ORIGINAL ARTICLE

Universal adhesive: the effect of different simulated pulpal pressure fluids and bonding modes to dentin

Lucélia Lemes Gonçalves[1] · Tânia Mara Da Silva[1] · Anuradha Prakki[2] · Daphne Camara Barcellos[3] ·
Taciana Marco Ferraz Caneppele[4] · Hueder Paulo Moisés De Oliveira[5] · Sérgio Eduardo de Paiva Gonçalves[4,6]

Abstract

The aim of this study was to evaluate the effect of SPP with either fetal bovine serum (FBS) or deionized water (DW) on the bond strength (µTBS) of a Universal adhesive to dentin, in both etch-and-rinse (ER) and self-etch (SE) modes. The kinematic viscosity (cSt) of FBS and DW was measured at 25 °C ± 0.1 °C. Seventy-two sound human molars were sectioned and randomly divided into three groups according to the SPP conditions: (1) Control (0 cm H_2O), (2) SPP (15 cm H_2O) with FBS, (3) SPP (15 cm H_2O) with DW. Each group was subdivided ($n = 10$) based on the bonding modes: ER (37% phosphoric acid + ScothBond Universal Adhesive) or SE (ScothBond Universal Adhesive). Samples were then submitted to µTBS. Data were analyzed by Student's t test, two-way ANOVA and Tukey tests ($p < 0.05$). The cSt results showed that DW (23.59 ± 0.39) had significantly higher values than FBS (22.33 ± 0.06). With regard to SPP, the control group (36.1 MPa) had significantly higher values of µTBS when compared to the SPP using FBS (31.06 MPa) and SPP with DW (26.55 MPa). According to ANOVA, the bonding modes and the interaction of simulated pulpal pressure (SPP) did not statistically influence the results ($p < 0.05$). The presence of SPP reduced the bond strength of Universal adhesive to dentin. DW during SPP had significantly reduced bonding values when compared to FBS. Bonding strategies were not affected by SPP when evaluated in a short period of time (24 h).

Keywords Dental pulp · Dental bonding · Adhesives

✉ Sérgio Eduardo de Paiva Gonçalves
  sergio.e.goncalves@unesp.br

  Lucélia Lemes Gonçalves
  dralucelialemes@hotmail.com

  Tânia Mara Da Silva
  taniamara.odonto@gmail.com

  Anuradha Prakki
  anuradha.prakki@dentistry.utoronto.ca

  Daphne Camara Barcellos
  daphnecbarcellos@hotmail.com

  Taciana Marco Ferraz Caneppele
  taciana.caneppele@unesp.br

  Hueder Paulo Moisés De Oliveira
  hueder.paulo@ufabc.edu.br

[1] Anhanguera Faculty of São José dos Campos,
   São José dos Campos, SP, Brazil

[2] Department of Clinical Sciences Restorative, Faculty
   of Dentistry, University of Toronto, Toronto, ON, Canada

[3] Department of Prosthodontics, Federal University of Espírito
   Santo, Vitória, ES, Brazil

[4] Department Restorative Dentistry, Institute of Science
   and Technology, UNESP–Univ Estadual Paulista,
   São José dos Campos, SP, Brazil

[5] Center for Natural and Human Sciences, Federal University
   of ABC, Santo André, SP, Brazil

[6] Departamento de Odontologia Restauradora, Instituto de
   Ciência e Tecnologia, UNESP–Univ Estadual Paulista,
   Campus de São José dos Campos, Av. Engenheiro Francisco
   José Longo, 777, Jardim São Dimas, São José dos Campos,
   SP 12245-000, Brazil

Introduction

After six decades since Buonocore [1] introduced the fundamental concepts for adhesion to enamel, several adhesives were developed. Nowadays, two main adhesive systems are available on dental market, the etch-and-rinse and self-etch modes [2]. In etch-and-rinse bonding, phosphoric acid etching removes the smear layer, demineralizes the dentin surface and exposes collagen fibrils to allow monomer resins infiltration leading to the formation of hybrid layer [3, 4]. However, during this process, dentin permeability and the hydraulic conductance increase upon removing the smear layer and opening of the dentinal tubules, which affects the degree of moisture on the etched dentin surface [5, 6]. On the other hand, in self-etch bonding demineralization and infiltration of adhesive into the substrate occurs simultaneously, without complete removal of the smear layer; hence, the dentin permeability and the hydraulic conductance do not increase [7].

The latest adhesive system combines the etchant, primer and adhesive in a single-bottle solution that can be used in both etch-and-rinse or self-etch [5, 8] bonding modes on dentin, and is known as "universal" or "multimode" adhesives. Universal adhesives distinguish from the classical 1-step self-etch adhesives because they contain acidic functional monomers, such as methacryloyloxydecyl dihydrogen phosphate (MDP) and polyalkenoic acid co-polymer in their composition [9]. The molecule MDP and polyalkenoate co-polymer are responsible for chemical bonding of the adhesive with dentin. They interact with calcium present in hydroxyapatite crystals, creating hydrolytically stable calcium salts at the interface with hydroxyapatite, termed 'hydroxyapatite nano-layering' [9–11].

