant:* 37% phosphoric acid *Bond:* MDP, HEMA, dimethacrylate resins, methacrylate-modified polyalkenoic acid copolymer, ethanol, water, filler, initiator, silica	Self-etching	1. Actively apply adhesive to the entire surface for 20 s; if necessary, rewet the disposable applicator. 2. Direct a gentle air stream over the adhesive for 5 s or until it no longer moves and the solvent is completely evaporated. 3. Light cure for 10 s.

Abbreviations: BHT, butylated hydroxytoluene; Bis-GMA, bisphenol A glycidyl methacrylate; EDAB, ethyl-4-dimethylamino benzoate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; PENTA, dipentaerythritol penta-acrylate monophosphate; UDMA, urethane dimethacrylate.

[a]According to the manufacturer's instructions.

accelerated aging by pH cycling is effective in assessing bonding degradation. Therefore, the present study compared the dentin bond strength of 4 adhesive systems, including a universal adhesive, submitted to 2 aging methods and a negative control condition (immediate bond strength). Two null hypotheses were tested: (1) The aging processes would result in bond strengths similar to the immediate bond strength, and (2) the adhesive systems tested would exhibit similar bond strengths regardless of the aging method used.

Materials and methods

Tooth selection and preparation

The study protocol was reviewed and approved by the Institutional Ethics Committee at Federal University of Santa Maria, Santa Maria, Brazil. Seventy-two caries-free third molars were obtained from an institutional tooth repository and stored in distilled water at 4°C for up to 3 months after being disinfected in aqueous 0.5% chloramine-T solution.

A slow-speed diamond saw (Labcut 1010, Extec) was used under copious water irrigation to section each tooth in the middle of the crown, perpendicular to the long axis, to obtain flat midcoronal dentin surface. The surrounding enamel was removed with a diamond bur in a high-speed handpiece under copious water irrigation (No. 3146, KG Sorensen). A standardized smear layer was obtained by polishing the tooth specimen with a 600-grit silicon carbide paper under running water for 60 seconds.[14]

Experimental design

Teeth were randomly allocated into 12 groups (n = 6 each) according to the adhesive system tested (Adper Single Bond 2, 3M ESPE; Clearfil SE Bond, Kuraray America; Prime & Bond 2.1, Dentsply Sirona Brasil; or Scotchbond Universal, 3M ESPE) and aging method (conventional aging by means of 6 months' water storage, accelerated aging by pH cycling, or negative control [24 hours' water storage to assess immediate bond strength]).

Bonding protocols

All adhesives were applied by a single trained operator according to the manufacturers' instructions (Table 1). Three layers of composite resin (Filtek Z250, 3M ESPE), 2.0 mm each, were then applied to the tooth specimen. Each layer was light cured for 40 seconds with a monitored LED curing unit (Emitter C, Schuster) at 600 mW/cm^2. All specimens were stored in distilled water at 37°C for 24 hours before the sticks were obtained.

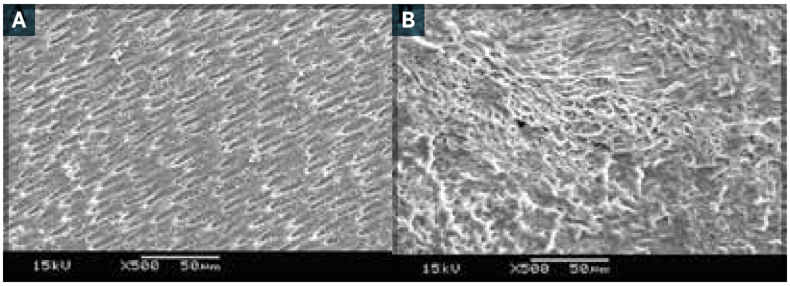


Figure. Representative fractured specimens treated with Adper Single Bond 2 adhesive system. A. Specimen from the negative control group exhibiting a regular surface and normal tubule opening. B. Specimen from the pH cycling group exhibiting intertubular porous zones with irregular surfaces resulting from the demineralization process.

