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Bond strength of two universal adhesive systems to human dentin using different strategies

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ABSTRACT

The objective of this study was to evaluate the microtensile bond strength (µTBS) to dentin of two universal adhesive systems: Single Bond Universal (SBU) and Ambar Universal (AU), used in different adhesion strategies. ***Materials and Method:*** *Thirty-six human teeth were prepared (n=6) and treated following different adhesive strategies: G1: SBU-etch-and-rinse, applied on dry dentin; G2: SBU-etch-and-rinse, applied on moist dentin; G3: SBU-self-etching; G4: AU-etch-and-rinse, applied on dry dentin; G5: AU-etch-and-rinse, applied on moist dentin; G6: AU-self-etching. The specimens were submitted to µTBS test, failure analysis, and scanning electron microscopy (SEM). Data were analyzed with ANOVA and Tukey's tests (p <0.05).* ***Results:*** *Microtensile bond strength was significantly lower in G1 than G2 and G3. AU adhesive performed worse than the SBU system, except in G5. Cohesive and mixed failures predominated in G1 and G2, while adhesive failures predominated in G3 and G5.* ***Conclusions:*** *Universal adhesives are an interesting innovation, but there are still doubts about their performance, mainly regarding the different protocols provided by the manufacturers. The conventional adhesive strategy on moist dentin demonstrated higher µTBS for both adhesives. The use of the self-etching strategy with the SBU showed promising results.*

Keywords: *dental bonding - dentin - adhesives*

Resistência de união de sistemas adesivos universais à dentina humana usando diferentes estratégias

RESUMO

O objetivo deste estudo foi avaliar a resistência de união à microtração (µTBS) de dois sistemas adesivos universais: Single Bond Universal (SBU) e Ambar Universal (AU), utilizados em diferentes estratégias de adesão. ***Materiais e método:*** *36 dentes humanos foram preparados (n=6) e tratados seguindo diferentes estratégias adesivas: G1: SBU-condicionamento e enxágue, aplicado sobre dentina seca; G2: SBU-condicionamento e enxágue, aplicado sobre dentina úmida; G3: SBU-autocondicionante; G4: AU-condicionamento e enxágue, aplicado em dentina seca; G5: AU-condicionamento e enxágue, aplicado sobre dentina úmida; G6: AU-autocondicionante. Os espécimes foram submetidos ao teste de µTBS, análise de falhas e microscopia eletrônica de varredura (SEM). Os dados foram analisados com os testes ANOVA e Tukey (p<0,05).* ***Resultados:*** *A resistência de união à microtração de G1 foi significativamente menor que G2 e G3. O adesivo AU teve um desempenho pior que o sistema SBU, com exceção do G5. Falhas coesivas e mistas predominaram em G1 e G2 enquanto G3 e G5 apresentaram predominância de falhas adesivas.* ***Conclusões:*** *Os adesivos universais representam uma inovação interessante, mas ainda há dúvidas sobre seu desempenho, principalmente em relação aos diferentes protocolos fornecidos pelos fabricantes. A estratégia adesiva convencional em dentina úmida demonstrou maior µTBS para ambos os adesivos. O uso da estratégia autocondicionante com a SBU apresentou resultados promissores.*

Palabras clave: *adesivos dentinários - dentina - adesivos*

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INTRODUCTION

Adhesion in dentistry is a critical process dependent on numerous factors such as the type of substrate, environment humidity, the adhesive system used, and professional operating capability[1,2]. An adhesive must be able to promote an equally effective bond on both enamel and dentin, even though they are entirely different tissues. On enamel, adhesion occurs due to micro-retention produced on the acid-etched surface filled by the resin monomers. On the heterogeneous dentin substrate, adhesion is challenging because ideal moisture conditions must be maintained to enable adequate infiltration of the adhesive into the demineralized substrate[1-3].

