Brazilian Dental Science
unesp
UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE DOI: https://doi.org/10.4322/bds.2025.e4782

Universal dentin bonding system: influence of handling conditions on the determination of adhesive properties on dentin

Sistema adesivo universal: influência das condições de manuseio na determinação de suas propriedades adesivas em dentina

Marina Ciccone GIACOMINI[1], Rafaela PASCON[2], Mariana Ribeiro dos SANTOS[3], Camila Moreira MACHADO[4], Victor MOSQUIM[1], Juliana Carvalho JACOMINE[1], Linda WANG[1]

1 - Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Odontológicos. Bauru, SP, Brazil.
2 - Autônoma. Rio Claro, SP, Brazil.
3 - Autônoma. Bauru, SP, Brazil.
4 - Prefeitura Municipal de Bauru. Bauru, SP, Brazil.

How to cite: Giacomini MC, Pascon R, Santos MR, Machado CM, Mosquim V, Jacomine JC, et al. Universal dentin bonding system: influence of handling conditions on the determination of adhesive properties on dentin. Braz Dent Sci. 2025;28(3):e4782. https://doi.org/10.4322/bds.2025.e4782

ABSTRACT

Objective: Despite advances on dentin bonding systems (DBSs), their adhesive potential can still be significantly affected by storage conditions and handling. The present study aimed to evaluate the influence of DBS manipulation on the physical-mechanical properties of these materials. **Material and Methods:** Three DBSs were tested: Adper Scotchbond Multi-Purpose [MP] (3-step etch-and-rinse), Clearfil SE Bond [SE] (2-step self-etch), and Adper Single Bond Universal [SU] (universal adhesive). The experimental conditions were Control [CTRL] – recommended storage; Laboratory-aged [LAB] – artificially aged under controlled laboratory conditions; and Clinically used [CLIN] – exposed to a 2-week clinical routine. Degree of conversion (DC) was assessed using Fourier Transform Infrared Spectroscopy (FTIR) on 3 μL samples (n = 5), while the surface microhardness (SM) was tested on disc specimens (KHN 10kgf/5s/n=6). For microtensile bond strength (μTBS), 90 human molars were randomized according to the DBS, restored with a two 2 mm-increment of composite resin and sectioned into beams (0.64 mm^2 cross-section) after 24 h and tested at 0.5 mm/min under a 500 N load (n = 10). Data were analyzed using two-way ANOVA and the Tukey`s tests (p = 0.05). **Results:** SE presented the highest DC values, while SU presented the lowest. MP reached the highest SM under clinical conditions, while SU exhibited consistently low values among all conditions. For the μTBS, SU decreased under LAB and CLIN, while MP remained stable throughout. **Conclusion:** SU was more affected by handling conditions than MP and SE. Proper storage and clinical use protocols are essential to preserve DBS performance.

KEYWORDS

Dentin; Dentin-bonding agents; Dental bonding; Mechanical tests; Surface properties.

RESUMO

Objetivo: Apesar dos avanços nos sistemas adesivos (SAs), seu potencial adesivo ainda pode ser significativamente afetado por condições de armazenamento e manipulação. Este estudo avaliou a influência da manipulação dos SAs em suas propriedades físico-mecânicas. **Material e Métodos:** Três SAs foram testados: Adper Scotchbond Multi-Purpose [MP] (três passos - convencional), Clearfil SE Bond [SE] (dois passos - autocondicionante) e Adper Single Bond Universal [SU] (universal). As condições experimentais foram: Controle [CTRL] - armazenamento recomendado; Envelhecimento laboratorial [LAB] – artificial controlado e Clínico [CLIN] – rotina clínica por duas semanas. O grau de conversão (GC) foi avaliado por espectroscopia de infravermelho com transformada de Fourier (FTIR) em amostras de 3 μL (n = 5). A microdureza superficial (MS) foi mensurada em discos

(KHN, 10 kgf, 5 s, n = 6). Para a microtração (μTBS), 90 molares humanos foram restaurados com 2mm de resina composta, seccionados em palitos (0,64 mm^2) e testados após 24 h (0,5 mm/min, 500 N, n = 10). Os dados foram analisados por ANOVA de dois fatores e teste de Tukey ($p < 0,05$). **Resultados:** O SE apresentou os maiores valores de DC e SU os menores. O MP obteve os maiores valores de MS sob a condição CLIN, enquanto o SU mostrou valores consistentemente baixos em todas as condições. Para μTBS, SU apresentou redução nas condições LAB e CLIN, enquanto o MP manteve-se estável. **Conclusão:** O SU foi mais afetado pelas condições de manipulação do que o MP e o SE. Protocolos adequados de armazenamento e uso clínico são essenciais para preservar o desempenho dos SAs.

PALAVRAS-CHAVE

Dentina; Sistemas adesivos; Adesão dentária; Testes mecânicos; Propriedades de superfície.