Table 2. Microtensile bond strength (MPa) for all adhesives and aging methods (n = 6 per group).

	Adper Single Bond 2		Scotchbond Universal		Clearfil SE Bond		Prime & Bond 2.1	
Aging method	Mean (SD)	PF/TS	Mean (SD)	PF/TS	Mean (SD)	PF/TS	Mean (SD)	PF/TS
Negative control	51.38 (11.88)^A	0/67	31.54 (7.08)^B	4/56	31.84 (7.47)^C	3/76	28.38 (10.66)^C	1/88
pH cycling	49.71 (7.72)^A	0/60	36.45 (4.41)^B	0/75	27.77 (8.73)^C	6/86	26.40 (10.15)^C	2/70
Conventional aging	45.60 (15.07)^A	0/66	41.34 (4.07)^B	0/51	23.31 (6.40)^C	2/78	28.52 (10.74)^C	0/76

Abbreviation: PF/TS, number of pretest failures/number of specimens tested.
Different letters indicate statistically significant differences between groups ($P < 0.05$; Tukey test).

Specimen preparation

After the 24-hour storage period, all bonded specimens were sectioned in 2 directions, perpendicular to the adhesive interface, with a water-cooled diamond saw (Labcut 1010, Extec) to obtain sticks with a cross-sectional area of 0.8 mm^2, as measured with a digital calipers (Carbografite, Equipamentos Industriais).

Negative control group

The specimens in the negative control group underwent microtensile bond strength testing immediately after the 24-hour storage period.

Conventional aging group

After sectioning, the sticks from each tooth in the conventional aging group were placed in polypropylene centrifuge tubes and stored in distilled water at 37°C for 6 months. The water was not changed during the storage period.

Accelerated aging group

After sectioning, the sticks from each tooth in the accelerated aging group were placed in polypropylene centrifuge tubes and underwent pH cycling for 14 days. The sticks were immersed in a demineralizing solution (2.2mM calcium dichloride [CaCl2], 2.2mM monosodium phosphate [NaH2PO4], and 0.05M acetic acid; pH = 4.5) for 8 hours and then in a remineralizing solution (1.5mM CaCl2, 0.9mM NaH2PO4, and 0.15mM potassium chloride; pH = 7.0) for 16 hours.[15,16] After each cycle, the solutions were changed, and the sticks were rinsed with deionized water and blotted dry. Solutions were measured periodically with a pH meter.

Microtensile bond strength testing

After undergoing the assigned aging method, each resin-dentin stick was attached to a testing jig with cyanoacrylate and stressed at a crosshead speed of 1 mm/min until failure in a universal testing machine (EMIC DL 1000, Instron Brasil Equipamentos Científicos). The microtensile bond strength values (MPa) were determined by dividing the measured force (N) registered at the failure point by the bonded area (mm^2).

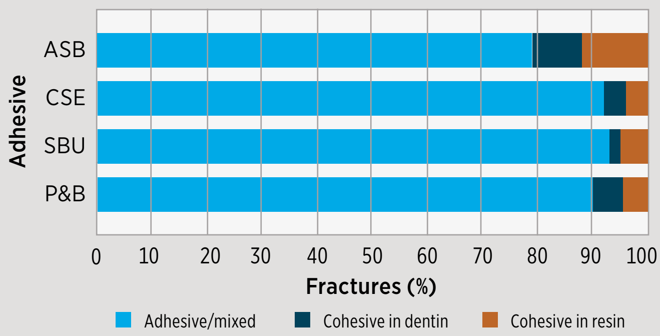
Failure mode analysis

Debonded specimens were evaluated under a stereomicroscope at 40× magnification to classify the failure as adhesive/mixed (failure at the resin-dentin interface or adhesive failure mixed with cohesive failure of the adjacent substrate) or cohesive (failure exclusively in dentin or composite resin).[16]

