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Effect of the Absence of HEMA on the Bonding Properties of Universal Adhesive Systems Containing 10-MDP: An *In Vitro* Study

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Clinical Relevance

This study supports clinicians in selecting the most adequate universal adhesive system, demonstrating that important chemical characteristics such as pH, type of solvent, and the presence or absence of HEMA ought to be taken into account.

SUMMARY

Objectives*:* To evaluate the absence of 2-hydroxyethyl methacrylate (HEMA) on the adhesive properties with enamel and dentin of universal adhesive systems containing 10-methacryloyloxydecyl dihydrogen phosphate (MDP).

Methods and Materials*:* One hundred and twelve caries-free third molars were used to test

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adhesion to dentin (n=64) and enamel (n=48). For each substrate, teeth were divided into eight experimental groups: four different adhesives each using two adhesive strategies. The adhesives used were: (1) Scotchbond Universal (SBU, 3M Oral Care, St Paul, MN, USA) as a HEMA-containing universal adhesive; (2) Gluma Bond Universal (GBU, Kulzer, Hanau, Germany); (3) Solare Universal Bond (SUB, GC, Tokyo, Japan); and (4) Zipbond Universal (ZIP, SDI, Victoria, Australia) as HEMA-free universal adhesives. The adhesive strategies used were etch-and-rinse (ER) and self-etch (SE). For dentin tests, the occlusal third of the crown of all teeth was removed and an adhesive protocol was applied. After completing the restoration, specimens were sectioned into bonded sticks (0.8 mm^2) and tested for microtensile bond strength (μTBS), *in situ* degree of conversion (DC), and nanoleakage (NL) by scanning electron microscopy. For enamel tests, teeth were sectioned into four parts (buccal, lingual, and proximal), and an adhesive protocol was applied. After completing the restoration, the specimens were tested for their microshear bond strength (μSBS). For *in situ* degree of conversion (DC) and nanoleakage (NL) evaluation of enamel, the specimens were sectioned in slices to be evaluated. The data for each substrate were subjected to two-way ANOVA and Tukey's test (α=0.05) for each property evaluated.

Results: The SBU and ZIP adhesives showed the highest μSBS, and DC (dentin and enamel) and lower NL (dentin) values compared to GBU and SUB (p=0.001). However, SBU showed better results in terms of μTBS and μSBS (SE strategy), and DC (dentin and enamel) than ZIP. Strategy ER presented higher values of μTBS and μSBS when compared to strategy SE (p=0.001), except for SBU.

Conclusion*:* The effect of the absence of HEMA in commercial universal adhesive systems on enamel and dentin adhesive properties appears to be material-dependent.

INTRODUCTION

The search for simplified and less sensitive techniques for dental adhesive application has resulted in countless research and development efforts by manufacturers.[1] The emergence of universal adhesive (UA) systems in 2011[2-4] brought a new perspective to this research area. The addition of functional resinous monomers to the adhesives provides chemical bonding, allowing them to be used in direct and indirect procedures to bond different substrates (enamel, dentin, ceramic, etc).[5,6]

Among the components of these adhesive systems, 10-methacryloyloxydecyl dihydrogen phosphate (MDP) has become the most used. This compound is so effective because it binds ionically to the hydroxyapatite (HAp) present in enamel and dentin, and forms nanolayers of MDP-Ca. These nanolayers enable chemical adhesion to the dental surface and protect the hybrid layer against hydrolysis.[7]

Unfortunately, several concerns have been raised in relation to the adhesive performance of MDP-containing adhesives.[8] For instance, while Muñoz and others and Cardoso and others[9,10] showed that most MDP-containing adhesives have stable bonds with dentin, Zhang and others[11] and Sai and others[12] showed that all universal adhesives, including those containing MDP, degraded after 6-12 months of water storage. Factors such as the concentration of MDP, MDP purity, and interactions with other monomers could be responsible for some of the conflicting results.[13,14] Recent evidence shows that the presence of 2-hydroxyethyl methacrylate (HEMA) may affect the capacity of MDP to interact with HAp powder.[15]

HEMA is a hydrophilic monomer, well-known to improve the wetting properties of adhesive systems on dental substrates. This makes it an excellent adhesion promoter because it increases the diffusion and miscibility of components, improves the formation of the hybrid layer[16], and minimizes phase separation.[17] It is also known that HEMA promotes the formation of an unstable aqueous gel that is susceptible to hydrolytic degradation.[18,19] Another drawback of its use in UAs is that HEMA has a negative interaction with MDP, which significantly reduces the demineralization of HAp. This decreases the formation of MDP Ca salts and partially inhibits the deposition of the nanolayers, which are necessary to obtain an adequate chemical interaction with the dentin substrate.[20] Recently, commercial MDP-containing, HEMA-free universal adhesives have been launched onto the market (Gluma Bond Universal, Kulzer, Hanau, Germany; Solare Universal Bond, GC Corp, Tokyo, Japan, and Zipbond Universal, SDI, Bayswater, Australia).