The adhesive systems currently marketed are classified into two categories: 1) conventional (*etch-and-rinse*) and 2) self-etching (*self-etch*)[4]. In conventional systems, the main drawback is the bonding deterioration that occurs in demineralized dentin incompletely filled by the resin monomers after acid etching, leading to microleakage and dentin hypersensitivity that may affect the longevity of a restoration. Self-etching systems were developed to minimize this problem through demineralization and co-occurring primer infiltration[5]. These innovative systems create a chemical interaction between the adhesive and the dental tissue, making the interface more resistant to biodegradation, especially at the dentin substrate[6-7]. Although self-etching adhesive systems do not require moisture to bond, water is included in their composition to ionize the hydrophilic acid monomers, which are responsible for the mineral ions available to the chemical bond with the dental substrate[8-10].

Approximately ten years ago, multi-mode adhesives were designed under the all-in-one concept, providing greater versatility than existing adhesives and enabling the clinician to decide which adhesive strategy to use[1,9,11-14].These universal adhesives tend to minimize dentin sensitivity because deep demineralization is not necessary. Some studies, however, have shown that in enamel, the selective etching technique improves bonding performance[6,8,9,15,16].

Although universal adhesives are an interesting innovation, there are still doubts about their performance, mainly regarding the different protocols provided by the manufacturers[7,17]. Recent systematic reviews concluded that the application of the universal adhesive by the conventional or self-etching method was satisfactory, especially for mild-acidic adhesives[18,19]. However, considering the difficulty of controlling dentin moisture, it is relevant to ascertain whether bond strength changes according to dentin moisture. The aim of this study was thus to evaluate the microtensile bonding strength (μTBS) of two universal adhesive systems applied to human dentin according to different adhesive strategies. This null hypothesis is that different adhesive strategies do not influence bonding strength to human dentin with two adhesive systems.

MATERIALS AND METHOD

Study design and sample number

Two universal adhesive systems, Single Bond Universal (SBU) (3M ESPE, St. Paul, MN, USA) and Ambar Universal (AU) (FGM, Joinville, SC, Brazil) were analyzed with three adhesive strategies: G1: SBU-etch-and-rinse mode and dry dentin; G2: SBU-etch-and-rinse mode and moist dentin; G3: SBU-self-etching; G4: AU-etch-and-rinse mode and dry dentin; G5: AU-etch-and-rinse mode and moist dentin; G6: AU-self-etching.

Considering the error probability of Type I (5%) and Type II (20%), six teeth per group were used, with a minimum of seven specimens per tooth. The teeth prepared were randomly divided into six groups of six teeth, achieving at least 42 specimens (sticks) per group.

Tooth preparation and bonding procedures

This study was approved by the Ethical Committee in Human Research (CEP) (CAAE – 68999817.4.0000.5149). Thirty-six recently removed intact human third molars were selected from the Human Teeth Biobank at the School of Dentistry of the Universidade Federal de Minas Gerais and stored in a 0.5% chloramine solution for 24 hours[20]. The teeth were kept under distilled water until the beginning of the experimental procedures, not exceeding one month after extraction.

A section of the crown was cut perpendicular to the axis of the teeth using a diamond saw blade (Diamond Wafer Blade, Series 15 HC, Lake Bluff, IL, USA) in a cutting machine (IsoMet, Buehler, Lake Bluff, IL, USA) under water cooling, removing the occlusal third of the crown. The dentin surfaces were verified under an optical microscope (Stemi DV4, Zeiss, Oberkochen, Germany) to ensure complete removal of the enamel. To obtain a flat surface of the dental substrate and create a standard smear layer, all dentin surfaces were polished in a metallographic polisher (Arotec Industry e-Commerce, Cotia, SP, Brazil) with #600 grit silicon carbide abrasive paper (3M, Nova Veneza, SP, Brazil) under water irrigation for 60 s for each tooth before performing the adhesive procedures[21].

The teeth were randomly divided into six groups of six teeth (n = 6), to which were applied the three different adhesive strategies using the two adhesive systems. The adhesives were applied to the flat dentin surfaces according to the manufacturer's instructions (Table 1).