Universal adhesives contain up to 20% water in its composition, which is required to ionize the respective monomers for classical self-etch mode [9]. Due to high hydrophilicity, studies suggested that 1-step self-etch adhesives behave as semi-permeable membrane allowing water diffusion even after polymerization, being ineffective in reducing dentin permeability during bonding procedures under intrapulpal pressure [12–15]; however, this assumption has not yet been confirmed to universal adhesives [9]. Nevertheless, adhesive permeability and pulpal fluid flow may all together hamper the achievement of durable bonds between substrate and adhesive, allowing subsequent degradation of the resin-dentin interface by hydrolysis. Though, it has also been speculated that universal adhesives present better tolerance to variations in dentin humidity (moist or dry) [9, 16], in which case SPP may not be so detrimental to dentin bond strength of universal adhesives.

Despite the fact that simulated intrapulpal pressure (SPP) test has been considered a relevant tool to assess the interaction between adhesive and dentin, the majority of studies in the literature have used in vitro tests with no SPP. Previous studies suggested that the dentin bond strength was reduced when SPP was applied during bonding procedure [12, 17–20].

Different fluids have been used to simulate dentin fluid flow, which have differently affected the bonding performance [15, 17, 20]. Although water is the most commonly fluid used [21], it cannot simulate the real features of the pulpal fluid [18]. Fetal bovine serum (FBS) maybe considered a more clinically relevant intrapulpal fluid due to the presence of proteins in its composition [14].

Thus, the aim of this "in vitro" study was to evaluate the effect of SPP with either FBS or deionized water (DW) in the bond strength (µTBS) of a universal adhesive to human dentin. SBU was evaluated in both etch-and-rinse and self-etch modes. The null hypotheses tested were: (I) different fluids used to SPP do not affect the bond strength of a universal adhesive to dentin; (II) different bonding modes do not affect the bond strength of a universal adhesive to dentin under SPP.

Materials and methods

Specimen preparation

Seventy-two sound human molars that were extracted for therapeutic reasons under approval of Local Institutional Review Board were used in this study (protocol #754.624). The teeth were cleaned using periodontal curettes and stored in DW at 4 °C for no more than 6 months after extraction [19].

The teeth were fixed in an acrylic cylindrical holder (2.5 cm/diameter and 2 cm/height) with dental wax and sectioned by cutting machine under water cooling (Labcut, Extec; Enfield, CT, USA). Two horizontal sections were performed: (1) parallel to the occlusal surface, to expose dentin; (2) 1 mm below the enamel-cementum junction, to separate the crown from the roots, which were discarded. The pulpal tissues were removed using curettes.

Dentin specimens were worn with 400-grit aluminum oxide abrasive disks (Extec Corp., CT, USA) in a polishing device (DP-10, Panambra, São Paulo, Brazil) under water cooling. A caliper (Otto-Arminger & Cia Ltda., Rio Grande do Sul, Brazil) was used to measure the thickness of the remaining dentin. Dentin specimens were standardized at approximately 2 mm thickness from the highest pulp horn [22]. Dentin surface was polished using 600-grit aluminum oxide abrasive disks (Extec Corp., CT, USA) in a polishing

device (DP-10, Panambra, São Paulo, Brazil) under water cooling to produce and standardize the smear layer.

The 72 specimens were randomly divided into three groups according to the SPP conditions: Absence SPP (control), SPP with FBS, and SPP with DW. The FBS solution (Gibco Lifetech, Sao Paulo, Brazil) was used in a 1:3 dilution ratio with physiological saline [18].

Kinematic viscosity of solutions (cSt)

To determine the cSt of the fluids used for SPP, the Ford Cup number 4 (Scientific, commercial Hipperquímica Ltda., Santo André, SP, Brazil) was used. The glass tank was completely filled with the solutions to be tested (Table 1) and cSt was calculated based on the flow time, in seconds (t), measured with a digital timer, using the following equation: (cSt) $v = 3.85$ (t -4.49). The time of disposal was assessed three times, with the liquid kept at a controlled temperature of 25 °C $\pm$ 0.1 °C.

Simulated intrapulpal pressure

Self-cured acrylic resin holders (Jet, Artigos Odontológico Clássico, São Paulo, Brazil) were built ($1.5 \times 1.5 \times 0.5$ cm) and two holes were drilled into these holders to allow fluid to flow inside the pulp chamber. SPP was achieved by positioning two hypodermic needles (0.7×10 mm) into the center of the acrylic holder, perpendicular to its base. The first needle was positioned so that its upper tip communicated with the pulp chamber; its lower tip was linked to a hydrostatic pressure device [21, 23]. The second needle was placed with its upper tip inside the pulp chamber; the lower tip was linked to silicone tubes enabling the fluid to flow toward the other reservoir. The hydrostatic pressure device had a reservoir filled with DW [23] or FBS, placed at 15 cm above the level of the pulp chamber [24]. Before simulating the intrapulpal pressure, DW or FBS was injected into the pulp chamber

to avoid air bubbles and to assure there was total filling by the liquids.

