Interfacial integrity of bonded restorations with self-etching adhesives: Water storage and thermo-mechanical cycling

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ABSTRACT

Objectives: Objective: To evaluate the effect of thermo-mechanical cycling (TMC) on the microleakage (μL) and axial gap width (AG) of Class V bonded restorations in premolars using self-etching adhesive systems. The bond strength of composite restorations to dentin (μTBS) using the same adhesives was also evaluated in third molars after water storage: 24 h and 6 months. The research hypotheses were tested for the results of two self-etching adhesives in comparison when a conventional two-step adhesive was used: (1) the μL and AG would be lower, regardless of TMC; (2) the μTBS of self-etching adhesives would be higher, irrespective of evaluation times.

Methods: Sixty Class V composite restorations were made in 30 premolars and bonded with Adper Single Bond 2 (ASB2), AdheSE (ASE), and Adper Prompt L-Pop (APL-P) (n=20). Dentin μL and AG were immediately measured for half of the sample. The other half was evaluated after TMC. Eighteen third molars were also selected and bonded using the same adhesives to test the μTBS to dentin. Specimens were evaluated after 24 h and 6 months of water storage.

Results: No differences in μL and AG were found among the groups (P>.05). The μTBS mean values were: ASB2>ASE>APL-P (P<.05); only Adper Single Bond 2 presented a significantly lower μTBS after water storage (P<.05).

Conclusions: The bonding approach does not influence the microleakage and interfacial gap extension. Despite the decrease in the mean values, the bond strength to dentin of the conventional, two-step adhesive remains high after 6 months of water storage. (Eur J Dent 2012;6:169-177)

Key words: Dentin-bonding agents; bond strength; durability; microleakage.

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INTRODUCTION

One of the main problems detected by dental clinicians after restoring a Class V cavity is the marginal quality, especially when the cervical margin is placed in dentin.[1] This marginal area is of particular interest, as it is more prone to marginal microleakage.[2] This problem occurs because of the morphological and dynamic aspects of dentin. A variety of composites and polymerization techniques have been tried to provide better marginal adaptation.[1] Another clinical approach to address this problem is the use of different adhesive systems.[3] Applying etch-and-rinse adhesives leads to dentin demineralization; as a consequence, the opening of dentin tubules and exposure of collagen fibers occur in this enamel-free area.[4] Increased tubular fluid flows might be impeditive to the monomers' ability to permeate around collagen fibers.[5] Another problem is the discrepancy between the depths of demineralization and monomer resin infiltration, leaving areas in which collagen fibers are exposed and unprotected. As a consequence, nanoleakage may occur within the hybrid layer in gap-free margins.[6] Uninfiltrated, exposed collagen fibers are considered the weakest link of the hybrid layer, as they are subjected to hydrolytic degradation by oral or dentinal fluid.[7] As a result, marginal and internal gaps develop.[8] On the other hand, the use of self-etching adhesives has been advocated for many clinical applications.[9] Their adhesion mechanism favors adhesion by simultaneously etching and infiltrating the adhesive monomer into the dentinal surface.[10] The original idea behind the use of this category of adhesives was an attempt to avoid not only tubular opening but also collagen fibril exposure. However, it has been reported that nanoleakage along resin/dentine interfaces is produced by self-etching systems, thus suggesting water movement within resin-dentine interfaces not only within voids left in uninfiltrated areas of the hybrid layer, but also within the adhesives.[11] It has also been claimed that the nanoleakage observed when self-etching adhesives are applied is not necessarily caused by disparities between the depths of demineralization and resin infiltration.[12] In fact, the nanoleakage highlighted areas of increased permeability within a polymerized resin matrix in which water was incompletely removed, resulting in regions of incomplete polymerization and/or hydrogel formation.[12]

Moreover, polymerization shrinkage, composite viscoelastic properties, and adhesion to and the flexibility of cavity walls are additional factors that influence the integrity of this area.[13] Clinically, stresses may also be generated at the interface during tooth function. These stresses are even more critical in Class V restorations because they may undergo flexure along with tooth mastication.[14] Thermal and/or mechanical stress concentration may lead to the deterioration of preexisting gaps or the formation of new ones.[15] It is believed that the presence of gaps, irrespective of their size/extension, adversely affects the longevity of bonded restorations.[16]

The aim of this in-vitro study was to evaluate the influence of water storage and thermo-mechanical cycles on the bond strength, microleakage, and internal gaps of composite restorations when various adhesive systems were applied to dentin-margin in Class V preparations (a two-step etch-and-rinse, a two-step self-etching, and a one-step self-etching adhesive). The research hypotheses tested were: (1) microleakage, (2) axial gap width, and (3) bond strength would demonstrate better results when self-etching adhesives are applied in comparison to a conventional etch-and-rinse adhesive system.