INTRODUCTION

The latest technologies supporting the development and application of dentin bonding systems rely on categories based on acidic monomers, including two-step etch-and-rinse and universal systems. The literature demonstrates that these systems can combine optimal bonding performance under various clinical conditions with user-friendly application, particularly in the case of universal systems. However, their apparent simplicity may lead clinicians to underestimate the importance of proper handling procedures.

Currently, DBSs based on 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate) have proven to be reliable in promoting chemical interaction with dentin under minimal conditioning. However, beyond proper indication, the key point for a successful performance mostly relies on how the professional handle them to determine the longevity of restorative adhesive dentistry onto dentin [1]. Due to the complexity of the dentin substrate, DBSs have intricated formulations composed of hydrophilic and hydrophobic resin monomers with varying molecular weights, viscosities, and organic solvents (acetone, ethanol, and/or water) [1-3]. In addition, additives such as solvents, photo-initiators, and stabilizers are included, making these formulations more susceptible to degradation [1]. Therefore, proper storage and handling of these systems are essential to ensure their quality and long-term performance since these factors are often underestimated by clinicians [4].

Currently, the preference for self-etching systems has been demonstrated since they do not require prior dentin conditioning, thus preserving the substrate and providing favorable outcomes. The same principle applies to universal systems used in self-etching mode [3-6]. In addition, the activity of proteolytic enzymes associated with acid conditioning has been shown to compromise bonding quality [5-7].

Due to the user-friendly nature of some systems, practitioners often underestimate their complexity and overlook the need for strict control, from storage conditions to proper clinical application [2,3,8]. Under such conditions, the main components of DBSs may degrade, compromising their bonding potential. Typically, non-simplified DBSs contain methacrylates such as HEMA (2-hydroxyethyl methacrylate) combined with hydrophobic monomers like Bis-GMA (bisphenol A glycidyl methacrylate). In self-etching and universal systems, 10-MDP is commonly used in both primer and bonding agents, which are often combined in a single bottle [8,9]. This formulation increases the risk of monomer solvation and degradation [10-12].

Moreover, improper storage at inadequate temperatures or prolonged bottle opening during clinical use facilitates solvent evaporation and increases oxygen exposure, accelerating material degradation and leading to reduced bonding efficacy and postoperative sensitivity [1,2,13-15]. Since bonding effectiveness depends on the integrity of the polymer chain, such environmental factors are critical for long-term performance [2,15].

Although the performance of DBSs has been widely studied under ideal conditions [1,2,4,15], the impact of handling such as storage or clinical use remains underexplored. The present study addresses this gap by evaluating three representative adhesives from different categories under varied manipulation scenarios. Clarifying these effects is essential for improving clinical protocols and ensuring long term restoration success. The selected adhesives represent distinct and widely used categories: Adper Scotchbond Multi-purpose (three-step etch-and-rinse – MP), Clearfil SE Bond (Two-step self-etch system - SE), and Adper Single Bond Universal (universal system in self-etch mode – SU).

Therefore, the aim of the present study was to investigate the impact of different manipulation conditions on the mechanical properties of three main DBS categories by evaluating the degree of conversion (DC), surface microhardness (SM), and microtensile bond strength (μTBS). The null hypotheses were: (1) There are no differences in DC, SM, and μTBS among the tested DBS categories (3-step etch-and-rinse, 2-step self-etch, and universal system in self-etch mode); and (2) There are no differences in DC, SM, and μTBS among the different manipulation conditions (control, artificially aged in the laboratory, and clinically used).

MATERIAL AND METHODS

The present study was approved by the Local Ethical Committee (Protocol Number: #49808515.1.0000.5417).

Experimental design

The present *in vitro*, parallel, and blinded study employed a two-factor design:

(1) Dentin bonding systems (DBSs) at three levels — Adper Scotchbond Multi-purpose [MP], Clearfil SE Bond [SE], and Adper Single Bond Universal [SU] (used in the etch-and-rinse mode); (2) Manipulation conditions at three levels — Control [CTRL], in which adhesives were used immediately after bottle opening; Laboratory-aged [LAB], in which adhesives were subjected to simulated aging under controlled conditions; and Clinically used [CLIN], in which adhesives were used in a 2-week clinical routine.

Three response variables were assessed: degree of conversion (DC), surface microhardness (SM) and microtensile bond strength test (μTBS). All tests were performed under temperature and humidity control (23+2°C/ 80+10% of humidity). All procedures were performed by a single calibrated operator, and all tests were conducted by a second calibrated operator to minimize bias.

Sample size calculation

Sample size calculation was based on data from a pilot study, considering 5% as alpha error, 80% for minimum acceptable power and considering the analysis for 9 independent groups. Sample size of 3 and 6 were calculated for degree of conversion and surface microhardness, respectively. For the microtensile bond strength test, the tooth was considered the experimental unit [5,7,16], and a sample size of 10 was calculated.

Table I summarizes the composition and application protocol of each DBS, while Table II details the manipulation conditions.

The 2-week period was selected based on previous studies that demonstrated measurable changes in the composition and performance of the DBSs after short-term clinical exposure or simulated aging, without compromising the integrity of the materials [13,14].