Restorative procedures

Each group was randomly divided into two subgroups ($n = 10$) based on the bonding modes: etch-and-rinse or self-etch. The ScotchBond Universal adhesive (3 M ESPE, Saint Paul, MN, USA) adhesive was applied according to the manufacturer's recommendations. Composite resin blocks (4 mm/diameter and 2 mm/height) were built up on the pre-treated dentin. All specimens were immersed in DW at 37 °C for 48 h prior to testing. Materials used in this study, chemical composition and manufacturers are presented in Table 1.

Microtensile bond strength

After 48 h, the specimens were sectioned into dentin-composite resin sticks (1 mm^2) suitable for µTBS, using a cutting machine (Labcut, Extec; Enfield, CT, USA) under water cooling. The sticks were stored for 24 h in individual and identified tubes (Eppendorf, São Paulo, Brazil), containing DW at 37 °C. Before the µTBS test, the exact dimensions of the beam sections were measured at the adhesive interface area with a digital caliper (Starret Industria e Comercio; Itu, SP, Brazil). Each stick was fixed with cyanoacrylate glue to a metal jig for conducting the mechanical test using a universal testing machine (EMIC DL-1000, Equipamentos e Sistemas Ltda., Paraná, Brazil) at a crosshead speed of 0.5 mm/min and a 10 kgf load cell. The µTBS values (MPa) from the beams of the same tooth sample were averaged and the mean bond strength was used as one unit for statistical analysis. The failure mode of each beam was classified as: Cohesive in dentin—Failure predominantly (about 75%) inside the dentin; Cohesive in resin—Failure predominantly (about 75%) inside the resin; Adhesive—Failure in the adhesive interface/dental structure or in the adhesive interface/

Table 1 Materials and their compositions

Material	Composition
Bovine Fetal Serum South America, Gibco, Lifetech, Saint Paul, Brazil. (210245 k)	Beta Globulin 35 g/100 mL, Gamma Globulin 125.62 µg/mL, Total Protein 4.17 g/100 mL, Hemoglobin 21.48 mg/100 mL, pH 6.4, Osmolarity 340 313 mOs/kg H2O, Albumin 2.21 g/100 mL, Alpha Globulin 1.61 g/100 mL
ADV®, New Odessa, Saint Paul, Brazil. (123010)	Physiologic solution of sodium chloride 0.9%
Fusion Duralink Orthophosphoric acid, Angelus, Londrina, Paraná, Brazil. (26824)	Orthophosphoric Acid, Water, Thickener and Pigments
Scotch Bond Universal 3 M ESPE, Sumaré, Saint Paul, Brazil. (507329)	15–25% BisGMA, 15–25% HEMA, 5–15% dimethacrylate resins, 5–15% silano, 1–10% monomer phosphate MDP, 1–5% Vitrebond™ Copolymer(polyalckenoic acid), 10–15% ethanol, 10–15% water, < 2% primers, in mass
Filtek Z350 XT, 3 M ESPE, Sumaré, Saint Paul, Brazil. (828241)	BisGMA, BisEMA UDMA, TEGDMA, PEGDMA, nanoparticles of zircônia/silica

composite resin, in more than 75% of the analyzed area; Mixed—Failure with no predominance greater than 75% of any type of failure [25].

Scanning electron microscopy (SEM)

Two specimens from each group were prepared for SEM analysis. The teeth were sectioned perpendicularly to the adhesive interface. The sections obtained were polished using 2400 and 4000-grit aluminum oxide abrasive disks (Extec Corp., CT, USA) in a polishing device (DP-10, Pan-ambra, São Paulo, Brazil) under water cooling. Then, the specimens were etched with 32% phosphoric acid without silica (32% Uni-Etch, Bisco) for 15 s and rinsed for 10 s. Next, specimens were dehydrated, sputter-coated with gold/palladium, and examined in a scanning electron microscope (Inspect S50, FEI, Hillsboro, Oregon, EUA) at 15 kV, using 2000× magnification [26].
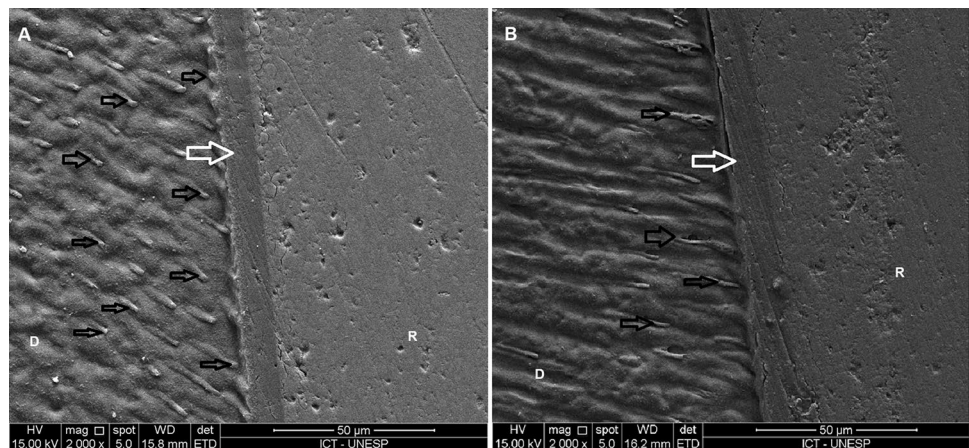
Statistical analysis