MATERIALS AND METHODS

Microleakage and adhesive gap extension evaluations

Thirty sound human premolars were scaled, cleaned with slurry of pumice and water, and stored in a 0.1% thymol solution at room temperature to prevent bacterial growth. Teeth were obtained and used in accordance with a protocol approved by the Human Assurance Committee (file number: 10-03-334). Teeth were selected and their roots were embedded in epoxy resin. Class V cavity preparations (2 mm wide x 3 mm long x 1.8 mm deep) were made on the buccal and lingual surfaces at the cement-enamel junction. Cavity dimensions were standardized using a digital caliper (Model CD-6BS; Mitutoyo, Tokyo, Japan). The composition and application modes of the adhesive systems studied are described in Table 1. The specimens were randomly divided into 3 groups (n=20) according to the adhesive system used: a two-step etch-and-rinse system [ASB2] (Adper Single Bond 2 - 3M/ESPE, St. Paul, MN, USA), a two-step self-etching system [ASE] (AdheSE, Ivoclar Vivadent, Schaan, Liechten-

stein), and a one-step self-etching system [APL-P] (Adper Prompt L-Pop, 3M/ESPE, St. Paul, MN, USA). Half of the sample (n=10) was submitted to thermo-mechanical cycles in distilled water for 500 cycles between 5 and 55° C, with a dwell time of 15 s (El Quip, MSCT-3, USA). Two coats of nail varnish were then applied, leaving a 1 mm-wide border around the margins of the restoration. Then the teeth were submitted to 50,000 mechanical cycles (El Quip, MSFM, USA). In this case, a perpendicular loading of 40-70 N was applied at the occlusal surface. The other half of the sample received no thermo-mechanical treatment. Control and thermo-mechanically cycled groups were then immersed in a 50 % $AgNO_3$ solution for 2 h in a dark room. After washing, the specimens were placed in a developer solution and exposed to fluorescent light for 6 h[17] (HC-110 Developer, Eastman Kodak Company, Rochester, NY, USA). The specimens were then sectioned and the microleakage at the dentin/cementum margin was analyzed using an optical microscope (Olympus BX41/Camera DP71, Japan) at 40 X magnification. The microleakage was rated using the following four classifications:

- Score 0 – absence of penetration of tracer agent;
- Score 1 – penetration to one-third of the cavity;
- Score 2 – penetration to two-thirds of the cavity, and
- Score 3 – penetration to more than two-thirds and in the axial wall of the cavity.

Finally, after marginal evaluation, the specimens were embedded in epoxy resin (Castin˙ Craft Clear Liquid Plastic, Environmental Technology Inc., Fields Landing, CA, USA), allowing each tooth to be mounted and sectioned using a water cooled rotating diamond blade (Isomet Low Speed Saw, Buehler Ltd., Evanston, IL, USA). Each restoration was sectioned resulting in two slices. Both sectioned surfaces were examined, but results of the two sections were taken as single data. After sectioning, each specimen was wet-polished with 600-, 1200-, and 2000-grit SiC papers. Then, the specimens were acid-etched (37% phosphoric acid gel) in order to remove the debris. The specimen preparation for SEM included a high vacuum silica-gel desiccation process for 48 h.[18] Then, the specimens were sputter-coated (Sputter Coater, model SCD 050, Balzers) with a thin palladium-gold film for 100 s at 40 mA (approximately 25 nm in thickness). The axial gaps were imaged using a magnification of 1000X. The gap width was measured in three different areas in each photomicrograph image and the mean value of each area was calculated.

Microtensile bond strength test

For the microtensile bond strength test, 18 sound human third molars were selected. The cusps were abraded using a water-cooled rotating diamond wheel (Isomet 1000, Buehler; Lake Bluff, IL, USA) to expose a flat surface free of enamel tissue in the mid-coronal dentin surface. A standard-

Table 1. Composition and application mode of the materials used.