After the adhesive procedure, the dentin surfaces were restored with a composite resin (Filtek Z350 XT, 3M ESPE, St. Paul, MN, USA) to a height of 6 mm, in increments of 2 mm for each layer. Each layer was light-cured for 20 s using a Bluephase (Ivoclar Vivadent, Schaan, Liechtenstein) light-curing device at an intensity of 1.200 mW/cm^2 controlled by a radiometer. At the end of the restorative procedure, the specimens were immersed in distilled water and stored in an incubator at 37 °C for 24 hours.

Specimen preparation

After 24 hours, a diamond saw blade 15.2 cm in diameter and 0.3 mm thick, mounted in the cutting machine, was used under constant water irrigation, with pressure 50 g, and rotational speed 250 rpm, to make sequential cuts in the vestibule/palatal direction, leaving sufficient thickness to obtain slices of approximately 1 mm each considering the

Table 1. Adhesive systems and strategies used

Adhesive System	Composition	Classification according to pH	Groups	Adhesive Strategy
Single Bond Universal (SBU)	10-MDP phosphate monomer, Vitrebond copolymer, HEMA, BISGMA, dimethacrylate resins filler, silane, initiators, ethanol, water	Mild (pH=2.7)	G1	Etch-and-rinse mode and dry dentin: Acid conditioning for 15s; rinse for 30s; air dry for 10s, keeping the dentin dry. Apply the adhesive for 20s with vigorous agitation; gently air for 10s and light-cure for 10s.
			G2	Etch-and-rinse mode and moist dentin: Acid conditioning for 15s; rinse for 30s; remove excess with absorbent paper, keeping dentin moist. Apply the adhesive for 20s with vigorous agitation; gently air for 10s and light-cure for 10s.
			G3	Self-etching: Without acid conditioning. Keep dentin dry, without overdrying it. Apply the adhesive for 20s with vigorous agitation; gently air for 10s and light-cure for 10s.
Ambar Universal (AU)	UDMA, HEMA, methacrylate hydrophilic monomers, methacrylate acid monomers, ethanol, water, silanized silicon dioxide, camphorquinone, ethyl 4-dimethylaminobenzoate, surfactant, sodium fluoride	Mild (pH=2.47)	G4	Etch-and-rinse mode and dry dentin: Acid conditioning for 15s; rinse for 30s; air dry for 10s, keeping the dentin dry. Apply the adhesive for 10s with vigorous agitation, followed by re-application for 10s; gently air for 10s and light-cure for 10s.
			G5	Etch-and-rinse mode and moist dentin: Acid conditioning for 15s; rinse for 30s; remove excess with absorbent paper, keeping dentin moist. Apply the adhesive for 10s with vigorous agitation, followed by re-application for 10s; gently air for 10s and light-cure for 10s.
			G6	Self-etching: Without acid conditioning. Keep dentin dry, without overdrying it. Apply the adhesive for 10s with vigorous agitation, followed by re-application for 10s; gently air for 10s and light-cure for 10s.

HEMA: 2-hydroxyethyl methacrylate; BISGMA: Bisphenol A-glycidyl methacrylate; UDMA: urethane dimethacrylate.

thickness of the disk. Subsequent cuts were made in the mesiodistal direction, maintaining a thickness of 1 mm. After that, cuts were made parallel to the occlusal plane, thereby obtaining stick-shaped specimens with an area of approximately 1.0 mm². The intact specimens (sticks) for each group were measured with a Mitutoyo digital electronic caliper (Kawasaki, Kanagawa, Japan) with a precision of 0.01 mm, confirming the total surface area of approximately 1.0 mm². Sticks with suspected adhesive failure were discarded. The composite resin portion on each stick was identified with a red marker and the dentin portion with a black marker. This procedure facilitated the identification of the parts after fracture. The sticks were stored in distilled water at room temperature until testing.

Microtensile bond strength test

The sticks were individually attached by their ends with a quick-curing cyanoacrylate-based gel adhesive (Super Bonder, Henkel Loctite Adesivos, São Paulo, SP, Brazil) to the Geraldeli's claw. This µTBS device adapts to the specific attachment used in the universal testing machine[22].