Mean cSt values were analyzed by Student t Test. For µTBS, specimens with cohesive and/or premature failures were discarded from statistical analysis. Data showed a normal distribution of the means that were submitted to two-way ANOVA (factors under study: pulpal pressure and bonding modes) and Tukey tests. Level of significance was set at 5%.

Table 2 Average values ± standard deviation of bond strength (MPa) in each group/subgroup

Pulpal pressure simulated	Etching	
	Conventional	Self-etch
Absence SPP	35.05 ± 6.69a[a]	37.25 ± 4.12a
SPP AD	27.86 ± 5.27b	26.12 ± 6.46b
SPP BFS	31.48 ± 5.30c	30.65 ± 5.30c

[a]Different letters mean significant differences among groups

Results

The cSt results showed that DW (23.59 ± 0.39) had significantly higher mean values than FBS (22.33 ± 0.06). The µTBS means (MPa) and results for Tukey test is presented in Table 2.

According to two-way ANOVA, the factor SPP did significantly influence the results ($p < 0.00001$). The bonding modes and the interaction of simulated pulpal pressure (SPP) did not statistically influence the results ($p < 0.05$).

The overall failure mode analysis showed 75% of adhesive failures, of which the majority of 55% corresponds to SPP groups. Cohesive failures occurred in 23% of specimens, of which the majority of 13% occurred in the control group. The mixed failures were 2%. The premature failures were less than 5%.

SEM images of the hybrid layer for the group with no SPP presented a thick hybrid layer and many resin tags (Figs. 1). In the groups using SPP (Figs. 2 and 3), a reduction of the hybrid layer thickness with fewer and shorter resin tags could be observed.

Discussion

Universal adhesives are composed of a complex chemical blend of solvents, acidic and functional monomers that promote bonding attempting to overcome substrate challenges. For instance, dentin permeability is one of the most important clinical conditions to be considered as adhesives can interact with pulpal fluid and this may influence the quality of adhesive–dentin interface [27].

Based on the present results, the first null hypothesis was rejected. Irrespective of the simulated pulpal fluid used, SPP negatively affected the performance of ScothBond Universal adhesive in comparison to the control group (36.15 MPa). These results corroborate with previous studies

Fig.1 SEM photomicrograph (2000×) of the bond interface for the absence of SPP associated to etch-and-rinse technique (**A**) and self-etch technique (**B**). D dentin; R resin; White arrow TAG; Black arrow adhesive interface
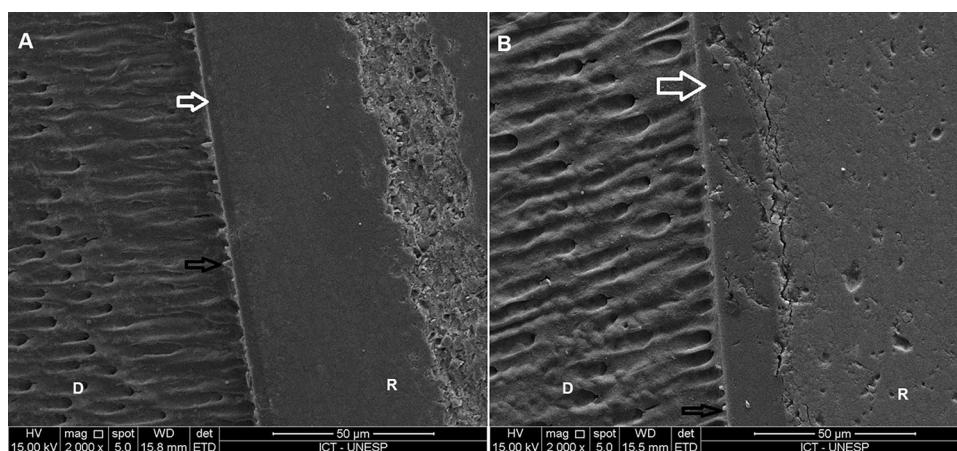
Fig. 2 SEM photomicrograph (2000×) of the bond interface for the presence of SPP with FBS associated to etch-and-rinse technique (**A**) and self-etch technique (**B**). D dentin; R resin; White arrow TAG; Black arrow adhesive interface