Product name	Ingredients	Application	Batch #
Adper Single Bond 2 (3M ESPE)	**Etch-and-rinse, conventional adhesive system;** Bis-GMA; polyalkenoic acid co-polymer; dimethacrylates; HEMA; photoinitiators; ethanol; water; nanofiller particles.	**Etching:** Apply Scotchbond™ Etchant to enamel and dentin for 15 s. Water-rinse for 10 s. Blot excess water using a cotton pellet or mini-sponge. **Adhesive:** Immediately after blotting, apply 2-3 consecutive coats of adhesive to etched enamel and dentin for 15 s with gentle agitation using a fully saturated applicator. Gently air thin for five seconds to evaporate solvents. Light cure for 10 s.	4BC
AdheSE (Ivoclar Vivadent)	**Primer:** phosphonic acid acrylate; Bis-acrylamide; water; initiators and stabilizers. **Bond:** dimethacrylates; hydroxyethyl methacrylate; highly dispersed silicon dioxide. **Initiators and stabilizers;** Activator; Solvent; Initiators.	Apply an adequate amount of primer assuring that the total reaction time should not be shorter than 30 s. Excess primer dispersed with a strong stream of air until the mobile liquid film is no longer visible. Application of bond, beginning with the dentin tissue. Bond dispersed with a very weak stream of air. Bond photoactivated for 10 s.	J09568
Adper Prompt-L-Pop (3M ESPE)	**Liquid 1 (red blister):** methacrylated phosphoric esters; Bis-GMA; Initiator: camphorquinone; stabilizers **Liquid 2 (yellow blister):** water; HEMA; Polyalkenoic acid; stabilizers	After mixing the content of the reservoirs, apply adhesive to the entire surface of the cavity, rubbing in the solution with moderate finger pressure for 15 s. Use a gentle stream of air to thoroughly dry the adhesive to a thin film. Light cure for 10 s.	293053

Bis-GMA: Bisfenol-A diglicidil dimethacrylate; HEMA: 2-Hydroxyethyl methacrylate

ized smear layer was produced using a wet-ground silicon carbide paper for 60 s and then finished to #600-grit. The specimens were then randomly divided into 3 groups (n = 6) according to the adhesive applied. Then, 6 layers of 1 mm-thick resin composite (Filtek Supreme XT – 3M/ESPE, St. Paul, MN, USA) were added to the surface of the restorations to obtain specimens with the same dentin/resin composite proportion. The roots were sectioned approximately 2 mm below the cement-enamel junction, perpendicular to the long axis of the tooth, using a diamond-impregnated disk (Extec, Enfield, CT, USA) in a specific cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) under water-cooling at 300 rpm. After each storage time (24 hours and 6 months), the teeth were longitudinally sectioned in both "x" and "y" directions across the bonded interface using the same cutting device under water-cooling. This resulted in bonded stick-shaped specimens with a cross-sectional area of 0.8 (±0.2 mm²). The squared sticks were then cemented to the testing device using cyanoacrylate cement (Zapit, DVA Inc., Corona, CA, USA). This device was attached to a universal testing machine (Instron Model 3342, Instron Corp., Canton, MA, USA) and stressed in tension at a cross-speed of 1 mm/min until failure. The sticks of each group were randomly divided to be tested immediately (24 h control groups) or after 6 months of storage in distilled water at room temperature. After testing, the fractured specimens were carefully removed from the apparatus and the cross-sectional area was measured with a digital caliper at the site of failure. The results were recorded and the debond stress values were converted into MPa.

Statistical methods

The two-way ANOVA and Bonferroni post-hoc tests were used to analyze the μTBS and adhesive gap width at a preset alpha of .05. The Kruskal-Wallis and Dunn post-hoc tests were used to compare the microleakage data present between groups (α =.05).

RESULTS

The results of the microleakage test in the dentin margins are presented in Table 2. Figure 1 presents the proportion between perfect sealing/presence of gaps according to experimental groups. Statistical analysis demonstrated no significance between the thermo-mechanically treated and untreated groups (P>.05). When the microleakage values were compared, no significance was found among the experimental groups (P>.05). Table 3 shows the results and statistical analysis for adhesive gap extension. No significance was found among the control groups (P>.05). When the mean values of the thermo-mechanically treated groups were compared, the same occurred. ASE presented the lowest gap extension mean value compared to the cases in which both Adper Single Bond 2 and Adper Prompt L-Pop were applied. Statistical analysis showed that the mean value noted when AdheSE was applied was significantly lower than that observed for Adper Single Bond 2 (P<.05). No significance was observed when the treated and untreated groups were compared in terms of adhesive gap width. None of the adhesives were able to provide perfect sealing of the axial walls (Figure 1). TCM treatment led to a reduction in the percentage of gap-free interfaces.