Although the negative interaction between these two monomers has been previously described for HAp powder,[13] to the best of the authors' knowledge, the effect of the presence of HEMA in commercial MDP-containing UAs on the bonding to enamel and dentin has not been fully clarified.

Therefore, this *in vitro* study aims to evaluate the effect of the removal of HEMA from UA systems containing MDP and its effect on adhesion to dentin and enamel.

One HEMA and MDP-containing UA was used as control. The null hypotheses tested were that the absence of HEMA in universal adhesives containing MDP would not influence (1) the bond strength, (2) the degree of conversion, and (3) the silver nitrate uptake in the hybrid layer formed by universal adhesives when applied to dentin and enamel.

METHODS AND MATERIALS

Tooth Selection

One hundred and twelve caries-free, human third molars were used in this study. The teeth were collected according to the guidelines of the ethics committee. The samples were disinfected with 0.5% chloramine and stored in distilled water until use. Sixty-four teeth were used for dentin evaluation, and 48 teeth were used for enamel evaluation.

Sample Size Calculation

For microtensile bond strengths (μTBS) to dentin, the sample size was determined by considering literature μTBS mean and standard deviation values of Scotchbond Universal (SBU) on the sound dentin stated to be 53.6 ± 5.0 MPa.[4,21,22] Using alfa of 0.05, a power of 80%, to detect a difference of 8 MPa, and considering possible losses, the minimum sample size was eight teeth per group. For microshear bond strengths (SBS) to enamel, the sample size was determined by considering literature μSBS mean and standard deviation values of SBU on the enamel stated to be 18.4 ± 4.5 MPa.[9,23,24] To detect a difference of 3.3 MPa among the tested groups using a two-sided test and a significance level and power of 5 and 80%, respectively, the minimum sample size was four teeth per group. Both sample sizes were calculated using a website (www.sealedenvelope.com).

Experimental Design and Groups

The occlusal third of the crown of the 64 human molars (n=8 per group) selected for dentin tests was removed with a diamond saw under water cooling in a cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) to obtain a flat dentin coronal surface. The enamel around the margins was removed using a diamond bur (#3195, KG Sorensen, Barueri, SP, Brazil). Subsequently, the exposed dentin surfaces were polished for 60 seconds on wet #600-grit silicon carbide paper; thus, the smear layer was standardized.

For enamel, the roots of the 48 molars selected (n=6 per group) were removed by cutting at the cemento-enamel junction, and the crowns were sectioned diagonally along the long axes of the teeth.[9] One hundred ninety-two enamel specimens, which originated from 48 teeth, were used for evaluation of μSBS, *in situ* degree of conversion, and nanoleakage at the resin–enamel interfaces.

After substrate preparation, the specimens of dentin and enamel were randomly assigned to eight experimental groups: four different adhesives, each using two adhesive strategies. The adhesives tested were: Scotchbond Universal (SBU, 3M Oral Care, St Paul, MN, USA, also known as Single Bond Universal in some countries) as a HEMA-containing universal adhesive; and Gluma Bond Universal (GBU, Kulzer, Hanau, Germany, also known as iBond Universal in some countries); Solare Universal Bond (SUB, GC, Tokyo, Japan, also known as G-Premio Bond and G-aenial Bond in some countries); and Zipbond Universal (ZIP, SDI, Victoria, Australia) as HEMA-free universal adhesives. The adhesive strategies used were etch-and-rinse (ER) and self-etch (SE).

Specimen Preparation and Adhesive Application for Dentin

Universal adhesives were applied to dentin following the manufacturer's instructions (Table 1). For dentin, 64 teeth were randomly assigned to eight experimental groups (n=8). After dentin restoration and preparation, all resin-dentin bonded sticks from each tooth were used for microtensile bond strength tests (μTBS), except for six sticks from each tooth, which were randomly divided for measurement of the degree of conversion (DC, n=3) and nanoleakage (NL, n=3).