Fig. 3 SEM photomicrograph (2000×) of the bond interface for the presence of SPP with DW associated to etch-and-rinse technique (**A**) and self-etch technique (**B**). D dentin; R resin; White arrow TAG; Black arrow adhesive interface
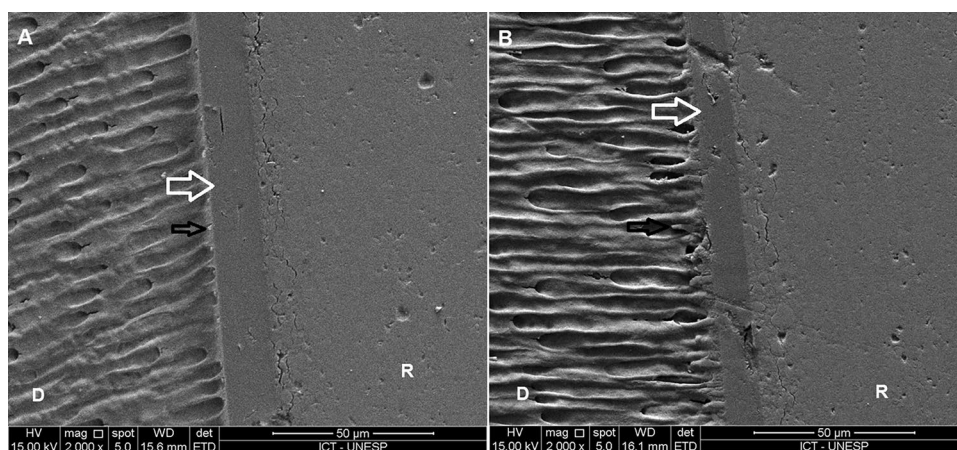
that observed a negative influence of SPP on bond strength values [12, 17–20]. However, the results showed that SBU behaved differently according to the type of fluid used, as FBS showed higher bond strength means (31.06 MPa) than water (26.55 MPa). Similar results were observed in other studies [17, 18].

Although the bond strength values obtained were statistically different, for each group, the prevalent failure mode was adhesive (55%). The results are in agreement with previous studies that show a decrease in bond strength under SPP [12, 17]. Apart from the actual fluid composition, the presence of moisture only, during the restorative procedure may jeopardize adhesive interface which is highlighted by the considerable percentage (13%) of cohesive failures in the control group.

The Kinematic viscosity measures the resistance to flow of a fluid under the influence of gravitational force, by the ratio of dynamic viscosity to density, and could be affected by composition, shape and molecular weight of its particles [28]. The higher the density, the lower it takes to flow through the dentinal tubules, and FBS has higher density (1.02 g/ml) compared to DW (0.99 g/mL), confirming the finds of this study, in which DW cSt was significantly higher

than FBS cSt. FBS has proteins in its composition, which may be responsible for the lower cSt. The use of FBS during SPP may have reduced dentin permeability due to coagulation and precipitation of proteins in the dentinal tubules [17]. It may have prevented excess moisture indirectly protecting the adhesive interface, justifying higher bond strength values in comparison to DW group. Comparable to FBS, the dentin fluid is composed of an extracellular pulpal fluid which is an ultrafiltrate of blood plasma [29]. Blood plasma is composed primarily of 91% water and 7% protein (Albumin-60% of the plasma proteins; 36% immunoglobulin and 4% of fibrinogen). The plasma is also composed of salts, electrolytes (sodium, potassium, magnesium, chloride and bicarbonate), enzymes and hormones [18, 23, 30]. Moreover, ScothBond Universal adhesive has HEMA in its composition which is known by perform protein coagulating effect [18]. Previous studies related higher bond strength when adhesives containing protein coagulation components were used with protein containing fluids [17, 29, 31].

ScothBond Universal adhesive is highly hydrophilic and behaves like a semi-permeable membrane; thus, SPP with water could have compromised the adhesive performance by promoting formation of water trees and poly-HEMA

hydrogels which reduces adhesive bond strength [32, 33]. The excessive wetness of dentin can lead to: (1) dilution of the adhesive monomers, (2) interference with the polymerization conversion of monomer [32], and (3) phase separation of the adhesive [34]. In spite of DW still being widely used in SPP studies [12, 20, 35], the interaction between adhesives and water may not be comparable to a clinical situation due to the different characteristics of water and dentinal fluid.

In this study, the bonding mode did not influence the bond strength of ScothBond Universal adhesive. There was no significant difference between etch-and-rinse and self-etch, which corroborates previous studies which found that immediate bond strengths are similar for both modes [36, 37]. Thus, the second null hypothesis was accepted. Moreover, no significant interaction between the bonding modes and SPP was found (Table 2).