The results of the bond strength test are summarized in Figure 2. The immediate results showed that when Adper Single Bond 2 was applied, the highest bond strength values (49.1 ± 11.6 MPa) were observed, while the lowest bond strength values were observed for Adper Prompt L-Pop (18.4 ± 6.6 MPa). Statistical analysis demonstrated that the bond strength mean value of Adper Single Bond 2 was significantly higher in comparison to the mean values observed when both AdheSE and Adper Prompt L-Pop were applied (P<.05). After 6 months, a significant reduction was only found for ASB2 (40.3 ± 8.7 MPa) (P<.05). Despite this decrease, the bond strength mean value of Adper Single Bond 2 was significantly higher than those noted at any evaluation time for both AdheSE and Adper Prompt L-Pop (P<.05).

DISCUSSION

The first hypothesis, that the application of self-etching adhesives would provide lower microleakage values in comparison to a conventional etch-and-rinse adhesive system, was not accepted. The microleakage values when the various adhesives were used were statistically equivalent (P>.05). Adhesive systems were selected to represent a variety of commonly used classifications: a conventional two-step adhesive; a two-step self-etching adhesive, and a one-step self-etching adhesive. The first approach (conventional adhesive) completely re-

moves the smear layer by conditioning the dentin tissue (Adper Single Bond 2). After rinsing, the adhesive system is applied to the demineralized dentin. The second approach (self-etching adhesive) is based on the simultaneous etching and priming of the smear-covered dentin. The two-step self-etching adhesive (AdheSE) contains an acidic primer that eliminates the separate acid-etching and rinsing steps, simplifying the bonding technique and reducing its technique sensitivity.[19] The all-in-one self-etching adhesive system (Adper Prompt L-Pop) simplifies the bonding procedure even more by dissolving the smear layer with acidic resins while simultaneously promoting monomer impregnation throughout the exposed collagen network.[11] In the present study, the research hypothesis raised assumed that the microleakage would be lower because of the reduced technique sensitivity when applying the self-etching adhesives tested. Conversely, in the present study, no significant difference was observed among the groups, irrespective of the thermo-mechanical treatment. These results were in accordance to recent studies that revealed that the microleakage in composite restorations is not influenced by the high degree of technique sensitivity associated with the use of different categories of adhesive systems,[20, 21] despite the thermo-mechanical treatment.

The thermal mechanism that causes microleakage in bonded restorations is claimed to be due to the linear thermal expansion coefficient (LTEC) among enamel (16.9 x 10-6 °C-1), dentine (10.6 x 10-6 °C-1), and restoration (17 to 83.5 x 10-6 °C-1).[22] LTEC is defined as the change in density when a material undergoes a change in temperature.[23] The differences in LTEC generate a negative interface

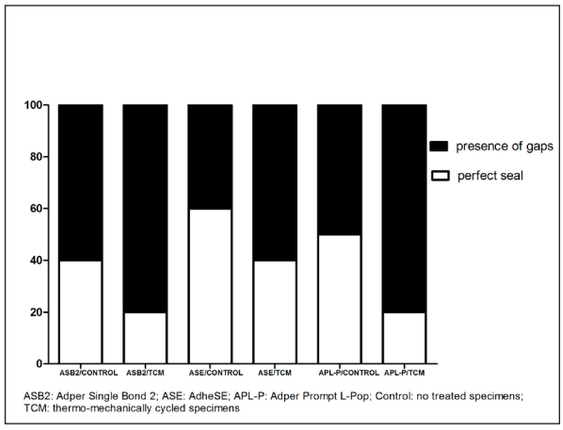

Figure 1. Percentage (%) of gap-free interfaces according to the experimental groups.