Degree of conversion (DC)

The degree of conversion (DC) of the dentin bonding systems (DBSs) was assessed according to ISO 4049:2019 using Fourier Transform Infrared Spectroscopy (FTIR) (Prestige 21, Shimadzu, Tokyo, Japan), equipped with an attenuated total reflectance (ATR) crystal composed of a horizontal diamond device and a 45° tilted mirror.

For each test, a drop of bonding agent (3 μL; n = 5) was placed onto the ATR crystal. The sample was light-cured (Radii-cal, SDI, Bayswater, Victoria, Australia) using an intensity of 1,000 mW/cm^2 for the time recommended by the manufacturer.

Spectra were collected before and after light activation using 32 scans (8 cm^{-1} resolution; 2.8 mm/s mirror speed) in transmission mode. The percentage of unreacted carbon–carbon double bonds (%C=C) was calculated from the absorbance ratio of the aliphatic C=C peak (1638 cm^{-1}) to the internal standard peak of aromatic C=C bonds (1608 cm^{-1}). The degree of conversion was determined by subtracting the %C=C from 100%.

All analyses were performed in triplicate.

Surface microhardness (SM)

Disc-shaped specimens were prepared by dispensing the bonding agents into two-part Teflon molds (5 mm diameter × 1 mm thickness), ensuring standardization in volume and area. Each sample was light-cured (Radii-cal CX, SDI, Bayswater, Victoria, Australia) at an intensity of

Table I - Composition and protocols of application of each DBS recommended by the manufacturer

Dentin bonding system	Manufacturer	Composition	Application protocol	Category
Adper Scotchbond Multi-purpose **[MP]**	Solventum, St. Paul, MN, USA	Primer: HEMA, water, Vitrebond™ Copolymer (polyalkenolic acid) Bond agent: Bis-GMA, HEMA, photoinitiator	37% phosphoric acid etching for 15s + Rinse for 15s + Drying with absorbent paper + Primer application + Slight air blast for 5s + Bond application + Light cured for 10s	3-step etch-and-rinse system
Clearfil™ SE Bond **[SE]**	Kuraray Co. Ltda., Osaka, Japan	Primer: MDP, HEMA, DMA; Catalyst Water Bond agent: MDP, HEMA, Bis-GMA; DMA, Camphorquinone, silanized colloidal silica	Application of self-etch primer + Wait for 20s + Solvent evaporation for 5s + Bond application followed by slight air blast + Light cured for 10s	2-step self-etch system
Adper Single Bond Universal **[SU]**	Solventum, St. Paul, MN, USA	Bis-GMA, MDP, HEMA, Vitrebond™ Copolymer, itaconic acid, camphorquinone; Ethyl alcohol, Water, Silica, Silane.	37% phosphoric acid etching for 15s + Rinse for 15s + Drying with absorbent paper + Active DBS application for 20s + Solvent evaporation for 5s + Light cure for 10s	Universal system

Bis-GMA: Bisphenol-A glycidyl methacrylate; DMA: dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; MDP: methacryloyloxydecyl dihydrogen phosphate.

Table II - Protocol of manipulation and aging conditions

Groups	Manipulation conditions
Control **[CTRL]**	Immediately after opening the bottle, the DBS was stored at standard room temperature (23 + 2 °C), as recommended by the manufacturers.
Artificially aged in laboratory **[LAB]**	During laboratory aging, bottles were opened twice daily (morning and afternoon), for two hours per session. Within each session, bottles were opened every 30 minutes. Between sessions, adhesives were stored at 6-8 °C in a refrigerator.
Aged in clinical routine **[CLIN]**	Regular use in clinical routine for two weeks, totaling a mean of 8 attendances of 4 continuous hours each by undergraduate students.

1,000 mW/cm^2 for the time recommended by the manufacturer.

Surface microhardness (SM) was evaluated using a Knoop indenter (Buehler Ltd, MicroMet 6040, Lake Bluff, IL, USA) under a static load of 10 kgf applied for 5 s. Three indentations were performed on each specimen, spaced 100 μm apart. The SM value was calculated as the mean of the three measurements.

Specimen preparation and bonding protocols

Ninety caries-free third human molars were collected (n = 10 per group) and stored in 0.1% thymol saline solution at 4°C for a maximum of two months.

The crowns and roots were separated through a section 3 mm below the cement–enamel junction using a water-cooled diamond-impregnated disc (Extec Corp, Enfield, CT, USA) in a digital cutting machine (ISOMET 1000, Buehler, Lake Bluff, IL, USA). A second cut was performed to remove the occlusal enamel and expose the mid-coronal dentin surface, which was subsequently polished using 600-grit SiC paper under running water for 30s (APL-4 Arotec, Cotia, SP, Brazil). This protocol allows for obtaining a standardized parameter closer to the clinical condition.

The specimens were randomly allocated into three groups according to the DBS: Adper Scotchbond Multi-Purpose [MP], Clearfil SE Bond [SE] and Adper Single Bond Universal – etch and rinse mode [SU]. The randomization was performed using Microsoft Excel software (Microsoft corporation, Redmond, WA, USA). After aging manipulations, the specimens were randomized according to the groups shown in Table II.