SPP causes the flow of dentin fluid from dentin tubules to the cavity surface. The increase of transdential permeability after smear layer removal during etching with phosphoric acid tends to impair bonding, because dentin wetness and fluid movement through bonded interfaces may hinder optimal resin seal [29, 38]. Also, excessive transudation of fluids from dentinal tubules can counteract adhesive penetration and exert an inhibitory effect on polymerization [38]. The etch-and-rinse mode was expected to affect negatively the bond strength because it leads to high permeability and moist on the dentin surface in comparison to the self-etch mode, and SPP should not significantly affect the behavior of self-etch mode compared to etch-and-rinse mode. The physiological pulpal pressure corresponds to a hydrostatic pressure ranging from 8 to 20 cm H_2O [39], and most researchers investigated SPP within 15–20 cm H_2O [17–20, 39]. However, the SPP employed in this study (15 cm H_2O) was likely high enough to remove smear plugs, leading to excessive water flow toward surface and interaction with the adhesive material [22, 38, 39]. This would reduce the concentration of monomers in the adhesive material composition to a threshold enough to impair bonding in self-etch mode.

Also, Feitosa et al., [39] observed that simplified adhesives such as 1-step self-etch adhesives, i.e., universal adhesives, were the most affected by pulp pressure when compared to multi-step dental bonding agents. The high permeability of simplified adhesives remains after photopolymerization, because they have high amounts of hydrophilic monomer and non-evaporated solvent in its composition [39]. This can lead to a decrease in microtensile bond strength, as noted in the results of our study.

In the SEM analysis of the hybrid layer, the control etch-and-rinse subgroup showed numerous resin tags and a thick hybrid layer (Fig. 1a) [40, 41], due to the removal of the smear layer and smear plugs by phosphoric acid [42]. On the other hand, the control self-etch subgroup exhibited less resin tags and a thinner hybrid layer (Fig. 1b). This could be related to the ultra-mild acidity of ScothBond Universal adhesive (pH 2.7), with its reduced ability to penetrate smear layer [42, 43]. SEM analysis for SPP showed similar adhesion patterns for both bonding modes, a thin hybrid layer and less resin tags (Figs. 2 and 3). A positive physiological hydrostatic pressure through the dentin tubules, and consequently excess moisture can hinder the monomer penetration into demineralized dentin and its polymerization impairing the formation of resin tags and the hybrid layer [22, 38, 39, 44]. Also, the excess water in the adhesive layer can dilute the monomers and reduce conversion of the monomers, negatively affecting adhesion [34, 45] due to the incorporation of water bubbles in the hybrid layer [38, 46]. However, despite the different thickness patterns, no difference in bond strength values was observed between bonding modes.

MDP and polyalkenoic acid co-polymer are responsible for stable ionic bonding of the adhesive to calcium at the interface with hydroxyapatite [9, 11]. Depletion of calcium from dentinal surface by phosphoric acid may also preclude any potential chemical bonding with 10-MDP [8] and polyalkenoic acid co-polymer from ScothBond Universal adhesive [47]. Consequently, etch-and-rinse strategy can trigger lower bond strengths and an increase in nanoleakage in comparison with the self-etch strategy [5, 47]. Therefore, the clinical use of ScothBond Universal adhesive in self-etch mode over etch-and-rinse mode in dentin is recommended.

Universal adhesives are versatile systems, however, the clinician must keep in mind that these systems may behave as permeable membranes after polymerization, which can compromise their long-term clinical behavior. Simulated pulpal pressure remains an important variable in testing the efficacy of adhesives, especially in the case of universal adhesives, which are highly hydrophilic. DW does not seem to be a suitable substitute for dentinal fluid and may lead to errors during bonding analysis, not representing clinical conditions. Thus, solutions with protein contents such as FBS should be preferred to perform SPP.

Conclusions

It can be concluded that the SPP affect the bond strength of ScothBond Universal adhesive to dentin. The use of DW in SPP significantly reduced the bond strength when compared to the use of FBS. Bonding modes were not affected by SPP when evaluated in a short period of time.

Declarations

Conflict of interest Authors declare that have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

References

1. Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res. 1955;34(6):849–53. https://doi.org/10.1177/00220345550340060801.

2. Gonçalves SE, Cruz N, Brayner R, Huhtala MF, Borges AB, Barcellos DC. Grander system: a new technology to reduce surface tension of adhesive systems in dentistry. Acta Odontol Scand. 2014;72(1):31–5. https://doi.org/10.3109/00016357.2013.794953.

3. Torres GB, da Silva TM, Basting RT, Bridi EC, França FMG, Turssi CP, do Amaral FLB, de Depaiva SE, Basting RT. Resin-dentin bond stability and physical characterization of a two-step self-etching adhesive system associated with TiF$_4$. Dent Mater. 2017;33(10):1157–70. https://doi.org/10.1016/j.dental.2017.07.016.

4. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater. 2011;27(1):17–28. https://doi.org/10.1016/j.dental.2010.10.023.

5. Loguercio AD, de Paula EA, Hass V, Luque-Martinez I, Reis A, Perdigão J. A new universal simplified adhesive: 36 month randomized double-blind clinical trial. J Dent. 2015;43(9):1083–92. https://doi.org/10.1016/j.jdent.2015.07.005.

6. Pashley EL, Tao L, Derkson G, Pashley DH. Dentin permeability and bond strengths after various surface treatments. Dent Mater. 1989;5(6):375–8. https://doi.org/10.1016/0109-5641(89)90103-6.

7. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, Pashley DH. Fluid movement across the resin-dentin interface during and after bonding. J Dent Res. 2004;83(11):843–8. https://doi.org/10.1177/154405910408301104.

8. Van Meerbeek B, Yoshihara K, van Landuyt K, Yoshida Y, Peumans M. 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.

9. Perdigão J. Current perspectives on dental adhesion: (1) Dentin adhesion—not there yet. Jap Dent Sci Review. 2020;56(1):190–207.

10. Yoshihara K, Yoshida Y, Nagaoka N, Hayakawa S, Okihara T, De Munck J, et al. Adhesive interfacial interaction affected by different carbon-chain monomers. Dent Mater. 2013;29:888–97.

11. Leite MLA, Costa CAS, Duarte RM, Andrade AKM, Soares DG. Bond strength and cytotoxicity of a universal adhesive according to the hybridization strategies to dentin. Braz Dent J. 2018;29(1):68–75.

12. Hosaka K, Nakajima M, Yamauti M, Aksornmuang J, Ikeda M, Foxton RM, Pashley DH, Tagami J. Effect of simulated pulpal pressure on all-in-one adhesive bond strengths to dentine. J Dent. 2007;35(3):207–13. https://doi.org/10.1016/j.jdent.2006.08.001.

13. Tay FR, Pashley DH, Garcìa-Godoy F, Yiu CK. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part II. Silver tracer penetration evidence. Am J Dent. 2004;17(5):315–22.

14. Tay FR, Pashley DH, Suh B, Carvalho R, Miller M. Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part I. Bond strength and morphologic evidence. Am J Dent. 2004;17(4):271–8.

15. Silva TM, Gonçalves LL, Siqueira EP, Pereira TC, Pontes SO, Grecca AR, Lopes SR, Gonçalves SEP. Influence of simulated pulpal pressure on the variation of intrapulpal temperature during adhesive system light-curing. Braz Dent Sci. 2017;20(2):55–61. https://doi.org/10.1007/s10103-016-2098-1.

16. Paris Matos T, Perdigão J, de Paula E, Coppla F, Hass V, Scheffer RF, et al. Five-year clinical evaluation of a universal adhesive: a randomized double-blind trial. Dent Mater. 2020. https://doi.org/10.1016/j.dental.2020.08.007.

17. Nikaido T, Burrow MF, Tagami J, Takatsu T. Effect of pulpal pressure on adhesion of resin composite to dentin: bovine serum versus saline. Quintessence Int. 1995;26(3):221–6.

18. Mobarak EH, El-Deeb HA, Yousry MM. Influence of different intrapulpal pressure simulation liquids on the microtensile bond strength of adhesive systems to dentin. J Adhes Dent. 2013;15(6):519–26. https://doi.org/10.3290/j.jad.a29719.

19. Sauro S, Pashley DH, Montanari M, Chersoni S, Carvalho RM, Toledano M, et al. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater. 2007;23(6):705–13. https://doi.org/10.1016/j.dental.2006.06.010.

20. Hosaka K, Nakajima M, Monticelli F, Carrilho M, Yamauti M, Aksornmuang J, et al. Influence of hydrostatic pulpal pressure on the microtensile bond strength of all-in-one self-etching adhesives. J Adhes Dent. 2007;9(5):437–42.

21. Santis LR, Silva TM, Haddad BA, Gonçalves LL, Gonçalves SEP. Influence of dentin thickness on intrapulpal temperature under simulated pulpal pressure during Nd:YAG laser irradiation. Lasers Med Sci. 2017;32(1):161–7. https://doi.org/10.1007/s10103-016-2098-1.

22. Belli R, Sartori N, Peruchi LD, Guimarães JC, Araújo E, Monteiro S Jr, Baratieri LN, Lohbauer U. Slow progression of dentin bond degradation during one-year water storage under simulated pulpal pressure. J Dent. 2010;38(10):802–10. https://doi.org/10.1016/j.jdent.2010.06.012.

23. Goodis HE, White JM, Marshall GW Jr, Yee K, Fuller N, Gee L, Marshall SJ. Effects of Nd: and Ho:yttrium-aluminium-garnet lasers on human dentine fluid flow and dental pulp-chamber temperature in vitro. Arch Oral Biol. 1997;42(12):845–54. https://doi.org/10.1016/s0003-9969(97)00076-9.

24. Özok AR, Wu MK, De Gee AJ, Wesselink PR. Effect of dentin perfusion on the sealing ability and microtensile bond strengths of a total-etch versus an all-in-one adhesive. Dent Mater. 2004;20(5):479–86. https://doi.org/10.1016/j.dental.2003.07.004.

25. Roulet JF, Van Meerbeek B. Statistics: a nuisance, a tool, or a must? J Adhes Dent. 2007;9(3):287–8.

26. Perote LCCC, Kamozaki MBB, Gutierrez NC, Tay FR, Pucci CR. Effect of matrix metalloproteinase-inhibiting solutions and aging methods on dentin bond strength. J Adhes Dent. 2015;17(4):347–52. https://doi.org/10.3290/j.jad.a34594.

