Universal adhesives applied to deep dentin with different bonding treatments

Germán Mirotti[1], Mónica P Lutri[1], María E Kraemer[1], Natalia Monserrat[1], María C Piconi[1], Alicia L Caballero[1], Carlos A Rozas[1], Luis M Croharé[2], Mario Sezin[1]

1. Universidad Nacional de Córdoba, Facultad de Odontología, Departamento de Rehabilitación Bucal, Cátedra de Operatoria I "A", Córdoba, Argentina.
2. Universidad Nacional de Córdoba, Facultad de Odontología, Área de Biología Odontológica, Córdoba, Argentina.

ABSTRACT

*Eighth-generation adhesives may be applied with total etch, selective-etch or self-conditioning, and serve as primers for non-dental substrates. **Aim:** To determine the bonding characteristics of universal adhesives applied to the deep pulp wall with different strategies, by means of shear bond strength and laser microscopy. **Materials and Method:** Cavities 4 mm deep and maximum width were carved in 36 extracted molars. Nine groups were formed according to dental substrate treatment and adhesives, as follows: **Total-etch:** <u>group 1-</u>Monobond 7 self-etch, <u>group 2-</u>One coat 7 universal, and <u>group 3-</u>Single bond universal; **Adamantine etch:** <u>group 4-</u>Monobond 7 self-etch, <u>group 5-</u>One coat 7 universal, and <u>group 6-</u>Single bond universal; **Self-conditioning:** <u>group 7-</u>Monobond 7 self-etch, <u>group 8-</u>One coat 7 universal, and <u>group 9-</u>Single bond universal. Molars were filled following the manufacturer's instructions. Three specimens per group (27 altogether) were used to determine shear bond strength using a universal testing machine, while layer thicknesses were measured on the remaining specimens using microscope images and Olympus LEXT 3D Software. Analysis of variance was used to compare data. **Results:** Mean (standard deviation) bond strength in megapascals (MPa) was: group 1: 7.06±3.01; group 2: 10.74±4.36; group 3: 8.20±3.92; group 4: 7.41±2.23; group 5: 6.84±1.50; group 6: 5.86±2.10; group 7: 5.83±1.94; group 8: 7.14±2.37; group 9: 8.06±3.51. Bond strength was higher (p=0.049) for total-etch (8.61±3.96) than for selective etch (6.71±1.98) and self-conditioning (6.91±2.68). No significant difference was found among the three adhesives (p=0.205). Adhesive layer in micrometers (µm) was total-etch 8.71±4.93, selective etch 5.49±1.70 and self-conditioning 6.27±3.01, with no significant difference. **Conclusions:** There were significant differences among bonding strategies, with the highest values for total-etch. No significant difference was observed between self-conditioning and selective etch. No significant difference was found among the adhesives, which all behaved similarly. The greatest adhesive layer thicknesses were recorded in the total-etch group, with no significant difference among the various adhesive approaches.*

Keywords: dentin bonding agents - microscopy confocal

Adhesivos universales aplicados a dentina profunda con diferentes tratamientos de unión

RESUMEN

*Los adhesivos universales de octava generación pueden ser aplicados con diferentes estrategias de unión: grabado total, grabado selectivo o autoacondicionamiento. Además, imprimen sustratos no dentales. **Objetivo:** Determinar las características de unión de adhesivos universales con diferentes estrategias en pared pulpar profunda mediante resistencia adhesiva al corte y microscopía laser. **Materiales y Método:** En 36 molares se tallaron cavidades de 4 mm de profundidad y ancho máximo. Se dividieron en 9 grupos según tratamientos y adhesivos. **Grabado total:** <u>grupo 1-</u>Monobond 7 self-etching, <u>grupo 2-</u>One coat 7 universal y <u>grupo 3-</u>Single bond universal; **Grabado selectivo:** <u>grupo 4-</u>Monobond 7 self-etching; <u>grupo 5-</u>One coat 7 universal y <u>grupo 6-</u>Single bond universal y **Autoacondicionamiento:** <u>grupo 7-</u>Monobond 7 self-etching; <u>grupo 8-</u>One coat 7 universal y <u>grupo 9-</u>Single bond universal. Las obturaciones se realizaron siguiendo las instrucciones del fabricante. La resistencia adhesiva al corte se determinó utilizando una máquina de ensayo universal sobre 27 especímenes mientras que los restantes fueron empleados para evaluar los espesores de la capa generado sobre imágenes obtenidas con microscopía y con el software Olympus LEXT 3D. Se ultilizó análisis de varianza. **Resultados:** Resistencia adhesiva en megapascal (MPa) media (desviación estándar): grupo 1: 7,06±3,01; grupo 2: 10,74±4,36; grupo 3: 8,20±3,92; grupo 4: 7,41±2,23; grupo 5: 6,84±1,50; grupo 6: 5,86±2,10; grupo 7: 5,83±1,94; grupo 8: 7,14±2,37; grupo 9: 8,06±3,51. Grabado total (8,61±3,96) registró los valores mayores (p=0,049) en comparación a grabado selectivo (6,71±1,98) y autoacondicionamiento (6,91±2,68). Los adhesivos no tuvieron diferencias significativas (p=0,205). Capa adhesiva en µm: Grabado total (8,71±4,93); grabado selectivo (5,49±1,70) y autoacondicionamiento (6,27±3,01) sin diferencias significativas (p=0,073). **Conclusiones:** Las estrategias de unión mostraron diferencias significativas; los valores más altos se obtuvieron con grabado total y entre autoacondicionamiento y grabado selectivo no hubo significancia. Los adhesivos evidenciaron comportamientos similares sin registrar diferencias significativas. Los mayores espesores de capa fueron con grabado total sin diferencias significativas entre las técnicas.*

Palabras clave: adhesivos dentales - microscopía confocal

INTRODUCTION

Universal dental adhesives were introduced as versatile multifunctional systems with fewer application steps, compatible with all treatment modalities for mineralized dental tissues[1]. Universal adhesives, also known as multimode or multipurpose adhesives, have been used in clinical practice since 2011. They can be applied to enamel and dentin as self-conditioning adhesives, and to enamel as etch-rinse adhesives, a technique known as "selective enamel etching". The acid monomers in their composition act as primers for non-dental substrates (alloys and polycrystalline ceramics), making universal adhesives suitable for application with different bonding strategies[2,3]. These adhesives have been recently called eighth-generation adhesives, based on the historical perspective of dental adhesives[4].