The bonding protocol was performed following the manufacturers' instructions as described in Table I.

The specimens were restored with composite resin (Solventum, St. Paul, MN, USA) used in two increments of 2mm and light-cured for 20s each (Radii-cal, SDI, Bayswater, Victoria, Australia) The restored specimens were immersed in artificial saliva (1.5 mM Ca $[NO_3]2{\cdot}4H_2O$, 0.9 mM $NaH_2PO4{\cdot}2H_2O$, 150 mM KCl, 0.1 mol/L Tris, 0.03 ppmF, pH 7.0) for 24h at 37°C. Both the bonding and restorative procedures were performed by a single, standardized and trained operator.

Microtensile bond strength test (μTBS)

Preparation and microtensile tests were performed based on Armstrong et al. [16] as per guideline recommendations. The restored specimens were sectioned perpendicularly to the bonding interface to obtain beams of ≅0.64 mm^2 area (0.8 mm x 0.8 mm). Subsequently, beams were fixed to the test device (Odeme dental research, Luzerna, SC, Brazil) with cyanoacrylate glue (Super Bonder Flex Gel- Loctite, Henckel Ltda, Itapevi, SP, Brazil) and tested in a universal testing machine (Instron 3342, Instron Co., Canton, MA, USA) with a crosshead speed of 0.5 mm/min and 500 N-load cell.

μTBS means and standard deviations were calculated and expressed in MPa. The failure mode was analyzed by a hand-held digital microscope (DINO-LITEplus digital microscope, AnMo Electronics Corporation, Hsinchu, China) at 40x magnification and classified as adhesive, mixed, cohesive in dentin or composite resin.

Statistical analyses

Data were statistically analyzed using the Statistica software (Statsoft®, Tulsa, OK, USA). Normality assumptions were verified before applying two-way ANOVA followed by the Tukey's post hoc test ($\alpha = 0.05$).

RESULTS

Degree of conversion (DC)

For the DC data, only the dentin bonding system presented a statistically significant effect ($p = 0.0130$), whereas neither the manipulation condition ($p = 0.6524$) nor its interaction with the dentin bonding system ($p = 0.3467$) acquired a statistical significance.

Although Table III presents the mean DC values for all groups, statistical differences are limited to comparisons among DBSs within each manipulation condition, as indicated by the uppercase letters.

For all tested conditions, Clearfil SE Bond [SE] demonstrated the highest degree of conversion values, while the Adper Single Bond Universal [SU] had the lowest performance. The Adper Scotchbond Multi-purpose [MP] did not differ significantly from either the Adper Single Bond Universal [SU] or Clearfil SE Bond [SE].

Surface microhardness (SM)

For the SM, both the dentin bonding system ($p = 0.000008$) and the manipulation conditions ($p < 0.001$) were statistically significant, as was their interaction ($p = 0.0005$). Table IV presents the mean SM values and standard deviations for each DBS under all manipulation conditions.

While the Adper Scotchbond Multi-purpose [MP] aged in the clinical routine yielded the highest values, the Adper Single Bond Universal [SU] presented a stable performance under all conditions. On the other hand, the lowest values were observed for the SU in the control group. Adper Scotchbond Multi-purpose [MP] and Clearfil SE Bond [SE] achieved their highest values when

Table III - Degree of conversion (%) and standard deviation according to the dentin bonding system and manipulation condition

MANIPULATION CONDITIONS	DENTIN BONDING SYSTEMS		
	MP	SE	SU
CTRL	66.69 (0.37) **AB**	69.70 (0.32) **A**	68.47 (8.94) **B**
LAB	69.09 (0.55) **AB**	72.48 (0.77) **A**	61.40 (14.65) **B**
CLIN	77.16 (2.78) **AB**	77.29 (1.27) **A**	50.12 (10.18) **B**

N=5; CTRL: Control Group (no manipulation was executed); LAB: artificially aged in laboratory; CLIN: aged in clinical routine; MP: Adper Scotchbond Multi-purpose; SE: Clearfil SE Bond; SU: Adper Single Bond Universal. Different uppercase letters indicate differences between DBSs (columns) in each manipulation condition (row) ($p<0.05$).

aged in the clinical routine condition. No differences were observed for any of the DBSs between the control and artificially aged in laboratory conditions.

Microtensile bond strength test (μTBS)

Both the dentin bonding system (p = 0.0048) and the manipulation condition (p < 0.0001) were statistically significant, as was their interaction (p = 0.0038). Table V presents the μTBS mean values and standard deviations for each group.

According to the dentin bonding system in the control groups, the Adper Single Bond Universal [SU] and Adper Scotchbond Multi-purpose [MP] presented the highest μTBS values, while the Clearfil SE Bond [SE] exhibited the lowest. No statistically significant differences were observed among the DBSs in the laboratory and clinically aged conditions. When comparing the manipulation effects within each material, only the Adper Single Bond Universal [SU] presented a reduction in bond strength under both aged conditions, while the Adper Scotchbond Multi-purpose [MP] and Clearfil SE Bond [SE] maintained stable performances.