The panel of the universal testing machine (EZ -Test, Shimadzu, Japan) was set at a constant speed of 0.5 mm/min and adjusted to detect the maximum load value necessary to fracture the specimen (in kilonewton, kn) and return to the zero (initial) position, after which a new specimen could be positioned for the test. The µTBS results were expressed in MPa and recorded in a spreadsheet.

The number of prematurely detached sticks in each group was recorded, but these values were not included in the statistical analysis. All premature failures that occurred during the cutting procedure and did not exceed 3% of the total number of tested specimens and were similarly distributed among the various groups.

Failure mode analysis and scanning electron microscopy (SEM) analysis

The fractured specimens were observed under the light of a Stemi DV4 optical microscope (Zeiss, Oberkochen, Germany) at 32x magnification by two professionals other than the one who performed the µTBS test. The fracture mode was classified as adhesive (A), mixed (M), cohesive at the resin (CC), or cohesive at dentin (CD). The percentage of failure patterns was calculated according to the frequency observed in each experimental group.

Representative fractured specimens of each group were dehydrated in alcohol in an ascending series (25%, 50%, 75%, 90%, and absolute) for one hour in each solution, followed by immersion in Bis(trimethylsilyl)amine (HMDS) for 10 min. After dehydration, the specimens were fixed on stubs with the aid of a double-sided carbon tape, and sputter-coated with carbon a vacuum sputter-coater (SDC 050, Bal-tec AG, Balzers, Liechtenstein), and observed using a scanning electron microscope (Quanta Fei 200, Hillsboro, OR, USA) operating at an acceleration voltage of 5 kV.

Statistical analysis

For statistical analysis of the data, analysis of variance (ANOVA) ($p<0.05$) was performed to verify differences between the groups. Pairwise comparisons were conducted using Tukey's significant difference test (HSD) ($p<0.05$). GraphPad Prism 7 software (La Jolla, CA, USA) was used for statistical analysis.

RESULTS

Regarding µTBS values, there was a statistically significant difference between G1 (SBU on dry dentin) and G2 (SBU on moist dentin), and between G1 and G3 (SBU in self-etching mode). Table 2 shows the mean and standard deviation of the µTBS test for all groups. SBU applied on moist dentin presented the highest results, followed by the self-etching technique.

Table 2. Means and standard deviation of microtensile bond strength (MPa) of the experimental groups

Groups	Number of specimens	Mean ± SD
G1	71	41.12 (10.72) a
G2	77	48.05 (10.27) b
G3	61	46.83 (12.87) b
G4	8	2.95 (//)
G5	72	44.46 (12.36) ab
G6	0	0.00

G1: Single Bond Universal – conventional mode on dry dentin; G2: Single Bond Universal – conventional mode on moist dentin; G3: Single Bond Universal – self-etching mode; G4: Ambar Universal – conventional mode on dry dentin; G5: Ambar Universal – conventional mode on moist dentin; G6: Ambar Universal – self-etching mode

SD: standard deviation; MPa: megapascal; Different letters indicate statistically different means (ANOVA and Tukey's test; $p < 0.05$)

It was impossible to obtain specimens from G6 because the resin became detached from all the teeth during preparation. In G4, only eight specimens were obtained from one tooth, because the resin became detached while the other specimens were being prepared. Hence, G4 and G6 were not included in the statistical analysis. AU applied on moist dentin (G5) showed μTBS similar to SBU.