Universal adhesives contain mixed monomers with slight to moderate acidity in reduced concentrations, conventional dimethacrylate cross-linkers, non-acid emulsifying monomers, catalyzer for light and dual curing and an adequate selection of solvents to improve monomer spreading and substrate infiltration capacity[2,5].

An adhesive that may be used in different procedures enables the dentist to choose the technique that best suits the clinical case, thereby optimizing the final restorative result. For instance, when a restoration requires a strong bond to the enamel, or in case of sclerotic dentin, it may be advisable to perform previous etching. The etching stage can be graded according to the time for which the phosphoric acid gel is applied before rinsing. On the other hand, it may be preferable to use the self-conditioning technique when dealing with difficult surgical access, limited time, or non-collaborative very young patients[6].

Even though it has been scientifically documented that the etching technique using phosphoric acid enhances one-step self-conditioning bonding to enamel, it is advisable to take more care with the etching procedure using additional phosphoric acid on dentin[7]. This is probably the main reason why most universal adhesive contain 10-methacryloxydecyl dihydrogen phosphate (10-MDP), a functional group of phosphoric acid, as their main adhesive monomer which produces a limited decalcification effect on the dentin surface. This procedure is considered the most reliable treatment for dentin, since the universal adhesives containing MDP have slight acidity and the capacity to interact chemically with hydroxyapatite crystals and enable stable salt formation of calcium phosphate and calcium carboxylate insoluble in water[8,9].

This new philosophy of versatile bonding encourages the use of the simplest option for each strategy, i.e., one-step self-conditioning or two-step etch and rinse, in order to bond direct or indirect restorations to enamel and dentin[10]. The universal etch and rinse (E&R) bonding mode involves a phosphoric acid etching step followed by a thorough water rinsing phase prior to application of a primer/adhesive resin combination. Monomers diffuse into the micro-etch pits created on the enamel to form microtags and macrotags, and into the exposed collagen fibril network at dentin to form a 3-5 μm hybrid layer. While the E&R bonding mode is undoubtedly the best bonding strategy to enamel, the resultant thick and HAp-free hybrid layer formed on the dentin is highly sensitive to degradation over time. The universal self-etch (SE) bonding mode involves the use of monomers with an acidic functional group that in principle simultaneously etches and infiltrates dentin down to a depth of about 1 μm. In general, the SE bonding mode underperforms the E&R bonding mode on enamel, by which enamel remains to be selectively etched with phosphoric acid. SE bonding nevertheless possesses chemical bonding potential as an additional benefit to achieve durable bonding[4]. However, universal bonding systems may show the deficiencies of their predecessors, i.e., one-step systems[11], so their bonding performance to dentin should be evaluated using different variables that modify in-vitro bond strength[12]. In this context, the purpose of the present study was to use shear bond strength and laser microscopy tests to determine the bonding characteristics of universal adhesives using total enamel and dentin etching treatments, adamantine selective etching and self-conditioning on the pulp wall of deep cavity preparations.

MATERIALS AND METHOD

This study was approved by the Academic Committee for Health Research (*Comité Académico de Investigaciones en Salud*) of the School of Dentistry, National University of Cordoba (CIEIS-ODO-CASI 48 I).

Cavity preparation

Single occlusal cavities 4 mm deep and maximum

width were carved in 36 third molars which had been extracted for orthodontic reasons. Cavity depth standardization was checked using a periodontal millimeter probe, CP-11, Hu-Friedy, Illinois, United States. The molars were divided into nine groups according to dentin substrate treatment (total etching, selective adamantine etching or self-conditioning), and the universal adhesive applied. **Group 1:** Total-etch+Monobond 7 self-etch adhesive (Densell, Buenos Aires, Argentina). **Group 2:** Total-etch+One coat 7 universal adhesive (Coltene, Altstätten, Switzerland). **Group 3:** Total-etch+Single bond universal adhesive (3M ESPE, Neuss, Germany). **Group 4:** Adamantine etching+Monobond 7 self-etch adhesive (Densell, Buenos Aires, Argentina). **Group 5:** Adamantine etching+One coat 7 universal adhesive (Coltene, Altstätten, Switzerland). **Group 6:** Adamantine etching+Single bond universal adhesive (3M ESPE, Neuss, Germany). **Group 7:** Monobond 7 self-etch adhesive (Densell, Buenos Aires, Argentina). **Group 8:** One coat 7 universal adhesive (Coltene, Altstätten, Switzerland). **Group 9:** Single bond universal adhesive (3M ESPE, Neuss, Germany). In groups 1, 2 and 3, total-etch was performed on enamel and dentin using 35% phosphoric acid (Densell, Buenos Aires, Argentina) for 10 s, followed by spray washing and drying for 5 s. Finally, universal bonding was applied according to the manufacturer's instructions. In groups 4, 5 and 6, enamel was etched using 35% phosphoric acid (Densell, Buenos Aires, Argentina) for 10 s. In groups 7, 8 and 9, universal adhesives were applied directly on-to the enamel and dentin as indicated by the manufacturer.

All the cavities were filled with submicron-hybrid composite resin Brilliant EverGlow (Coltene, Altstätten, Switzerland), by means of oblique incremental technique, and each layer was polymerized for 20 s with a LED unit (Optilux LED, Coltene, Altstätten, Switzerland).