DISCUSSION

Overall, the results of the present study demonstrated that the type of dentin bonding system (DBS) was the most relevant factor influencing performances throughout all tests, leading to the rejection of the first null hypothesis. The clinical success and longevity of adhesive restorations depend significantly on the appropriate selection and proper handling of the DBS [4,8,17]. These systems are particularly vulnerable when bonding to dentin, a substrate prone to degradation [18]. Regarding hydrolytic degradation, recent studies have highlighted the key role of proteolytic enzymes, such as matrix metalloproteinases (MMPs) and cysteine cathepsins, compromising the hybrid layer. These enzymes are activated in acidic environments or in the presence of exposed collagen fibrils, accelerating the degradation process [4,5,7]. These results should certainly influence investigations to search for strategies that can refrain their enzymatic action, such as the use of chlorhexidine aqueous solution, dimethyl sulfoxide solvent and remineralization ingredients.

Regardless of the main mechanisms of action, all of them are addressed to promote better protection of the denuded collagen matrix and reduce the action of these intrinsic enzymes. Up to now, laboratory and clinical investigations have proven that these anti-proteolytic agents can serve to postpone the degradation but not refrain them [4,5]. In addition, these strategies usually introduce an additional step in which the concerns rely on the chance for operational

Table IV - Surface microhardness (KHN) mean values and standard deviations for each dentin bonding system under different manipulation conditions

MANIPULATION CONDITIONS	DENTIN BONDING SYSTEMS		
	MP	SE	SU
CTRL	13.50 (5.79) **BC**	10.83 (1.83) **C**	8.04 (8.03) **C**
LAB	10.62 (2.60) **C**	12.34 (3.67) **BC**	10.86 (2.43) **C**
CLIN	24.51 (3.91) **A**	18.99 (5.51) **AB**	10.09 (2.52) **C**

N=6; CTRL: Control Group (no manipulation was executed); LAB: artificially aged in laboratory; CLIN: aged in clinical routine; MP: Adper Scotchbond Multi-purpose; SE: Clearfil SE Bond; SU: Adper Single Bond Universal. Different uppercase letters indicate differences between adhesive systems in each manipulation condition (p<0.05).

Table V - Mean values (MPa) and standard deviation of bond strength values regarding DBS and manipulation conditions

MANIPULATION CONDITIONS	DENTIN BONDING SYSTEMS		
	MP	SE	SU
CTRL	29.35 (5.75) **Aa**	26.60 (5.22) **Ba**	35.33 (5.30) **Aa**
LAB	26.37 (5.27) **Aa**	21.52 (3.70) **Aa**	20.37 (3.95) **Ab**
CLIN	26.92 (5.08) **Aa**	21.79 (6.59) **Aa**	23.94 (4.31) **Ab**

N=10; CTRL: Control Group (no manipulation was executed); LAB: artificially aged in the laboratory; CLIN: aged in the clinical routine; MP: Adper Scotchbond Multi-purpose; SE: Clearfil SE Bond; SU: Adper Single Bond Universal. Different uppercase letters indicate differences between the DBSs (columns) in each manipulation condition (row) (p<0.05). Different lowercase letters indicate differences between manipulation conditions (rows) in each DBS (columns) (p<0.05).

mistakes by the professional rather than an increase of application time.

Among the DBSs, acidic functional monomer-based categories have demonstrated great performance under different circumstances since they seem to combine chemical interaction with the substrate with proper use. This balance is particularly relevant in clinical scenarios involving altered substrates, which pose greater challenges for adhesion [19,20].

Therefore, the complex chemical composition of DBSs requires a clear understanding of their mechanisms and strict adherence to the manufacturers' guidelines. However, these systems are often handled without due attention by clinicians. In the present study, a laboratory condition was simulated to mimic the extended bottle opening that may occur in clinical settings. This situation favors solvent evaporation, which can compromise the adhesive performance [10,12,21]. Solvents play a key role in transporting monomers into the collagen matrix and influencing the degree of conversion. Their evaporation can negatively affect dentin wettability and contribute to postoperative sensitivity [22]. The degree of conversion (DC) was influenced by the composition of the DBSs, affecting their polymerization behavior.

Considering the three evaluated properties, the Clearfil SE Bond [SE] exhibited the highest overall values, likely due in part to its composition based on 10-MDP. This monomer is more acidic than the one used in the Adper Scotchbond Multi-purpose [MP]. For the degree of conversion and surface microhardness tests, only the bonding agents of the Adper Scotchbond Multi-purpose [MP] and Clearfil SE Bond [SE] were assessed since their primers are not light-curable. Both systems are non-simplified DBSs and therefore solvent-free. On the other hand, the Adper Single Bond Universal [SU] presented the lowest values. It is also interesting that a higher standard deviation was seen, suggesting less homogeneous performance. The Adper Single Bond Universal [SU] was the only simplified system tested. Its greater vulnerability may be attributed to the combination of monomers, solvents, and additives used for polymerization and preservation, as confirmed by its behavior under different testing conditions.