Figure 1 shows the frequencies of failure modes for each group. Adhesive failure was predominant for SBU in the self-etching mode and for AU in the conventional mode in moist dentin. There were more cohesive and mixed fractures in the SBU specimens when the dentin was etched with phosphoric acid. The SEM images illustrate the predominant failure pattern found in G1, G2, G3 and G5 (Fig. 2)

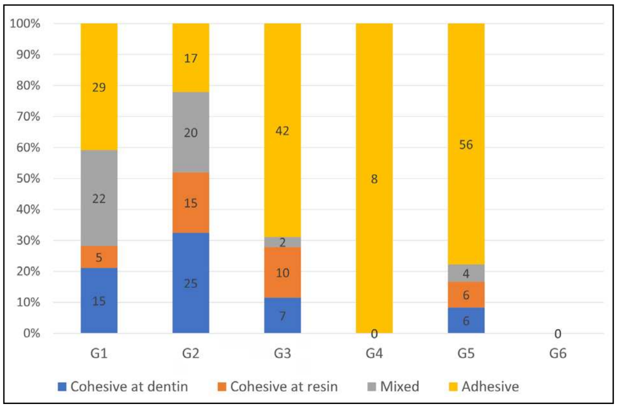

Fig. 1: *Number and percentage of specimens (%) according to the fracture mode of all experimental groups.*
G1= Single Bond Universal - conventional mode on dry dentin; G2= Single Bond Universal-conventional mode on moist dentin; G3= Single Bond Universal-self-etching mode; G4= Ambar Universal-conventional mode on dry dentin; G5= Ambar Universal-conventional mode on moist dentin; G6= Ambar Universal-self-etching mode.