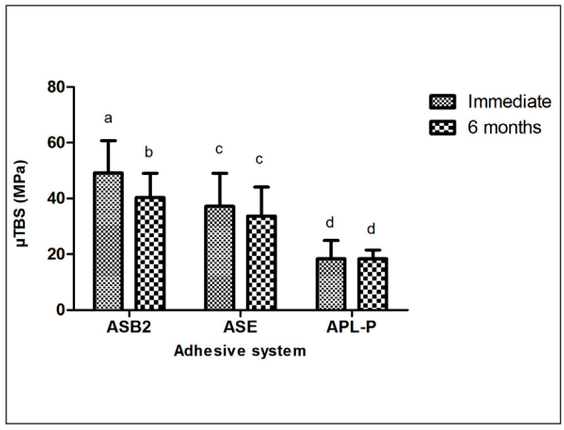

Figure 2. Microtensile bond strength measured immediately and after six months. ASB2: Adper Single Bond 2, ASE: AdheSE, APL-P: Adper Prompt L-Pop. Different letters: significant (P<.05). Vertical bars represent + 1 standard deviation.

Table 2. Median of microleakage observed at dentin margins.

		QUALITATIVE MICROLEAKAGE				
ADHESIVE SYSTEM	STRESS	0	1	2	3	Median
Adper Single Bond 2	C	8	2	0	0	0
	TMC	5	3	1	1	0.5
AdheSE	C	7	0	2	1	0
	TMC	5	4	1	0	0.5
Adper Prompt L-Pop	C	7	1	2	0	0
	TMC	4	5	1	0	1

Table 3. Adhesive axial gap width (standard deviation).

	ASB2	ASE	APL-P
CONTROL	1,83 (2,48)	0,69 (1,53)	2,16 (2,71)
TCM	6,94 (9,42)	1,63 (2,15)	5,91 (6,98)

ASB2: Adper Single Bond 2; ASE: AdheSE; APL-P: Adper Prompt L-Pop; Control: no treated specimens; TCM: thermo-mechanically cycled specimens
n=10
All values were statistically equivalent (ANOVA test, P>.05).

pressure that stimulates the penetration of oral fluids into the margins. If the temperature increases, the LTEC, pressure, and μL also increase.[24, 25] On the other hand, it has been advocated that the resin composites present a very slow rate of thermal diffusion.[26] Clinically, the short duration of thermocycles does not cause a dimensional change of the material and might not affect the microleakage.[26, 27]

The effect of mechanical stresses is related to the differing elastic modules of dentine (18-20 GPa), enamel (80 GPa), and resin (16 GPa). It has also been claimed that the combination of thermal changes and occlusal forces comprises approximately 95 % of the stresses applied to posterior restorations.[28] The consequence of the mechanical stress is usually the increase of gaps in the axial wall or in the enamel or dentin margins.[29, 30] According to the results of the present study, the percentage of gap-free axial interfaces was reduced after the thermo-mechanical treatment (Figure 1), irrespective of the adhesive applied. On the other hand, statistically equivalent gap widths were observed among the experimental groups (Table 3). Thus, the second hypothesis was not accepted, as no difference was noticed regarding the axial gap width.

The third hypothesis, that the bond strength to dentin of self-etching adhesives would be similar to that exhibited by a conventional etch-and-rinse adhesive, was not accepted. Both the immediate and the 6-month μTBS mean values found when the self-etching adhesives were used demonstrated significantly inferior results. When the conventional etch-and-rinse adhesive system was applied to dentin, significantly higher values were obtained ($P<.05$). Therefore, after 6 months, a significant reduction was observed for the ASB2 adhesive ($P<.05$). On the other hand, despite the lower mean values, the bonding to dentin observed after 6 months was statistically equivalent for both ASE and APL-P compared to the immediate values ($P>.05$).

The results expressed in the present study were consistent with the findings of other authors who found that the efficacy of the conventional etch-and-rinse adhesive system decreased with time when it was submitted to an artificial aging.[7, 31, 32] Water molecules degrade the dentin/restoration interface by hydrolysis, as a plasticizer agent or by increasing the coefficient of thermal expansion.[22] The consequences are the lixiviation of matrix molecules such as diluents, additives, plasticizers, initiators, and bonding agents.[33] Also, the water sorption and solubility of composites as well as adhesives increase progressively with time.[34] Furthermore, the etching procedure can subject collagen fibers to degradation, which can also be produced by the activation of host-derived matrix metalloproteinases (MMPs).[35] Despite the reduction in the μTBS with time, the mean μTBS values observed for ASB2 were higher than the mean values seen for the two self-etching adhesives.