Although the presence of solvents in the Adper Single Bond Universal [SU] may reduce its degree of conversion, both the Adper Single Bond Universal [SU] and Clearfil SE Bond [SE] are based on the acidic functional monomer 10-MDP. This monomer requires calcium neutralization to effectively bond to dentin, which represents a limitation in this type of assessment. Since the specimens were tested solely for DC, no neutralization was possible [12,23], and DC is known to be material-dependent, even under varying air-drying conditions [11]. Under this condition, no statistically significant differences were observed among manipulation conditions for the same material. However, the Adper Scotchbond Multi-purpose [MP-Clin] and Clearfil SE Bond in clinical conditions [SE-Clin] presented higher DC values than their respective controls, while the Adper Single Bond Universal in a clinical condition [SU-Clin] revealed a decrease. In the control group, all DBSs performed similarly, but under the LAB condition, the Adper Single Bond Universal [SU] values decreased.

A common clinical strategy is to dispense a drop of DBS into a disposable container for use throughout the procedure which should be covered to minimize oxygen exposure [12]. In the present study, the aged in clinical routine condition simulated a routine undergraduate clinical setting, where the adhesive bottle remained open for longer periods, resulting in increased oxygen contact.

On the other hand, distinct performance was observed for the surface microhardness (SM) and microtensile bond strength (μTBS). Once again, the type of DBS, the manipulation condition, and their interaction were statistically significant factors. Therefore, the second null hypothesis was also rejected. These differences may be attributed to the composition of each DBS, which influenced their performance. Acetone, ethanol, and water were the most common solvents used in the formulations [3,17,24]. During bonding procedures, these solvents must be adequately evaporated. However, low molecular and hydrophilic monomers such as HEMA are also volatile compounds [11,24] acting as a co-solvent that help prevent water/monomer phase separation [17]. Therefore, when bottles are left open for extended periods, these compounds may evaporate easily, compromising the performance of the bonding agent. In the present study, all DBSs tested contained HEMA in their formulations, as well as solvents such as water and/or ethyl alcohol.

Caution is necessary to interpret the performance regarding surface microhardness (SM), since aging in the clinical routine condition seems to determine the greatest values for all DBSs, where oxygen could impair the polymerization process and affect the superficial microhardness. In this scenario, the molecular chains can assist in determining more resistant materials, which favors the Adper Scotchbond Multi-purpose [MP] and Clearfil SE Bond [SE]. In the case of Clearfil SE Bond [SE], since it was composed of 10-MDP as an acidic monomer, it is more dependent on calcium to stabilize, which again favored the Adper Scotchbond Multi-purpose [MP] with the highest detected values compared to the Clearfil SE Bond [SE]. No statistical difference was detected for each material in all tested conditions. Among the tested groups, the Adper Single Bond Universal [SU] presented the lowest values, even though no differences regarding the condition were evidenced. Despite the information described above, this DBS is solvated, which became more vulnerable to solvation. It is again suggested that the solvent content and the functional monomer combination increase the complexity of the DBS [3,8,25].

Clinical aging [CLIN] followed a spontaneous routine for 2-weeks with no intention to establish a standard protocol as LAB groups. In this case, it can be speculated that the clinical condition might cause intense exposure to oxygen, therefore, the LAB protocol was limited.

In terms of μTBS assessment, it was evidenced that the Adper Scotchbond Multi-purpose [MP] and Clearfil SE Bond [SE] individually were able to maintain the same performance pattern, regardless of the manipulation condition. In addition, the Adper Single Bond Universal [SU] varied mostly when both laboratory and clinical aging were performed, which is interesting and expected. Among the three performed tests of the present investigation, the bond strength was the only one related to the interaction of the dentin. In this case, the role of the solvent from the primer component, and more likely, its evaporation can be noted. Since only the Adper Single Bond Universal [SU] is solvated, regardless of LAB or CLIN aging, both compromised its performance drastically compared to its control condition.

Even the Clearfil SE Bond [SE] presented the lowest bonding strength values, in accordance with the literature. The most important observation is related to their similar values regardless of the condition. The 10-MDP based two-step self-etching system acts through chemical reaction to calcium from dentin. Moreover, Kinder *et al.* (2022)[26] demonstrated that experimental adhesives containing 6 wt% and 12 wt% MDP maintained stable bond strength values even after 12 months of water storage, highlighting the critical role of an optimal MDP concentration in ensuring long-term adhesion durability. In this case, the rationale of stable values is more relevant than the absolute data.

Bis-GMA is a high weight molecular monomer component observed in all DBSs tested in different concentrations, restricting its wetting performance [3,17]. Therefore, the combination of other monomers and/or diluents are common to adhesive systems. Among the 10-MDP monomer disadvantages, it could be more susceptible to modification conditions [8,25]. For example, when it is associated with a solvent such as the Adper Single Bond Universal [SU], when applied to the dentin, it can be more vulnerable, as evidenced by the bond strength tests.