Shear bond strength

Three specimens from each group (27 altogether) were first sectioned longitudinally in lingual-buccal direction into sections 2.5 mm and 3 mm thick on average; then transversally, using a hard tissue microtome Isomet (Buehler Co., Illinois, United States) at 300 rpm and 50 grams pressure, under continuous water cooling. Two test specimens averaging 2.6 mm wide by 2.6 mm high were thus

formed, which included dentin pulp wall, universal adhesive system and composite resin. Thus, each group consisted of six test specimens (n=6). These samples were stored at 37 ºC for 24 h in a 100% humidity atmosphere.

Shear bond strength tests were performed using a universal testing machine with a crosshead speed of 0.5 mm per minute. The sections were fixed with a dental press and placed on the plate of the device such that the composite resin/dentin pulp wall union remained next to the edge. The beveled edge of the shear was placed at 0.5 mm from the material/substrate joint for cutting. A standard 0.5 mm thick layer was used to corroborate the separation. Analysis of variance was applied to determine the efficacy of the substrate treatment with each universal adhesive used.

Laser confocal microscopy study

One specimen from each group (9 altogether) was sectioned longitudinally using a hard tissue microtome Isomet (Buehler Co., Illinois, United States) at 300 rpm and 50 g pressure under continuous water cooling, to obtain test specimens 1.5 mm to 2 mm thick for each group (n=3 for each group). The sections were polished using a metallographic polisher (Praxis, Buenos Aires, Argentina) with tapered granulometry disks and felt cloths, washed with ultrasound and stored in a stove at 37 ºC for 24 h in a 100% humidity atmosphere. The sections were observed with laser confocal microscopy, Lext 3D Measuring Laser Microscope OLS4000 (Olympus, Japan). The thicknesses of the adhesive layer on the deep pulp walls were measured using the same software. Ten measurements were performed by microphotography at regular intervals on 50 μm long paths (Fig. 1). The data were analyzed using analysis of variance. Statistical significance was set at 0.05 for all tests.

RESULTS
Shear bond strength

Considering each adhesive with its respective treatment, shear bond strength was significantly higher (p=0.024) for One coat 7 universal using total-etch procedure than for any of the other three treatments performed on the dental substrate. Monobond 7 self-etch had the highest values with selective enamel etching, with no significant difference (p=0.384). Single bond universal with
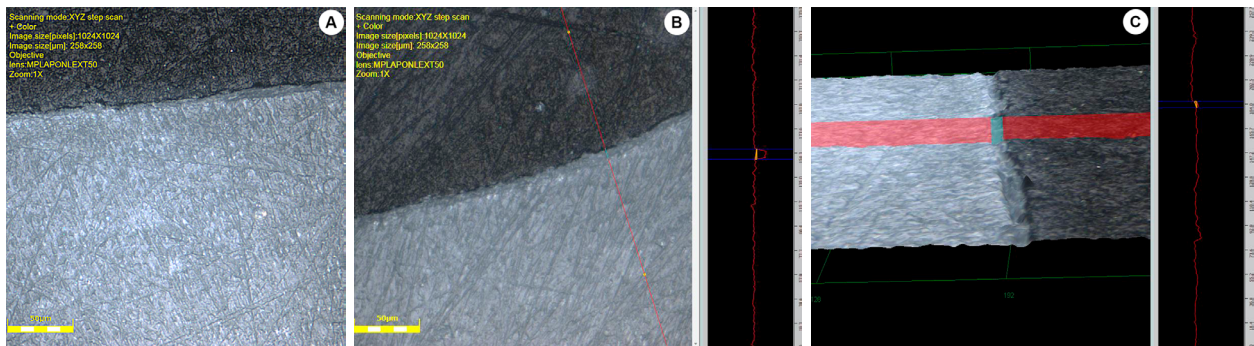
Fig. 1: *B and C show profile sections that were measured.* **A)** *Group 1: Simultaneous etching technique of enamel and dentin and application of Universal Adhesive Monobond 7 self-etch. Mean layer thickness between the pulp wall and the restoration material was 8.71±4.93 µm.* **B)** *Group 5: Application of One Coat Universal Adhesive by means of selective enamel etching. Mean layer thickness was 5.49±1.70 µm, and* **C)** *Application of Single Bond Universal Adhesive through self-etch mode. Mean layer thickness was 6.27±3.01 µm.*

total-etch protocol had higher values than the other treatments, with no statistically significant difference (p=0.299) (Fig. 2).

In general, bond strength for the total-etch treatment (8.61±3.96) was significantly greater than for the other treatments: selective enamel etching (6.71±1.98) and self-conditioning (6.91±2.68); p=0.049 (Fig. 3).

There was no statistically significant difference (p=0.205) among the different universal adhesives used. One coat 7 universal (8.33±3.45) mean values were higher than those of the other universal adhesives, Single bond universal (7.38±3.33) and Monobond 7 self-etch (6.75±2.47) (Fig. 4).

Laser confocal microscopy analysis: Adhesive layer thickness

Adhesive layer thicknesses were greatest for the total-etch treatment group, followed by self-conditioning, and lowest for the selective enamel etching treatment group. There was no significant difference among the different adhesive techniques (Fig. 5).