Morphologic analysis

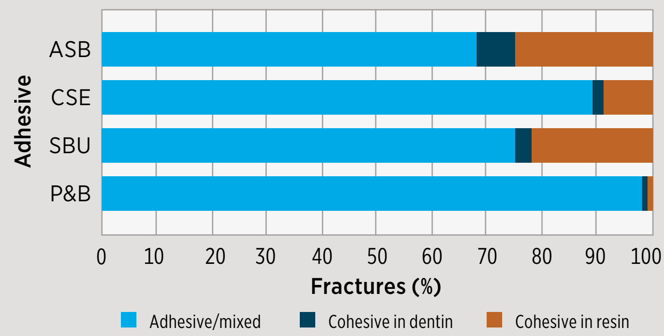
A scanning electron microscopic (SEM) analysis was performed to confirm dentin demineralization in the specimens submitted to artificial caries induction via pH cycling. One stick from each experimental group was selected and prepared for SEM analysis. Sticks were immersed in ethylenediaminetetraacetic acid solution (0.7M; pH = 7.4) for 5 minutes, followed by immersion in sodium hypochlorite (0.34M; pH = 12.3) for 3 minutes.[17] The sticks were then dehydrated in ascending

Chart 1. Fracture pattern distributions in the negative control group.



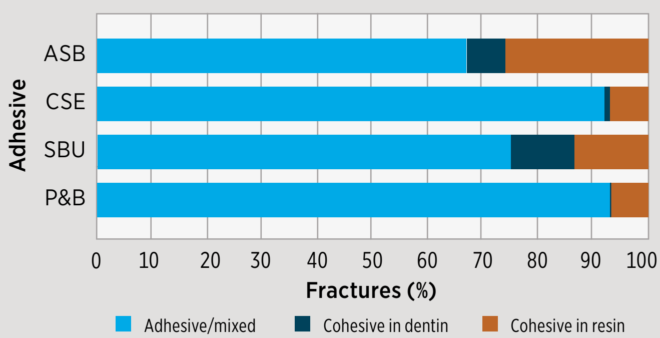
Abbreviations: ASB, Adper Single Bond 2; CSE, Clearfil SE Bond; P&B, Prime & Bond 2.1; SBU, Scotchbond Universal.

Chart 2. Fracture pattern distributions in the pH cycling group.



Abbreviations: ASB, Adper Single Bond 2; CSE, Clearfil SE Bond; P&B, Prime & Bond 2.1; SBU, Scotchbond Universal.

Chart 3. Fracture pattern distributions in the conventional aging group.



Abbreviations: ASB, Adper Single Bond 2; CSE, Clearfil SE Bond; P&B, Prime & Bond 2.1; SBU, Scotchbond Universal.

degrees of ethanol (30%, 50%, 70%, and 80% for 5 minutes each and 90%, 95%, and 100% for 10 minutes each). The sticks were gold sputtered and underwent SEM analysis (JSM-T330A, JEOL) at 15 kV and 500× magnification.

Statistical analysis

The experimental unit in this study was the tooth. Thus, the microtensile bond strength values of all the sticks from the same tooth were averaged for statistical analysis. The mean microtensile bond strength for all groups was expressed as the mean of the 6 teeth in each group. The sample size of 6 teeth per group was estimated before the study based on 80% power, a coefficient of variation of 20%, and an assumption of a 2-sided 5% significance level for comparisons.

Only specimens with adhesive/mixed failures were included in the statistical analysis. Pretest failures that occurred during specimen preparation were included in the statistical analysis with a value of 0 MPa.

The microtensile bond strengths were analyzed by a general linear model 2-way analysis of variance (adhesive systems vs aging method) and a post hoc Tukey test at a significance level of 0.05. Statistical analysis was performed using Minitab software (version 17, Minitab).