Based on the dynamics of all these components, the manipulation can determine some differences. Longer studies are welcome to follow these procedures. Investigations are continuously looking for monomer-based systems that can be used under clinical services without comprising distinct surfaces such as dental tissues and ceramics [20]. Regardless of the DBS, it is mandatory that professionals be aware and encouraged to follow the manufacturers' instructions for adequate conditions and manipulation.

The present study demonstrated that all tested systems are susceptible to degradation and compromised performance when improperly handled. Clinically, inadequate manipulation can jeopardize bonding effectiveness, increase postoperative sensitivity, and lead to marginal failures. Therefore, special attention is required when solvents and functional monomers are combined in a single bottle, since their interaction increases vulnerability under clinical conditions.

CONCLUSION

The mechanical performance of dentin bonding systems is directly influenced by their composition, storage and handling conditions. The universal adhesive showed greater susceptibility to degradation, likely due to its complex formulation and solvent content. Improper bottle manipulation led to reductions in degree of conversion, surface microhardness, and bond strength, compromising bonding effectiveness. Therefore, optimal storage and handling protocols are essential to ensure long-term clinical performances of DBSs.

Author's Contributions

MCG: Data Curation, Formal Analysis, Investigation, Project Administration, Writing – Original Draft Preparation, Writing – Review & Editing. RP: Formal Analysis, Methodology, Writing – Original Draft Preparation. MRS: Formal Analysis, Methodology, Writing – Original Draft Preparation. CMM: Conceptualization, Formal Analysis, Investigation, Methodology. VM: Data Curation, formal analysis. JCJ: Data Curation, Formal Analysis, Writing – Original Draft Preparation. LW: Conceptualization, Data Curation, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Validation, Writing – Review & Editing.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding

The present study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001 and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) – Grants 2014/17225-8 and 2014/23430-3.

Regulatory Statement

The present study was approved by the Local Research Ethics Committee on Human Beings of the Bauru School of Dentistry – University of São Paulo (Protocol Number #49808515.1.0000.5417) and conducted in accordance with National Health Council Resolution Nº. 466/2012.

REFERENCES

1. van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. Biomaterials. 2007;28(26):3757-85. http://doi.org/10.1016/j.biomaterials.2007.04.044. PMid:17543382.
2. van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, et al. Relationship between bond-strength tests and clinical outcomes. Dent Mater. 2010;26(2):e100-21. http://doi.org/10.1016/j.dental.2009.11.148. PMid:20006379.
3. Perdigão J, Araujo E, Ramos RQ, Gomes G, Pizzolotto L. Adhesive dentistry: current concepts and clinical considerations. J Esthet Restor Dent. 2021;33(1):51-68. http://doi.org/10.1111/jerd.12692. PMid:33264490.
4. Breschi L, Maravic T, Cunha SR, Comba A, Cadenaro M, Tjäderhane L, et al. Dentin bonding systems: from dentin collagen structure to bond preservation and clinical applications. Dent Mater. 2018;34(1):78-96. http://doi.org/10.1016/j.dental.2017.11.005. PMid:29179971.
5. Giacomini MC, Scaffa PMC, Gonçalves RS, Zabeu GS, Vidal CMP, Carrilho MRO, et al. Profile of a 10-MDP-based universal adhesive system associated with chlorhexidine: dentin bond strength and in situ zymography performance. J Mech Behav Biomed Mater. 2020;110:103925. http://doi.org/10.1016/j.jmbbm.2020.103925. PMid:32957220.
6. Agulhari MAS, Froio NL, Jacomine JC, Giacomini MC, Borges AFS, Honório HM, et al. Profile of MDP-chlorhexidine for universal dentin bonding systems: A calcium-competition interference? Int J Adhes Adhes. 2022;116:1031-40. http://doi.org/10.1016/j.ijadhadh.2022.103140.
7. Zabeu GS, Scaffa PMC, Giacomini MC, Vidal CMP, Tjäderhane L, Wang L. Gelatinolytic activity after dentin pretreatment with dimethyl sulfoxide (DMSO) combined to dental bonding systems: perspectives for biological responses. J Mech Behav Biomed Mater. 2022;130:105188. http://doi.org/10.1016/j.jmbbm.2022.105188. PMid:35344756.
8. Pashley DH, Tay FR, Imazato S. How to increase the durability of resin-dentin bonds. Compend Contin Educ Dent. 2011;32(7):60-4. PMid:21910364.
9. Feitosa VP, Ogliari FA, van Meerbeek B, Watson TF, Yoshihara K, Ogliari AO, et al. Can the hydrophilicity of functional monomers affect chemical interaction? J Dent Res. 2014;93(2):201-6. http://doi.org/10.1177/0022034513514587. PMid:24284259.
10. Garcia FC, Wang L, Pereira LC, Andrade e Silva SM, Marquezini L Jr, Carrilho MR. Influences of surface and solvent on retention of HEMA/mixture components after evaporation. J Dent. 2010;38(1):44-9. http://doi.org/10.1016/j.jdent.2009.09.003. PMid:19737594.
11. Carvalho CN, Lanza MD, Dourado LG, Carvalho EM, Bauer J. Impact of solvent evaporation and curing protocol on degree of conversion of etch-and-rinse and multimode adhesives systems. Int J Dent. 2019;2019:5496784. http://doi.org/10.1155/2019/5496784. PMid:31097965.
12. Balkaya H, Demirbuğa S. Evaluation of six different one-step universal adhesive systems in terms of dentin bond strength, adhesive interface characterization, surface tension, contact angle, degree of conversion and solvent evaporation after immediate and delayed use. J Esthet Restor Dent. 2023;35(3):479-92. http://doi.org/10.1111/jerd.12973. PMid:36194081.
13. Frankenberger R, Reinelt C, Petschelt A, Krämer N. Operator vs. material influence on clinical outcome of bonded ceramic inlays. Dent Mater. 2009;25(8):960-8. http://doi.org/10.1016/j.dental.2009.02.002. PMid:19344946.
14. Unlu N, Gunal S, Ulker M, Ozer F, Blatz MB. Influence of operator experience on in vitro bond strength of dentin adhesives. J Adhes Dent. 2012;14(3):223-7. http://doi.org/10.3290/j.jad.a22191. PMid:22043471.
15. Peumans M, De Munck J, Van Landuyt KL, Poitevin A, Lambrechts P, van Meerbeek B. Eight-year clinical evaluation of a 2-step self-etch adhesive with and without selective enamel etching.