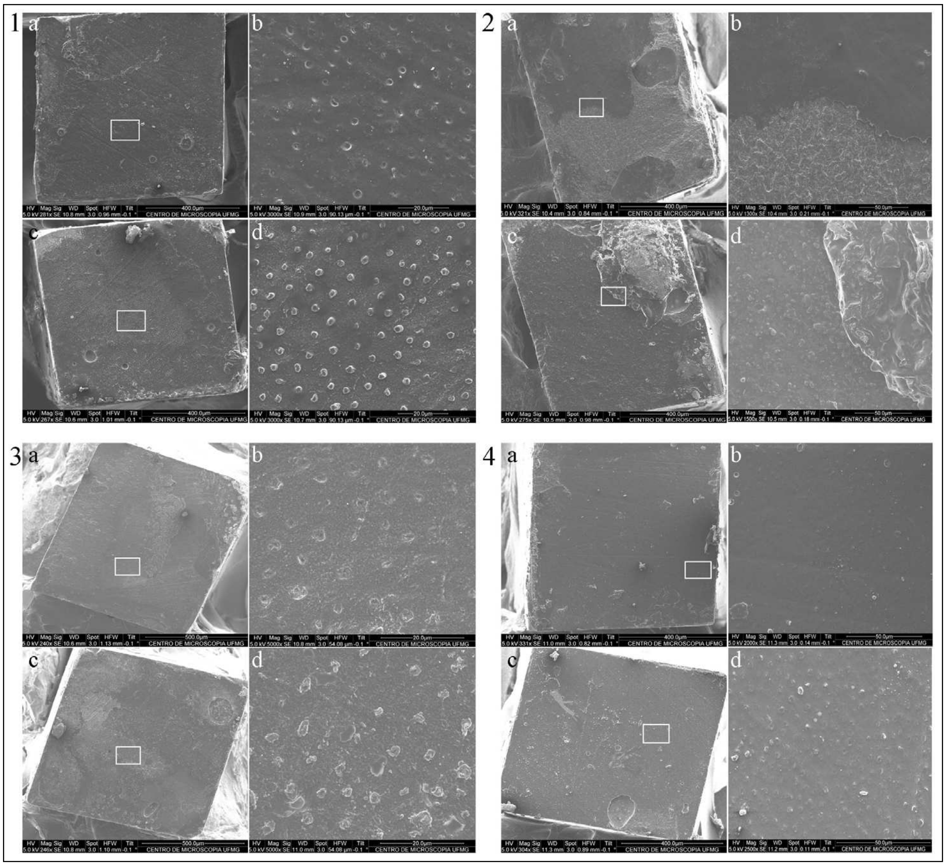

Fig. 2: 1) *SEM images of Single Bond Universal - conventional mode on dry dentin:* ***a-*** *top of the stick, dentin portion, after adhesive-type fracture at 50x and* ***b-****1000x magnifications;* ***c-*** *top of the stick, resin portion at 50x and* ***d-*** *1000x magnifications.* ***2)*** *SEM images of Single Bond Universal - conventional mode on moist dentin:* ***a-*** *top of the stick, dentin portion, after mixed-type fracture at 50x and* ***b-*** *1000x magnifications;* ***c-*** *top of the stick, resin portion at 50x and* ***d-*** *1000x magnifications.* ***3)*** *SEM images of Single Bond Universal - self-etching mode:* ***a-*** *top of the stick, dentin portion, after adhesive-type fracture at 50x and* ***b-*** *1000x magnifications;* ***c-*** *top of the stick, resin portion at 50x and* ***d-*** *1000x magnifications.* ***4)*** *SEM images of Ambar Universal - conventional mode on moist dentin:* ***a-*** *top of the stick, dentin portion, after adhesive-type fracture at 50x and* ***b-*** *1000x magnifications;* ***c-*** *top of the stick, resin portion at 50x and* ***d-*** *1000x magnifications.*

DISCUSSION

This study examined the μTBS of two universal adhesives, Single Bond Universal (SBU) and Ambar Universal (AU). In addition to the self-etching protocol, their behavior was analyzed using the conventional adhesive protocol, with previous conditioning with phosphoric acid (37%) and varying the humidity of the dentin substrate. Although there are systematic reviews of universal adhesive performance, different brands are rarely used, increasing the risk of bias[19]. Thus, we decided to evaluate one system that is used around the world and another Brazilian system that is widely used within the country, mainly due to its low cost, though there are still few studies on it in the literature.

Microtensile tests enable the analysis of the bond between surfaces in small areas[23] using a small number of teeth, considering the possibility of obtaining several replicas, and good customization of the study design. The quantitative analysis of the materials' bond strength to the point of failure can be combined with microscopy techniques to identify the fracture mode at the adhesive interface[24].

During the sectioning of the dentin/resin blocks to produce the specimens (sticks), dentin/resin detachment was observed in the AU self-etch group (G6) and in the AU etch-and-rinse on dry dentin (G4), resulting in zero and eight specimens, respectively. It is worth noting that that research protocols were performed carefully, as described above. Randomization and blocking principles were also followed, ensuring that the sources of variation would be acting comparably in all groups.

We found a statistically significant difference between G1 (SBU on dry dentin) and G2 (SBU on moist dentin), and between G1 and G3 (self-etching SBU). In both comparisons, μTBS results were lower for SBU on dry dentin. These allow us to partially reject the null hypothesis that using different adhesive strategies would not affect μTBS values. However, it is worth highlighting that all results from these adhesives were considered acceptable.

The present study corroborated previous reports that SBU did not differ in self-etching and conventional protocols and had bond strength values similar to ours[25-27]. In contrast, lower μTBS values were found using SBU in the self-etching strategy than in the conventional modes in wet and dry dentin[28]. This can be explained by the higher testing speed applied for the μTBS test (5.0 mm/min) compared to our study and others that used 0.5 to 1.0 mm/min. A clinical trial showed that after 5 years, the clinical behavior of SBU in the etch and rinse strategy was better than in the self-etch strategy, even considering different dentin moisture levels[29]. However, in the current in vitro study, no difference was found between moist and dry dentin.

It should be emphasized that AU did not promote satisfactory bonding using either adhesive strategy, so it was not possible to perform the bond strength test. G5 was the exception, since its results did not differ from those of SBU. Our results contradict other studies that found similar μTBS for the conventional and self-etching protocols with AU[27,30]. Additionally, in these studies, AU did not differ from SBU. However, in contrast to our study, one of them used eroded dentin[30], while another used bovine teeth and considered only adhesive and mixed failures in the calculation of bond strength values[27]. No other study on Ambar Universal was found, but regarding longevity, AU had lower dentinal bond strength after 6 months, and a more stable dentin bond when applied in the etch-and-rinse mode[27].