Results

The Figure shows representative fractured specimens from the negative control and accelerated aging (pH cycling) groups (Adper Single Bond 2 adhesive). A uniform surface and normal tubule opening can be seen in the noncycled specimen. In the pH cycled group, disorganization and loss of continuity in dentinal areas are observed, clearly reflecting the demineralization process.

Specimens that underwent accelerated aging by pH cycling or 6 months of water storage exhibited bond strength values that were statistically similar to those in the negative control group (ie, specimens tested immediately after being sectioned) (P = 0.917). Although there was no interaction between the adhesive system and aging method (P = 0.314), a statistically significant difference was observed among the adhesive systems ($P < 0.001$). Of the 4 adhesive systems, Adper Single Bond 2 had the highest bond strength values ($P < 0.05$). Scotchbond Universal exhibited statistically significantly higher bond strength values ($P < 0.05$) than Clearfil SE Bond and Prime & Bond 2.1, whose values were statistically similar (Table 2).

The 3 failure modes are shown in Charts 1-3. Adhesive/mixed failures were predominant; however, cohesive failures in dentin and resin were observed in all tested groups.

Discussion

Restorative materials are continually challenged in the oral environment by saliva, caries, and erosion as well as other chemical, mechanical, and thermal processes. One frequently occurring process is pH fluctuation resulting from alternating cycles of demineralization and remineralization. Thus, 2 aging methods were used in this study, with the aim of potentially accelerating bonding degradation and comparing the results with those of a negative control group (immediate bond strength values). The results revealed that neither accelerated aging by pH cycling nor 6 months of water storage (conventional aging) generated sufficient degradation on the adhesive interface to jeopardize the bond strength for any of the adhesive systems tested. Thus, the first null hypothesis could not be rejected.

The literature describes various aging methods and solutions to accelerate bonding degradation, such as thermocycling, water storage, sodium hypochlorite, propionic acid, acetic acid, and ethanol.[5,7,9] Although these methods differ in several aspects, all attempt to produce bond stress along the interface, leading to microdefects that might induce the breakdown of the adhesive interface. However, no consensus exists regarding study methodology, especially for thermocycling and water storage, as different numbers of cycles and months of water storage have been suggested. In addition, it is not well established if the water must be changed regularly to induce aging or if it can be left unchanged during the aging period.

Moreover, researchers have proposed alternating cycles of pH change as an accelerated aging method in an attempt to induce degradation near the adhesive interface.[12,13] In this study, no difference was observed in the bond strength values of specimens in the pH cycling group and those in the negative control group; however, dentin demineralization was observed near the adhesive interface, confirming the effectiveness of artificial caries induction, although it was not sufficient to jeopardize the dentin bond strength of the adhesive systems.

The use of pH cycling or other artificial caries induction models as an aging method has produced inconsistent results; however, its use is based on the premise that the acid challenge could produce erosion and crack formation on the adhesive surface, leading to its deterioration.[18] Deng et al compared several artificial aging methods, including pH cycling, and observed that after 15 cycles of demineralization and remineralization, a statistically significant difference was found for Adper Single Bond 2 and G-Bond.[7] Although the study consisted of 1 more cycle than that in the present study, less time (6 hours) was used for demineralization than in the current protocol (8 hours). On the other hand, Pedrosa et al did not find statistically significant differences between One-Up Bond F Plus, Clearfil SE Bond, and Clearfil Protect Bond, which is in accordance with the present results.[13] Hence, uncertainty remains with respect to whether more cycles of pH challenge would induce more degradation of the interface, as no study, to the authors' knowledge, has assessed more than 15 pH cycles.