Dent Mater. 2010;26(12):1176-84. http://doi.org/10.1016/j.dental.2010.08.190. PMid:20947155.

16. Armstrong S, Breschi L, Özcan M, Pfefferkorn F, Ferrari M, van Meerbeek B. Academy of Dental Materials guidance on in vitro testing of dental composite bonding effectiveness to dentin/enamel using micro-tensile bond strength (μTBS) approach. Dent Mater. 2017;33(2):133-43. http://doi.org/10.1016/j.dental.2016.11.015. PMid:28007396.

17. 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. http://doi.org/10.3290/j.jad.a43994. PMid:32030373.

18. Perdigão J, Ramose JC, Lambrechts P. In vitro interfacial relationship between human dentin and one-bottle dental adhesives. Dent Mater. 1997;13(4):218-27. http://doi.org/10.1016/S0109-5641(97)80032-2. PMid:11696900.

19. Jacomine JC, Giacomini MC, Agulhari M, Honório HM, Wang L. Twenty-month performance of a universal bonding system on simulated-challenged dentin substrates pretreated with chlorhexidine. Oper Dent. 2023;48(2):196-206. http://doi.org/10.2341/21-142-L. PMid:36656311.

20. Costa MP, Giacomini MC, Zabeu GS, Mosquim V, Dallavilla GG, Santos PSDS, et al. Impact of functional monomers, bioactive particles, and HEMA, on the adhesive performance of self-etch adhesive systems applied to simulated altered dentin. J Dent. 2024;151:105379. http://doi.org/10.1016/j.jdent.2024.105379. PMid:39341447.

21. Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, et al. Solvent and water retention in dental adhesive blends after evaporation. Biomaterials. 2005;26(34):6863-72. http://doi.org/10.1016/j.biomaterials.2005.05.011. PMid:15964621.

22. Aguilar-Mendoza JA, Rosales-Leal JI, Rodríguez-Valverde MA, González-López S, Cabrerizo-Vílchez MA. Wettability and bonding of self-etching dental adhesives. Influence of the smear layer. Dent Mater. 2008;24(7):994-1000. http://doi.org/10.1016/j.dental.2007.11.013. PMid:18295326.

23. Fabre HS, Fabre S, Cefaly DF, de Oliveira Carrilho MR, Garcia FC, Wang L. Water sorption and solubility of dentin bonding agents light-cured with different light sources. J Dent. 2007;35(3):253-8. http://doi.org/10.1016/j.jdent.2006.09.002. PMid:17045723.

24. Hashimoto M. Effects of air on volatile compound contents in resins. Nanobiomedicine. 2011;3(4):253-60.

25. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res. 2004;83(6):454-8. http://doi.org/10.1177/154405910408300604. PMid:15153451.

26. Kinder GR, Furuse AY, Costa RM, Lucena FS, Correr GM, Gonzaga CC. Effect of MDP concentration and addition of iodonium salt on the dentin bond strength of experimental adhesives. Braz Dent Sci. 2022;25(1):e2933. https://doi.org/10.4322/bds.2022.e2933.

Linda Wang
(Corresponding address)
Universidade de São Paulo, Faculdade de Odontologia de Bauru, Departamento de Dentística, Endodontia e Materiais Odontológicos, Bauru, SP, Brazil.
Email: wang.linda@usp.br

Editor: Sergio Eduardo de Paiva Gonçalves

Date submitted: 2025 Apr 21
Accept submission: 2025 Jul 19