DISCUSSION

The present study found that certain in-vitro conditions such as deep pulp wall and different universal adhesive bonding approaches determined low bonding values expressed in MPa in the shear bond strength tests when compared to those obtained,
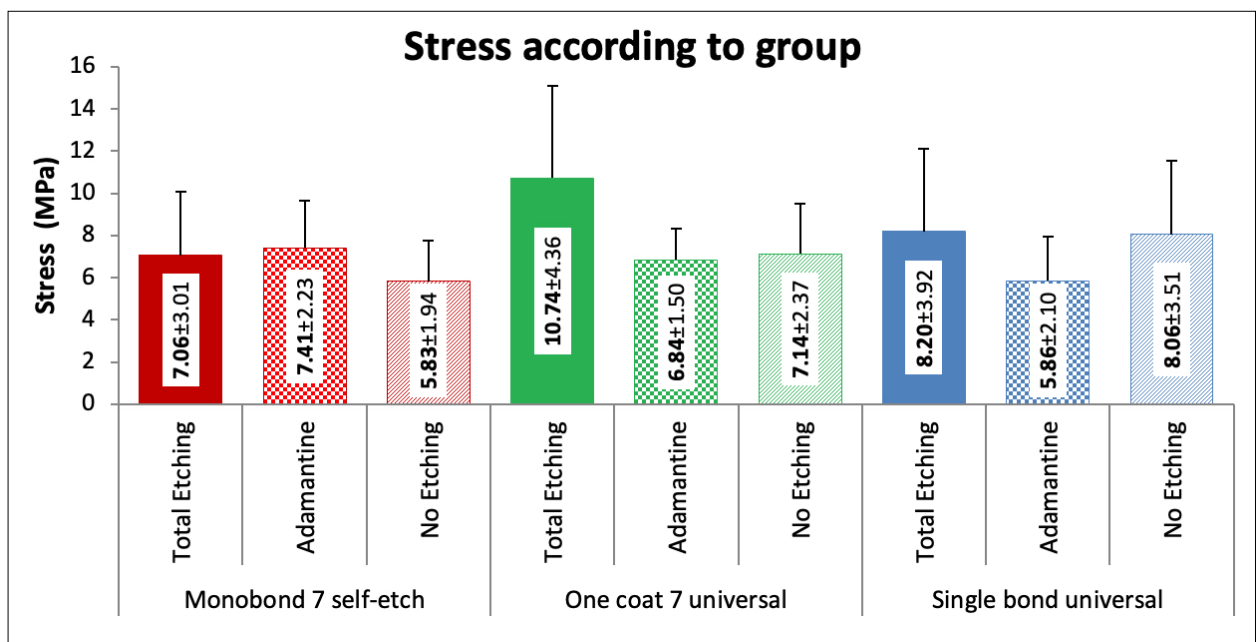

Fig. 2: *Shear stress according to adhesive and technique used. Mean and standard deviation values expressed in MPa.*
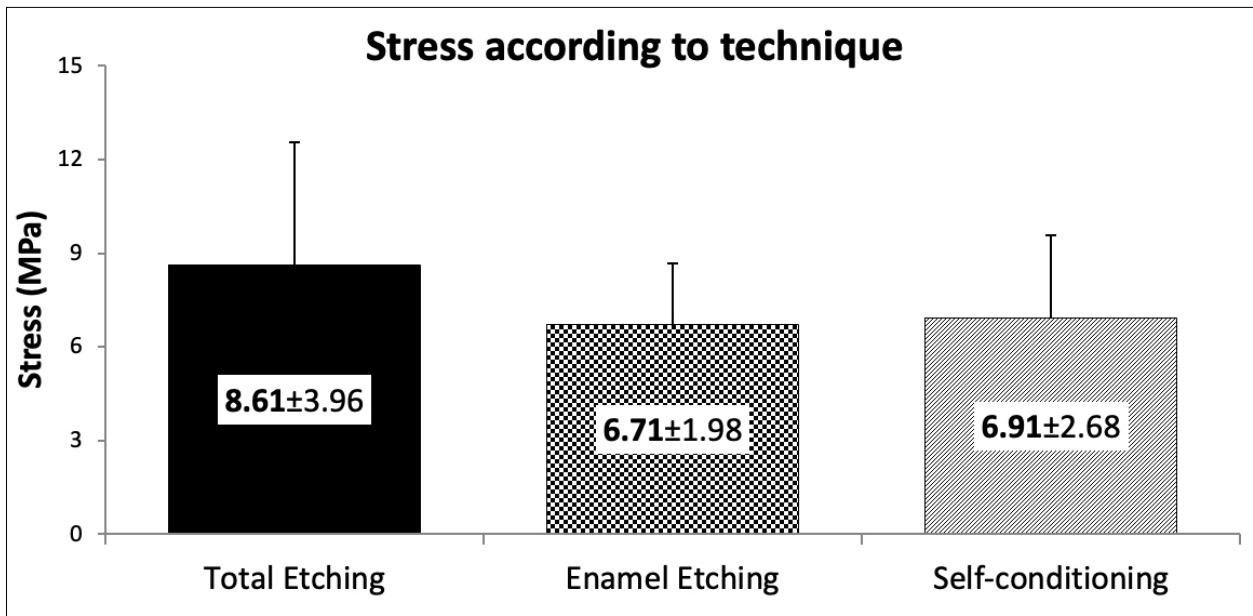
Stress according to technique

Fig. 3: *Shear stress according to technique. Mean and standard deviation values expressed in MPa.*