Water storage is a frequently used aging method, as it is an easy and inexpensive way to evaluate adhesive degradation. However, uncertainty remains regarding the amount of time needed or whether a daily water change is necessary to produce degradation. Storing dentin sticks in water may contribute to adhesive degradation as hydrolysis of the filler-matrix interface is expected over time, which may decrease the mechanical properties of the material.[19]

Another important factor is that some adhesive components may contribute to their stability and, thus, interfere in the degradation process; for example, the 10-methacryloyloxydecyl dihydrogen phosphate (MDP) monomer can produce stable chemical bonds over time.[20] Consequently, this study tested 4 adhesive systems composed of different monomers. None of the adhesives demonstrated statistically significantly lower bond strength values after 6 months of water storage. Some researchers have suggested that the water needs to be changed daily to accelerate degradation.[8,21] Although the results of the present study cannot confirm these findings, the authors speculate that, in the absence of frequent water changes, more than 6 months of water storage is needed to generate enough degradation with most adhesives.[19]

Antibacterial agents have been incorporated in some restorative materials because they contribute to remineralization of adjacent lesions in patients with high caries risk and reduce the occurrence of secondary caries that might weaken the adhesive interface.[22] Therefore, Prime & Bond 2.1 was included in this study to determine whether fluoride had a beneficial effect on bond strength after artificial carious lesions were induced. After 14 cycles of demineralization/remineralization, no statistically significant difference was observed for this adhesive, which is in accordance with other study findings.[23] However, because none of the adhesives in the study demonstrated diminished bond strength values, the effect of fluoride on the durability of adhesives requires further investigation.

Clearfil SE Bond and Scotchbond Universal contain the functional monomer MDP, which has been reported to establish a durable chemical bond to hydroxyapatite and, consequently, could lead to stable adhesion and less degradation over time.[20] Adper Single Bond 2 contains the polyalkenoic acid copolymer, which can establish a chemical bond to some degree but cannot self-assemble into nanolayers as does the MDP monomer.[24] Prime & Bond 2.1 is a fluoride-containing adhesive that can aid in remineralization of adjacent areas; however, the beneficial effect on the adhesive interface is still controversial.[12,13]

It appears that formation of an adequate hybrid layer is necessary for establishing immediate bond strength and for durability of the interface, as Adper Single Bond 2, the only adhesive system in this study with a separate acid conditioning step, had the highest bond strength of all adhesives tested; as a result, the second null hypothesis is rejected. Nevertheless, all of the adhesives tested maintained their bond strength after undergoing accelerated aging by pH cycling or 6 months of water storage.[13,25] Hence, all 4 adhesives are appropriate for clinical purposes.

Conclusion

Accelerated aging of bonded tooth specimens by means of pH cycling and 6 months of water storage did not degrade the adhesive interface or jeopardize the bonding stability of the 4 adhesive systems tested.

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The authors report no conflicts of interest pertaining to any of the products or companies discussed in this article.