Restorative Procedure and Resin-dentin Microtensile Bond Strength (μTBS)

After the bonding procedure, a composite restoration (Opallis, FGM, Joinville, SC, Brazil) was applied in 2-3 increments of 2 mm thickness, and each was light-cured for 40 seconds at 1 mm of distance, using an LED unit set at 1400 mW/cm^2 (Valo, High Power Mode, Ultradent Products, Inc, South Jordan, UT, USA) for all specimens. A single trained operator performed all the procedures.

The teeth were stored in distilled water at 37°C for 24 hours. Afterward, they were sectioned into mesio-distal and buccal-lingual segments using a cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) to obtain resin-dentin bonded sticks with a cross-sectional area of approximately 0.8 mm^2, as measured by a digital caliper (Digimatic Caliper, Mitutoyo, Tokyo, Japan).

To measure the microtensile bond strength values, the resin-dentin bonded sticks were attached to a Geraldeli's jig with cyanoacrylate resin and tested in

Table 1: *Adhesive Systems, Composition, and Application Mode*

Adhesive Systems (Batch Number)	pH	Composition	Self-etch Strategy	Etch-and-rinse Strategy
Scotchbond Universal (SBU; 3M Oral Care #1926900596) As HEMA-containing adhesive	2.5	10-MDP phosphate monomer, dimethacrylate resins, HEMA, methacrylate-modified polyalkenoic acid copolymer, filler, ethanol (10%-15%), water, initiators, silane	Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. If necessary, rewet the disposable applicator during treatment. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely. Light-cure for 10 s.	Apply etchant for 15 s. Rinse for 15 s. Air dry 2 s. Apply adhesive as for the self-etch mode.
Gluma Bond Universal (Kulzer, South América, #K010039) As HEMA-free adhesive	1.5	10-MDP, Bis-GMA, 4-ethyl dimethyl aminobenzoate, cetylamine hydrofluoride, initiator, acetone (25%-50%), water	Apply the adhesive to the entire preparation with a microbrush and rub it in for 20 s. Direct a gentle stream of air over the liquid for about 5 s until it no longer moves and the solvent has evaporated completely. Light-cure for 10 s.	Apply etchant for 15 s. Rinse for 15 s. Air dry 2 s. Apply adhesive as for the self-etch mode.
Solare Universal Bond (GC, Germany, #181252) As HEMA-free adhesive	1.3	4-META, UDMA, TEGDMA, 10-MDP, acetone (25-50%), water, silanated colloidal silica, initiators	Apply the adhesive to the entire preparation with a microbrush and rub it in for 10 s. Evaporate excess solvent by thoroughly air-drying with an air syringe for at least 10 s. Light-cure for 10 s.	Apply etchant 15 s. Rinse for 15 s. Air dry 2 s. Apply adhesive as for the self-etch mode.
Zipbond Universal (SDI, Germany, #190713) As Hema-free adhesive	2.5	10-MDP, ethanol (30%-35%), initiator, water, fluoride.	Apply the adhesive to the entire preparation with a microbrush and rub it in for 10 s. Wait another 10 s. Evaporate excess solvent by air-drying for 5 s. Light-cure for 10 s.	Apply etchant 15 s. Rinse for 15 s. Air dry 2 s. Apply adhesive as for the self-etch mode.

Abbreviations: Bis-GMA, bisphenol A diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; 4-META, 4-methacryloyloxyethy trimellitate anhydride; 10-MDP, 10-methacryloyloxdecyl dihydrogen phosphate; UDMA, urethane dimethacrylate; TEGDMA: triethylene glycol dimethacrylate.

a universal testing machine (Instron, São José dos Pinhais, PR, Brazil) at 0.5 mm/min until failure. The bond strengths were calculated by dividing the load at failure by the cross-sectional bonding area.

All fractured resin-detin bonded sticks were analyzed using a digital microscope at 100× magnification (Olympus SZ40, Tokyo, Japan), and the failure mode was classified as follows: (1) cohesive (C, failure exclusively within the resin composite or the dentin); (2) adhesive (A, failure at the resin-dentin interface); or (3) mixed (M, failure inside any of the bonded substrates).