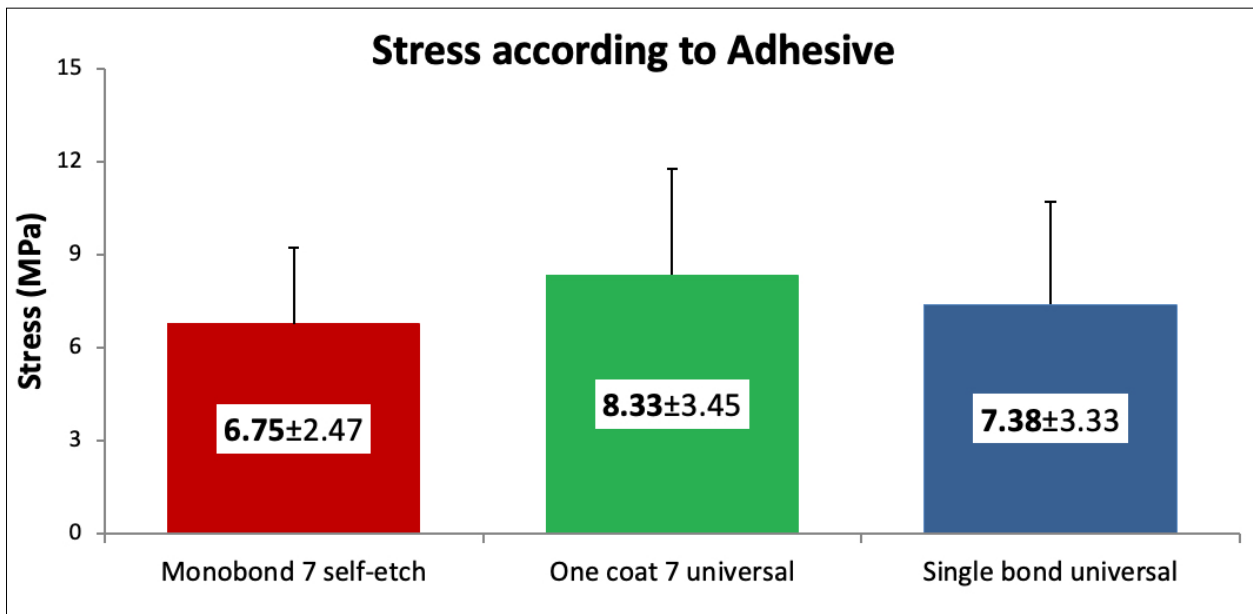## Stress according to Adhesive

Fig. 4: *Shear stress according to universal adhesive. Mean and standard deviation values expressed in MPa.*

for example, for lateral, median or superficial walls[13]. This means that cavity depth and wall type affect the results of the laboratory tests, regardless of the bonding approach chosen. We agree that it is more difficult to achieve bonding to the dentin closer to the pulp chamber roof than to the superficial dentin[4]. Adhesion to dentin remains a challenge, mainly because dentin has a more organic composition than enamel, and its wet, organic nature makes bonding difficult[13]. When dentin is etched, the acid demineralizes the intertubular dentin, resulting in the exposure of the superficial collagen network[14].

The network is infiltrated with the adhesive resin, leading to the formation of a hybrid layer which is responsible for the bond between the resin and the dentinal tissues[15]. To ensure ideal bonding conditions, the dentin demineralized with acid must be kept wet to prevent the collagen fibrils from collapsing, though not too wet, because over-wetting prevents resin impregnation of the collagen fibrils[16]. Smear layer removal with single-step etching before the dentin adhesive is applied (etch-rinse technique), or its modification with a self-etch monomer (self-conditioning technique) is crucial to form a hybrid
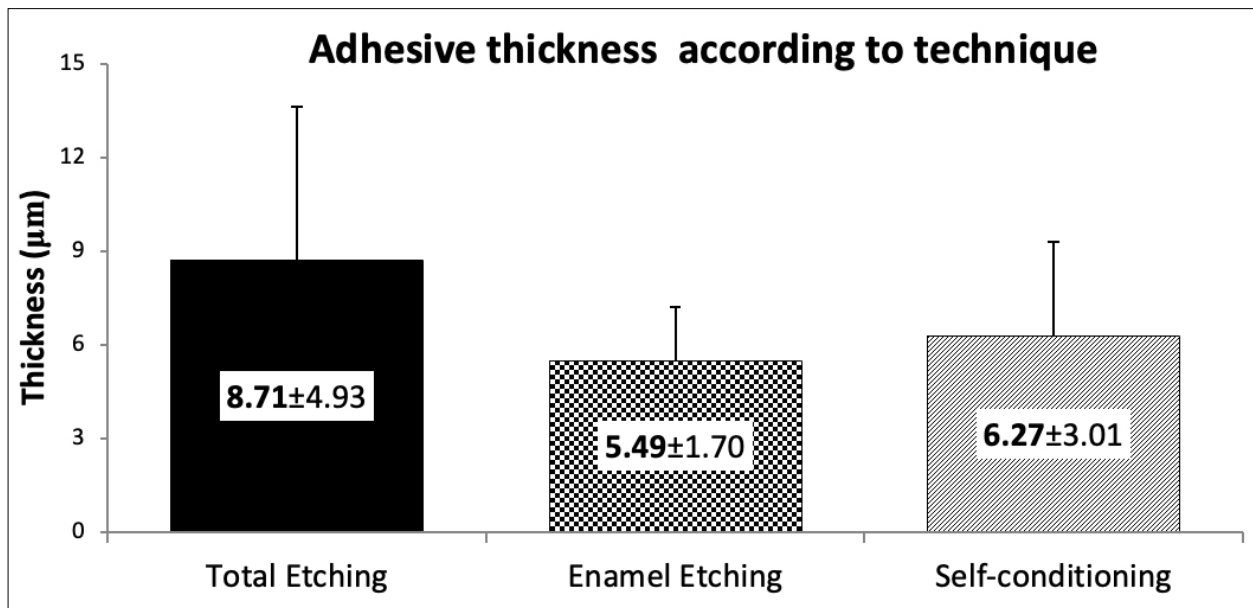
Fig. 5: *Adhesive layer thickness according to technique. Mean and standard deviation values expressed in μm.*

layer in order to ensure an effective bond between the adhesive resin and the dentin[17].

The present study found higher values for total-etch treatments in deep dentin than for the other two methods (selective etching and self-conditioning). However, during the total conditioning step, phosphoric acid eliminates the smear layer while demineralizing the dentin to a depth of 3 to 5 mm, thus exposing a collagen fibril scaffold deprived of hydroxyapatite[18,19]. The highly mineralized peritubular dentin dissolves almost completely and the dentin tubules widen. However, the literature reports that no statistically significant difference was recorded in the dentin regarding bonding efficacy when self-etch or etch-rinse approaches were used[2]. It has been suggested that the bonding techniques requiring smear layer removal are associated with greater postoperative sensitivity than systems that leave the smear layer in situ[20]. Even though it has been demonstrated that the bonding procedure may cause transitory pulp inflammation, especially in the deep cavities, it is likely that continuous bacterial irritation due to microgaps and microfiltration may cause damage to the pulp and postoperative pain[21].