The outcome of composite restorations is based on the adhesive interface integrity, and several polymers have been created to promote more resistance over time.[2] In oral conditions, the water environment, thermal changes, and fatigue determine the progressive degradation of adhesive interface components, increasing the potential failure of restorations.[1] The degree of resistance to degradation can also be related to the solvent added to the adhesive.[36] Water is a highly polar solvent of Adper Prompt L-Pop which presents a high dielectric constant, which allows the strong hydrogen bridges to be formed and to expand the collagen matrix.[3] Unfortunately, high ebullition temperature, low vapor pressure, and oral cavity humidity makes its evaporation more difficult.[37] Self-etching adhesives require a higher volume of solvent to keep the hydrophilic and hydrophobic monomers together and to contribute to the ionization process for self-etching activity. Thus, these systems behave as semi-permeable membranes that can lose their properties through the separation of components.[12] The main component of Adper Prompt L-Pop is HEMA-phosphate. HEMA phosphate and phosphoric acid are comparably aggressive in terms of etching potential.[38] However, HEMA-phosphate exhibited a lower apatite-dissolving capacity than H_3PO_4.[39] The reason for this factor is probably because HEMA-phosphate is considered an unstable molecule40 and because of its high molecular weight (322 g/mol), which gives it a lower molar concentration. Although water is required for ionization of acidic monomers, residual water also lowers their concentration.41 In water, HEMA-phosphate component degrades into HEMA and phosphoric acid molecules. This probably results in a resin-sparse, less highly polymerized region along the base of the hybrid layers.19 Despite its low pH (0.8), which can effectively seal enamel margins in Class V restorations,[42, 43] the instability of Adper Prompt L-Pop can form irregular

demineralization depths and heterogeneous monomer interdiffusion into the dentinal tissue.[43]

Besides water as a solvent, Adper Single Bond 2 contains ethanol, which has a higher vapor pressure and low dielectric constant and produces hydrogen bridges with water, resulting in a better evaporation rate.[3,37] Ethanol also produces a stiffening effect on demineralized collagen, which maintains interfibrillar spaces after evaporation.[44,45] However, the water molecules contained in the Adper Single Bond 2 adhesive are not sufficient to compensate for the collapsed collagen fibers, which requires previously wet dentin,[3] thus increasing the bonding sensibility. Adper Prompt L-Pop and Adper Single Bond 2 have polyalkenoic copolymers, which offer greater resistance to humidity.[43] However, this molecule cannot dissolve adequately in adhesive solution; rather it separates into phases, producing globular formations in the adhesive layer.[46] According to the manufacturer, the water present in the composition of AdheSE composition does not work as a solvent. ASE has bis-acrilamide molecules, which improve its resistance to degradation. Its methacrylamide molecule has a -CO-NH- or -CO-N- group, which differs from conventional acrilates and methacrylates in an ester group (-CO-O-R-). It has a similarity to collagen amino acids, which promote the formation of hydrogen bridges between carboxyl groups and the amide of the monomers with the carboxyl groups of the collagen.[47]

The present study indicated the importance of interface integrity over time. It was demonstrated that the conventional etch-and-rinse adhesive system achieved the highest bond strength values. It is true that the bonding strength values decreased with time, but the results observed for Adper Single Bond 2 after 6 months were still significantly higher than with self-etching systems. Also, despite the lower values, both self-etching adhesive systems demonstrated bond strength stability with time. It was also pointed out that AdheSE was able to exhibit lower gap widths under conditions of stress. Based on the results of the present study, the importance of obtaining high bond strength levels can be questioned in terms of longevity. It is true that most manufacturers claim higher bond strength values as one of their main advantages. It is true that the self-etching adhesives exhibited lower bond strength values, but the advantages of the simplicity of the technique and the elimination of the rinsing and drying steps cannot be ignored. In this way the possibility of over-wetting or over-drying, which are deleterious to the interface integrity, is reduced. Future clinical studies are necessary to confirm that these characteristics are important to restoration's longevity.

CONCLUSION

Within the limitations of this study, it was concluded that:

- Despite the higher values, the bonding effectiveness of the two-step etch-and-rinse Adper Single Bond 2 was reduced after water storage;
- The one-step, self-etching AdheSE and Adper Prompt L-Pop were not affected by water storage, presenting similar μTBS before and after water storage; and
- None of the adhesive systems tested were affected by thermo-mechanical cycling, showing similar microleakage and adhesive gap width before and after cycling stresses.

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