Adhesives used in self-etching mode are designed to bond to tooth substrates by self-conditioning and simultaneous replacement by resin, integrating the smear layer to the adhesive interface[31]. The ability to infiltrate the smear layer and hybridize the underlying dentin is a process dependent on both the aggressiveness of the self-etching adhesive and the thickness of the smear layer[32].

SBU and AU have similar compositions. They both contain 10-methacryloxydecyl dihydrogen phosphate (MDP) as a functional monomer. Despite sharing similarities in composition and versatility, they can differ in aspects such as the amount of water, solvent, MDP, resin dimethacrylates, and acidity. These differences may influence the viscosity and moisture of the adhesive, affecting its ability to penetrate and act on demineralized or non-demineralized dentin. In the present study, AU did not show positive results when applied in self-etching mode or conventional mode on dry dentin. It was assumed that the acidic monomers were not able to interact sufficiently with the dentin substrate in the self-etching mode to promote adequate demineralization and hybridization. Also, the amount of water may have been insufficient in the conventional mode in dry dentin to promote rehydration, preventing the adhesive from permeating the collagen network after etching[33].

For SBU, G1 results are compatible with previous reports that consider moisture maintenance essential to achieving successful bonding on conditioned dentin[34-37]. The μTBS proved to be satisfactory, as previous reports allowed us to infer its good capacity to promote rehydration of conditioned and dried dentin[12,28]. SBU contains an ethanol/water-based solvent system with 10–15 wt.% each. Thus, it has enough water to shape the collagen network, promoting re-expansion and re-opening of the interfibrillar spaces from the collapsed dentin, allowing the infiltration of resin monomers[38]. Moreover, the technical profile of SBU indicates that it contains a polyalkenoic acid copolymer (Vitrebond copolymer) capable of providing satisfactory adhesion to the dentin under different humidity levels[39]. The presence of such a substance in AU has not yet been reported. Clinical studies did not show significant differences in the performance of the conventional technique with SBU on dry and moist dentin but found similar marginal adaptation and discoloration for up to 36 months of follow-up[11,15]. Although the performance of SBU in dry dentin in our study was significantly worse, μTBS values were still considerably high. Thus, this may be a valuable option in clinical practice, considering that ideal moisture maintenance in demineralized dentin is challenging to achieve.

In this study, the groups tested with SBU in conventional dry dentin and self-etching modes had prevalence of adhesive- and/or mixed-fracture patterns, with data corroborated by previous reports[28,30]. When acid etching and dry dentin were used, one paper reported predominance of cohesive fracture[12], and another found predominance of cohesive failures for the SBU when tested in both adhesive strategies[27]. In general, studies that tested SBU in conventional mode with moist dentin found a predominance of cohesive failures in dentin or in the composite. These results are reasonable because the higher micromechanical retention obtained after acid etching explains the higher bond strength values, at least in the "immediate" condition. Considering that SBU has not been found to behave differently in the conventional approach in moist dentin and self-etching, its more superficial interaction with the dentin substrate without prior phosphoric acid etching may reduce the risk of postoperative sensitivity and degradation of the collagen fibrils, which could compromise adhesive stability over time[12]. It is believed that this long-term performance is a great advantage of these new adhesive systems. Although G5 (AU used in conventional mode with moist dentin) had μTBS values that were statistically similar to SBU groups, it is interesting that failures in G5 were predominantly adhesive. Curiously, this behavior is currently observed in the SBU adhesive in self-etching mode. It was expected that failure patterns would be mostly cohesive and/or mixed in the conventional mode, as reported previously[27]. AU bond strength longevity evaluated in bovine teeth showed an increase in the frequency of pre-test failures, especially when used in self-etching mode[27].

There is still no gold standard protocol for universal adhesives[40], which reinforces the importance of continuing to study tools and protocols that improve their use. Long-term clinical trials should be encouraged to confirm the outcomes of universal adhesives.

In conclusion, Single Bond Universal μTBS was higher in etch-and-rinse mode on moist dentin and in self-etching mode compared to etch-and-rinse on dry dentin. Ambar Universal, however, presented acceptable μTBS values only for etch-and-rinse mode on moist dentin.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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