Shear bond strength is a valid versatile method for assessing bonding effectiveness to dental substrates in laboratory tests[22]. The formation of smear layer in vitro has also been shown in laboratory tests. Chowdhury et al. assessed the effects of the smear layers formed by abrasives having similar coarseness to the fine-grit diamond stone. They established a model for dentin bonding tests, yielding clinically relevant significant results. They also performed micro-tensile bond strength tests (μTBS) of currently available universal adhesives and of a two-step self-etch adhesive. The presence of smear layer formed under these conditions had no significant effect on the resin-dentin bond strength of the adhesives tested. Moreover, the performance of the bond of the universal adhesives to the dentin may be improved duplicating its application time. The elimination or modification of the smear layers covering the dentin is critical to allow penetration of the adhesive molecules and to ensure a strong bond between the resin and the dentin[23]. Universal adhesives benefit from phosphoric acid etching because bond strengths increase, mainly on the enamel surface. The authors compared in vitro shear strength of four universal adhesives on enamel and dentin with and without additional phosphoric acid etching, finding mean bond strength values to enamel ranging from 13.4 and 21.9 MPa in the self-etch mode. When the etch-rinse protocol was used, the mean bond strength was over 30 MPa. Regarding the dentin, the significant differences in the self-etch mode depended on each adhesive used[24]. Stape et al. claim that selective dentine etching for 3 s improved the interaction depth of the tested universal adhesive without overexposing the demineralized collagen or reducing Ca availability

at the bonded interface. Nevertheless, the universal adhesives used in the self-etch mode produce superior long-term dentin bonding compared to the etch and rinse mode. Selective etching for 3 s with conventionally used H_3PO_4 improves dentin bonding effectiveness; nonetheless, longer etching times should be strictly avoided[25]. With regard to the self-conditioning protocol, Tsujimoto et al. compared universal adhesives in self-conditioning mode and two-step self-conditioning adhesives by means of initial shear bond strength tests and shear-fatigue strength test, at the level of the resin composite/ adhesive bond to dentin. Their results encourage the continued use of the two-step self-etch adhesive over some universal adhesives but suggest that changes to the composition of universal adhesives may lead to dentin bond fatigue durability similar to that of two-step self-etch adhesives[26]. Daneshkazemi et al. reported mean values in MPa between 35.74 and 18.09 in superficial dentin in micro-tensile with two adhesive protocols, self-etch and etch-rinse. Universal adhesives had the highest adhesive values considered as independent etching[27]. Lezaja Zebic et al. conducted microtensile bond strength tests of universal adhesives applied to dentin following total etch or self-etch protocols, direct or indirect water storing, using pulpal pressure simulation. Adhesives were applied to class 1 cavities and to mid-coronal dentin. Results obtained following the self-etch protocol were more stable in the long term than with total-etch protocol. Simulated pulpal pressure and Class 1 preparation may be recommended for adhesive strength tests. The values obtained were in the range of 19-42 MPa initially and 16-36 MPa after 6 months storage[28].

Concerning the effect of the chemical composition of the adhesives on the bond strength with dental substrates, Papadogiannis et al. performed bond strength tests of universal adhesives based on the adhesive monomer 10-MDP. Adhesive monomer, the inclusion of different comonomers (reticulating or bond promotors) catalyzers and solvents led to great variations in the properties of the adhesive film, thus affecting its reactivity with dentin and later its bond strength. By using infrared reflectance microscopy, the authors confirmed that the dentin surfaces treated with universal adhesives did not have a smear layer. Moreover, microscopic images exhibited gaps and porosities[29].

By using an electron microscope, Zhang et al. observed collagen degradation not seen on hybrid layers created by adhesives containing 10-MDP with the etch-rinse mode, which produced collagen fibers that were partially degraded with intact periphery[30]. In line with this, the results reported by Zecin-Deren et al. are attributed to the fact that the adhesive used contains 10-MDP as adhesive monomer in its chemical composition. In bond strength tests, these authors found higher mean values with Single Bond Universal than with other adhesives used in their study[31]. This phosphoric acid functional group also contains a polymerizable methacrylate group responsible for the curing potential, and a group of 10-carbon chain to separate the other two active groups[32].

The carbon separator affects the monomer flexibility, solubility, moisturizing, and hydrophilic-hydrophobic balance[33]. In order to further improve bond strength to dentin of Single Bond Universal, it may be advisable to apply it two or three times and to polymerize it after the final application[31].

Taking dentin depth and conditioning mode as variables, Yousry et al. reported that using etch-rinse adhesives, the shear bond strength values in superficial dentin were significantly higher than in deep dentin. Unlike the results obtained with the self-etch-systems, the performance in both dentins was similar. The authors concluded that bond strength to dentin depends on both the adhesive and the substrate. Contemporary adhesive systems may produce variable bond results to superficial and deep dentin owing to variations in their composition rather than to their application technique[34].

Similarly, Yoshihara et al. concluded that the 10-MDP monomer in high purity is essential to achieve long-lasting bonding, excellent hybridization with 10-MDP-Ca salts, and nanolayering. They suggested that the highest bond effectivity of 10-MDP-based adhesives reported are not only attributed to a stronger 10-MDP chemical bond, but also to higher etching potential[35].

Rosa et al. conducted a systematic review of 10 articles to determine whether etch-rinse mode or self-etch mode is the better protocol for enamel and dentin bonding by universal adhesives. The in vitro studies analyzed the bond strength of universal adhesives to dentine and/or enamel through the etch-rinse and self-etching strategies. They concluded that the bond strength to enamel of universal adhesives is enhanced by previous etching with phosphoric

acid. However, this effect was not evident on dentin with the use of mild universal adhesives with etch-rinse differences strategy. No statistically significant difference was found between etch-rinse and self-etch for mild universal adhesives[36].

Regarding the different protocols in which the universal adhesives can be used and the way these modes may affect and modify dentin wetness, Kumagai et al. claimed that universal adhesives may be applied with either self-etch or etch-rinse modes. Nevertheless, universal adhesives should be strictly applied to wet dentin for bonding in the etch-rinse mode. They observed a well-formed hybrid layer on wet dentin, in contrast to defects, pores and reduced hybridization thickness when the adhesives were applied to over-dried dentin[37].