References

1. Morresi AL, D'Amario M, Capogreco M, et al. Thermal cycling for restorative materials: does a standardized protocol exist in laboratory testing? A literature review. *J Mech Behav Biomed Mater.* 2014;29:295-308.
2. De Munck J, Van Landuyt K, Peumans M, et al. A critical review of durability of adhesion to tooth tissue: methods and results. *J Dent Res.* 2005;84(2):118-132.
3. Scherrer SS, Cesar PF, Swain MV. Direct comparison of the bond strength results of the different test methods: a critical literature review. *Dent Mater.* 2010;26(2):e78-e93.
4. Osorio R, Pisani-Proenca J, Erhardt MC, et al. Resistance of ten contemporary adhesives to resin-dentine bond degradation. *J Dent.* 2008;36(2):163-169.
5. Muñoz MA, Luque-Martinez I, Malaquias P, et al. In vitro longevity of bonding properties of universal adhesives to dentin. *Oper Dent.* 2015;40(3):282-292.
6. Marchesi G, Frassetto A, Mazzoni A, et al. Adhesive performance of a multi-mode adhesive system: 1-year in vitro study. *J Dent.* 2014;42(5):603-612.
7. Deng D, Yang H, Guo J, Chen X, Zhang W, Huang C. Effects of different artificial ageing methods on the degradation of adhesive-dentine interfaces. *J Dent.* 2014;42(12):1577-1585.
8. Kitasako Y, Burrow MF, Nikaido R, Tagami J. The influence of storage solution on dentin bond durability of resin cement. *Dent Mater.* 2000;16(1):1-6.
9. Reis A, Martins GC, Paula EA, Sanchez AD, Loguercio AD. Alternative aging solutions to accelerate resin-dentin bond degradation. *J Adhes Dent.* 2015;17(4):321-328.
10. Marquezan M, Corrêa FN, Sanabe ME, et al. Artificial methods of dentine caries induction: a hardness and morphological comparative study. *Arch Oral Biol.* 2009;54(12):1111-1117.
11. Torkabadi S, Nakajima M, Ikeda M, Foxton RM, Tagami J. Influence of bonded enamel margins on dentin bonding durability of one-step self-etching adhesives. *J Adhes Dent.* 2009;11(5):347-353.
12. Peris AR, Mitsui FH, Lobo MM, Bedran-russo AK, Marchi GM. Adhesive systems and secondary caries formation: assessment of dentin bond strength, caries lesions depth and fluoride release. *Dent Mater.* 2007;23(3):308-316.
13. Pedrosa VO, Flória FM, Turssi CP, Amaral FL, Basting RT, França FM. Influence of pH cycling on the microtensile bond strength of self-etching adhesives containing MDPB and fluoride to dentin and microhardness of enamel and dentin adjacent to restorations. *J Adhes Dent.* 2012;14(6):525-534.
14. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching adhesives, I: depth penetration beyond dentin smear layers. *Dent Mater.* 2001;17(4):430-444.
15. Antoniazzi BF, Nicoloso GF, Lenzi TL, Soares FZ, Rocha Rde O. Selective acid etching improves the bond strength of universal adhesive to sound and demineralized enamel of primary teeth. *J Adhes Dent.* 2016;18(4):311-316.
16. Nicoloso GF, Antoniazzi BF, Lenzi TL, Soares FZ, Rocha RO. Is there a best protocol to optimize bond strength of universal adhesive to artificially induced caries-affected primary or permanent dentin? *J Adhes Dent.* 2016;18(5):441-446.
17. Schilke R, Lisson JA, Bauss O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. *Arch Oral Biol.* 2000;45(5):355-361.
18. Sauro S, Watson TF, Tay FR, et al. Water uptake of bonding systems applied on root dentin surfaces: a SEM and confocal microscopic study. *Dent Mater.* 2006;22(7):671-680.
19. Makishi P, André CB, Ayres A, Martins AL, Giannini M. Effect of storage time on bond strength and nanoleakage expression of universal adhesives bonded to dentin and etched enamel. *Oper Dent.* 2016;41(3):305-317.
20. Yoshida Y, Yoshihara K, Nagaoka N, et al. Self-assembled nano-layering at the adhesive interface. *J Dent Res.* 2012;91(4):376-381.
21. Skovron L, Kogeo D, Gordillo LA, et al. Effects of immersion time and frequency of water exchange on durability of etch-and-rinse adhesive. *J Biomed Mater Res B Appl Biomater.* 2010;95(2):339-346.
22. Ten Cate JM, van Duinen RN. Hypermineralization of dentinal lesions adjacent to glass-ionomer cement restorations. *J Dent Res.* 1995;74(6):1266-1271.
23. Nakajima M, Okuda M, Ogata M, Pereira PN, Tagami J, Pashley DH. The durability of a fluoride-releasing resin-adhesive system to dentin. *Oper Dent.* 2003;28(2):186-192.
24. Yoshida Y, Van Meerbeek B, Nakayama Y, et al. Evidence of chemical bonding at biomaterial-hard tissue interfaces. *J Dent Res.* 2000;79(2):709-714.
25. Manfroi FB, Marcondes ML, Somacal DC, Borges GA, Júnior LH, Spohr AM. Bond strength of a novel one bottle multimode adhesive to human dentin after six months of storage. *Open Dent J.* 2016;10:268-277.