27. 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.

28. Özok AR, Wu MK, Wesselink PR. Comparison of the in vitro permeability of human dentine according to the dentinal region and the composition of the simulated dentinal fluid. J Dent. 2002;30(2–3):107–11. https://doi.org/10.1016/s0300-5712(02)00005-2.

29. Augustin C, Paul SJ, Lüthy H, Schärer P. Perfusing dentine with horse serum or physiologic saline: Its effect on adhesion of dentine bonding agents. J Oral Rehabil. 1998;25(8):596–602602. https://doi.org/10.1046/j.1365-2842.1998.00276.x.

30. Schaller J, Gerber S, Kämpfer U, Lejon S, Trachsel C. Human blood plasma proteins: structure and function. New Jersey: Wiley; 2008. https://doi.org/10.1002/9780470724378.

31. Gernhardt CR, Bekes K, Fechner K, Schaller HG. The influence of human plasma used for in vitro dentin perfusion on microtensile bond strength of 5 self-conditioning dentin adhesives. Quintessence Int. 2006;37(6):429–35.

32. Paul SJ, Leach M, Rueggeberg FA, Pashley DH. Effect of water content on the physical properties of model dentine primer and bonding resins. J Dent. 1999;27(3):209–14. https://doi.org/10.1016/s0300-5712(98)00042-6.

33. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, et al. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials. 2005;26(33):6449–59. https://doi.org/10.1016/j.biomaterials.2005.04.052.

34. Spencer P, Ye Q, Park J, Misra A, Bohaty BS, Singh V, Parthasarathy R, Sene F, Gonçalves SEP, Laurence J. Durable bonds at the adhesive/dentin interface: an impossible mission or simply a moving target? Braz Dent Sci. 2012;15:4–18. https://doi.org/10.14295/bds.2012.v15i1.790.

35. Escribano N, Del-Nero O, La MJCD. Sealing and dentin bond strength of adhesive systems in selected areas of perfused teeth. Dent Mater. 2001;17(2):149–55. https://doi.org/10.1016/s0109-5641(00)00057-9.

36. Muñoz MA, Luque-Martinez I, Malaquias P, Hass V, Reis A, Campanha NH, et al. In vitro longevity of bonding properties of universal adhesives to dentin. Oper Dent. 2015;40(3):282–92. https://doi.org/10.2341/14-055-L.

37. Da Rosa WLDO, Piva E, Da Silva AF. Bond strength of universal adhesives: a systematic review and meta-analysis. J Dent. 2015;43(7):765–76. https://doi.org/10.1016/j.jdent.2015.04.003.

38. Mazzitelli C, Monticelli F, Osorio R, Casucci A, Toledano M, Ferrari M. Effect of simulated pulpal pressure on self-adhesive cements bonding to dentin. Dent Mater. 2008;24(9):1156–63.

39. Feitosa VP, Correr AB, Correr-Sobrinho L, Sinhoreti MA. Effect of a new method to simulate pulpal pressure on bond strength

40. Ikeda M, Tsubota K, Takamizawa T, Yoshida T, Miyazaki M, Platt JA. Bonding durability of single-step adhesives to previously acid-etched dentin. Oper Dent. 2008;33(6):702–9. https://doi.org/10.2341/08-26.

41. Langer A, Ilie N. Dentin infiltration ability of different classes of adhesive systems. Clin Oral Investig. 2013;17(1):205–16. https://doi.org/10.1007/s00784-012-0694-4.

42. Wagner A, Wendler M, Petschelt A, Belli R, Lohbauer U. Bonding performance of universal adhesives in different etching modes. J Dent. 2014;42(7):800–7. https://doi.org/10.1016/j.jdent.2014.04.012.

43. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: depth of penetration beyond dentin smear layers. Dent Mater. 2001;17(4):296–308. https://doi.org/10.1016/s0109-5641(00)00087-7.

44. Feitosa VP, Leme AA, Sauro S, Correr Sobrinho L, Watson TF, Sinhoreti MA, Correr AB. Hydrolytic degradation of the resin-dentin interface induced by the simulated pulpal pressure, direct and indirect water aging. J Dent. 2012;40:1134–43.

45. Pucci CR, Gu LS, Zeng C, Gou YP, Tay FR, Niu LN. Susceptibility of contemporary single-bottle self-etch dentine adhesives to intrinsic water permeation. J Dent. 2017;66:52–61.

46. Cardoso GC, Nakanishi L, Isolan CP, Jardim PDS, Moraes RR. Bond stability of universal adhesives applied to dentin using etch-and-rinse or self-etch strategies. Braz Dent J. 2019;30(5):467–75.

47. Sezinando A, Serrano ML, Pérez VM, Muñoz RGA, Ceballos L, Perdigão J. Chemical adhesion of polyalkenoate-based adhesives to hydroxyapatite. J Adhes Dent. 2016;18(3):257–65. https://doi.org/10.3290/j.jad.a36222.