Choi et al. analyzed wetness on the dentin surface as a factor affecting the micro-tensile bond strength of universal adhesives. They suggest that the wetness of the dentin surface should be carefully controlled with special consideration for the application of universal adhesives[38]. Sugimura et al. published that some universal adhesives, with the addition of specific components and water content optimization, can achieve stable bonds irrespective of surface wetness, though they agreed that the moisture of the dentin surface is an important factor for universal adhesive bonding in the etch-rinse mode. In addition, dentin surface wetness did not influence the thickness of the adhesive layer or hybrid layer of the dentine-resin interfaces[39].

In the present study, laser microscopy measurements showed that the adhesive layer was thicker in the total-etch treatment group (8.71 μm), and thinner in both the self-etch approach (6.27 μm) and selective etching procedure in enamel (5.49 μm). There were no significant differences among the adhesive techniques proposed. These values agree with a publication which reports that the etch-rinse approach increases hybridization thickness (4 to 6 μm)[4]. Chen et al. observed hybrid layers of universal adhesives ~5 μm and <0.5 μm thick in the etch-rinse mode and self-conditioning mode[5]. By using electron microscopy, Takamizawa et al. observed similar adhesive layer thicknesses for single-step universal adhesives and self-conditioning adhesives[40].

Universal adhesives reflect manufacturers' efforts to provide versatility in product design by adapting a single bottle self-etch adhesive to other application modes, without compromising its bonding effectivity[37]. These adhesives may be applied in simplified clinical steps, are less technique-sensitive, require shorter application times, and cause less postoperative sensitivity. The present study showed that the highest values were obtained with the total etching mode. Moreover, if this protocol were used in deep dentin, universal adhesives would not fulfill the premise that they cause little or no postoperative sensitivity, a highly relevant clinical factor in selecting the best technique for dentinal substrate treatment.

In conclusion, in the present study, shear bond strength using various protocols for activation of deep dentine substrates differed significantly among the three conditioning procedures. The total etching treatment or total removal of the smear layer yielded the highest bond strength values analyzed in the deep pulp wall. The bonding performance of universal adhesives applied with self-conditioning approach was similar to that of selective enamel etching protocols. In general, bond strength values did not differ significantly among self-etch Monobond 7, One coat 7 universal and Single bond universal. The bond layer was thickest in the total etching treatment, without significant differences among the three bonding techniques applied. Regardless of the different results obtained in laboratory tests, it is the dentist who should decide on the most appropriate mode for the various clinical situations, especially when the deep dentin wall is involved.

REFERENCES

1. Nagarkar S, Theis-Mahon N, Perdigão J. Universal dental adhesives: Current status, laboratory testing, and clinical performance. J Biomed Mater Res B Appl Biomater. 2019;107:2121-2131. https://doi.org/10.1002/jbm.b.34305

2. Hanabusa M, Mine A, Kuboki T, Momoi Y et al. Bonding effectiveness of a new "multi-mode" adhesive to enamel and dentine. J Dent. 2012;40(6):475-484. https://doi.org/10.12691/ijdsr-6-5-3

3. Perdigão J, Sezinando A, Monteiro PC. Laboratory bonding ability of a multi-purpose dentin adhesive. Am J Dent. 2012; 25:153-158.

4. Van Meerbeek B, Yoshihara K, Van Landuyt K, Yoshida Y et al. From Buonocore's pioneering acid-etch technique to self-adhering restoratives. A status perspective of rapidly advancing dental adhesive technology. J Adhes Dent. 2020;22(1):7-34. https://doi.org/10.3290/j.jad.a43994

5. Chen C, Niu LN, Xie H, Zhang ZY et al. Bonding of universal adhesives to dentine-Old wine in new bottles? J Dent. 2015;43(5):525-536. https://doi.org/10.1016/j.jdent.2015.03.004

6. Sofan E, Sofan A, Palaia G, Tenore G et al. Classification review of dental adhesive systems: from the IV generation to the universal type. Ann Stomatol (Roma). 2017;8(1):1-17.

7. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A et al. State of the art of self-etch adhesives. Dent Mater. 2011;27:17-28. https://doi10.1016/J.dental.2010.10.023

8. Wang R, Shi Y, Pan Y, Cui Y et al. Adhesive interfacial characteristics and the related bonding performance of four self-etching adhesives with different functional monomers applied to dentin. J Dent. 2017;62:72-80. https://doi.org/10.1016/j.jdent.2017.05.010

9. Carrilho E, Cardoso M, Marques Ferreira M, Marto CM et al. 10-MDP based dental adhesives: Adhesive interface characterization and adhesive stability-A systematic review. Materials. 2019;12(5):790. https://doi.org/10.3390/ma12050790

10. Muñoz MA, Luque I, Hass V, Reis A et al. Immediate bonding properties of universal adhesives to dentine. J Dent. 2013;41(5):404-411. https://doi.org/10.1016/j.jdent.2013.03.001

11. Zhang ZY, Tian FC, Niu LN, Ochala K et al. Defying ageing: An expectation for dentine bonding with universal adhesives? J Dent. 2016;45:43-52. https://doi.org/10.1016/j.jdent.2015.11.008

12. Miyazaki K, Tsubota K, Takamizawa T, Kurokawa TH et al. Factors affecting the in vitro performance of dentin-bonding systems. Japanese Dental Science Review. 2012;48:53-60. https://doi.org/10.1016/j.jdsr.2011.11.002

13. Perdigão J. Dentin bonding-variables related to the clinical situation and the substrate treatment. Dent Mater. 2010;26(2):e24-37. https://doi.org/10.1016/j.dental.2009.11.149

14. Lapinska B, Klimek L, Sokolowski J, Lukomska-Szymanska M. Dentine surface morphology after chlorhexidine application-SEM study. Polymers (Basel). 2018; 10(8):905. https://doi.org/10.3390/polym10080905

15. Lukomska-Szymanska M, Sokolowski J, Lapinska B. Degradation of a hybrid layer-Review of literature. J Stoma. 2017;70(1):88-94.

16. Pashley DH, Tay FR, Breschi L, Tjäderhane L et al. State of the art etch-and-rinse adhesives. Dent Mater. 2011;27(1):1-16. https://doi.org/10.1016/j.dental.2010.10.016

17. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28(3):215-235. PMID: 12760693.

18. Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P et al. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res. 1992;71(8):1530-1540. https://doi.org/10.1177/00220345920710081301

19. Ferrari M, Goracci G, García-Godoy F. Bonding mechanism of three "one-bottle" systems to conditioned and unconditioned enamel and dentin. Am J Dent. 1997; 10(5):224-230.

20. Unemori M, Matsuya Y, Akashi A, Goto Y et al. Composite resin restoration and postoperative sensitivity: clinical follow-up in an undergraduate program. J Dent. 2001;29(1):7-13. https://doi.org/10.1016/S0300-5712(00)00037-3

21. Camps J, Déjou J, Rémusat M, About I. Factors influencing pulpal response to cavity restorations. Dent Mater. 2000;16(6):432-440. https://doi.org/10.1016/S0109-5641(00)00041-5

22. De Munck J, Luehrs AK, Poitevin A, Van Ende A et al. Fracture toughness versus micro-tensile bond strength testing of adhesive-dentin interfaces. Dent Mater. 2013;29(6):635-44. https://doi.org/10.1016/j.dental.2013.03.010

23. Chowdhury A, Islam R, Alam A, Matsumoto M et al. Variable smear layer and adhesive application: The pursuit of clinical relevance in bond strength testing. Int J Mol Sci. 2019;20(21):5381. https://doi.org/10.3390/ijms20215381

24. Jacker-Guhr S, Sander J, Luehrs AK. How "Universal" is adhesion? Shear bond strength of multi-mode adhesives to enamel and dentin. J Adhes Dent. 2019;21(1):87-95. https://DOI: 10.3290/j.jad.a41974

25. Stape THS, Wik P, Mutluay MM, Al-Ani AAS et al. Selective dentin etching: A potential method to improve bonding effectiveness of universal adhesives. J Mech Behav Biomed Mater. 2018;86:14-22. https://doi.org/10.1016/j.jmbbm.2018.06.015

26. Tsujimoto A, Barkmeier WW, Takamaizawa T, Watanabe H et al. Comparison between universal adhesives and two-step self etch adhesives in terms of dentin bond fatigue durability in self-etch mode. Eur J Oral Sci. 2017;125(3):215-222. https://doi10.1111/eos.12346

27. Daneshkazemi P, Ghasemi A, Daneshkazemi A, Shafiee F. Evaluation of micro shear bonding strength of two universal dentin bondings to superficial dentin by self-etch and etch-and-rinse strategies. J Clin Exp Dent. 2018;10(9):e837-e843. https://doi.org/10.4317/jced.54740

28. Lezaja Zebic M, Dzeletovic B, Miletic V. Microtensile bond strength of universal adhesives to flat versus class I cavity dentin with pulpal pressure simulation. J Esthet Restor Dent. 2018;30(3):240-248. https://doi.org/10.1111/jerd.12363

29. Papadogiannis D, Dimitriadi M, Zafiropoulou M, Gaintantzopoulou MD et al. Universal adhesives: Setting characteristics and reactivity with dentin. Materials (Basel). 2019;12(10):1720. https://doi.org/10.3390/ma12101720

30. Zhang ZY, Tian FC, Niu LN, Ochala K et al. Defying ageing: An expectation for dentine bonding with universal adhesives? J Dent. 2016;45:43-52. https://doi.org/10.1016/j.jdent.2015.11.008

31. Zecin-Deren A, Sokolowski J, Szczesio-Wlodarczyk A, Piwonski I et al. Multi-layer application of self-etch and universal adhesives and the effect on dentin bond strength. Molecules. 2019;24(2):345. https://doi10.3390/molecules24020345

32. Van Meerbeek B, Peumans M, Poitevin A, Mine A et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater. 2010;26(2):e100–e121. https://

doi10.1016/j.dental.2009.11.148

33. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007;28(26):3757-3785. https://doi.org/10.1016/j.biomaterials.2007.04.044

34. Yousry MM, ElNaga AA, Hafez RM, El-Badrawy W. Microshear bond strength and interfacial morphology of etch-and-rinse and self-etch adhesive systems to superficial and deep dentin. Quintessence Int. 2011;42(9):e96-e106.

35. Yoshihara K, Hayakawa S, Nagoaka N, Okihara T et al. Etching efficacy of self-etching functional monomers. J Dent Res. 2018;97(9):1010-1016. https://doi.org/10.1016/j.jdent.2015.04.003

36. Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: A systematic review and meta-analysis. J Dent. 2015;43:765-776. https://doi.org/10.1016/j.jdent.2015.04.003

37. Kumagai RY, Hirata R, Pereira PNR, Reis AF. Moist vs over-dried etched dentin: FE-SEM/TEM and bond strength evaluation of resin-dentin interfaces produced by universal adhesives. J Esthet Restor Dent. 2020;32(3):325-332. https://doi.org/10.1111/jerd.12537

38. Choi AN, Lee JH, Son SA, Jung KH et al. Effect of dentin wetness on the bond strength of universal adhesives. Materials (Basel). 2017;10(11):1224. https://doi.org/10.3390/ma10111224

39. Sugimura R, Tsujimoto A, Hosoya Y, Fischer NG et al. Surface moisture influence on etch-and-rinse universal adhesive bonding. Am J Dent. 2019;32(1):33-38.

40. Takamizawa T, Imai A, Hirokane E, Tsujimoto A et al. SEM observation of novel characteristic of the dentin bond interfaces of universal adhesives. Dent Mater. 2019;35(12):1791-1804. https://doi.org/10.1016/j.dental.2019.10.006