Specimen Preparation and Adhesive Application for Enamel

For enamel, 92 specimens (n=24 per group) were embedded in a PVC ring filled with acrylic resin, displaying the enamel surface on the top of the cylinder. Acid-resistant, double-faced adhesive tape

(Adelbras Ind e Com Adesivos Ltda, SP, Brazil) which had been perforated using a hygienic Ainsworth-style rubber-dam punch with a known surface area (Coltene, Alstätten, Switzerland) was placed on the enamel surface. The enamel surfaces were then bonded according to the manufacturer's instructions (Table 1).

A polyethylene Tygon tube (Tygon Medical Tubing Formulations 54-HL, Saint Gobain Performance Plastics, Akron, OH, USA), with a length of 0.5 mm and the same internal diameter as the perforations, was positioned over the double-faced tape, with the lumen coincident with the perforations, to obtain a cross-sectional area of 0.5 mm^2. Using a digital microscope (Olympus SZ40, Tokyo, Japan), a trained operator positioned six to eight tubes per surface and packed resin composite inside each tube. A clear Mylar matrix strip was placed over the filled Tygon tube and pressed gently into place and light-cured for 20 seconds using an LED unit set at 1400 mW/cm^2 (Valo, High Power Mode, Ultradent Products).

After storage in distilled water for 24 hours at 37°C, the tygon tubes and double-faced tape were removed using a surgical blade to expose the resin cylinders. Each restored specimen was examined under a stereomicroscope at 10× magnification. The bonded cylinder was discarded if there was any evidence of gaps or porosity at the interface.

After enamel restoration, the 92 restored enamel specimens (n=24 per group) were divided according to the following tests: 16 for resin-enamel bond strength (μSBS, n=16), 4 for the degree of conversion (DC, n=4), and 4 for the nanoleakage (NL, n=4).

Resin-enamel Microshear Bond Strength (μSBS)

For shear testing, the specimens were attached to a specific accessory (Odeme Biotechnology) and tested using a universal testing machine (Instron, São José dos Pinhais). The specimens were placed onto the machine, and a thin orthodontic wire (0.2 mm diameter) was looped around the base of each composite cylinder. The setup was kept aligned (resin–enamel interface, wire loop, and center of the load cell) to ensure the correct orientation of the shear forces, and the shear load was applied at 1 mm/min until failure. The μSBS values were calculated by dividing the load at failure by the surface area (mm^2). All fractured bonded specimens were analyzed using a digital microscope at 100× (Olympus SZ40, Tokyo, Japan), and the failure mode was classified in the manner described in the previous section.

In situ Degree of Conversion (DC) for Dentin and Enamel

The previously obtained resin-dentin bonded sticks were used for the dentin measurements. For the enamel measurements, the resin-enamel bonded specimens were sectioned longitudinally across the bonded interface with a low-speed diamond saw (Isomet, Buehler) to obtain two slices of the resin-enamel bonded interfaces.

All specimens to be evaluated were polished on wet #1500- and #2000-grit silicon carbide paper, ultrasonically cleaned, and measured by a micro-Raman spectrometer (XploRA ONE Raman microscope, HORIBA Scientific, Piscataway, NJ, USA), which was previously calibrated to zero with the following configuration: a 785-nm diode laser; 100× objective; 600-lines/mm grafting centered between 500 and 2000 cm^{-1}; 100 mW power; 3 μm spatial resolution; 1 cm^{-1} of spectral resolution; 30 seconds of accumulation time; and six co-additions. First, the spectra of non-polymerized adhesives were measured. Then three spectra at random sites were measured for each resin-dentin bonded stick within the hybrid layer in intertubular-infiltrated dentin, as well as resin-enamel bonded slices within the hybrid layer in the enamel interface. The ratio of the aliphatic and aromatic peak intensities, at 1638 cm^{-1} and 1608 cm^{-1}, respectively, in the cured and uncured adhesives were used to determine the DC.

Nanoleakage Evaluation (NL) for Dentin and Enamel

The dentin and enamel specimens were prepared in the same way as for the degree of conversion measurements. All specimens to be evaluated were placed in an ammonical silver nitrate solution in the dark for 24 hours, rinsed in distilled water, and immersed in a photo-developing solution under fluorescent light for 8 hours. The specimens were then polished with 2500-grit SiC paper, ultrasonically cleaned, air dried, mounted on stubs, and coated with carbon-gold (MED 010, Balzers Union, Balzers, Liechtenstein). The silver penetration levels were analyzed using a field-emission scanning electron microscope (FE-SEM) operated in the backscattering mode (VEGA 3 TESCAN, Shimadzu, Tokyo, Japan). Three SEM images of each specimen were captured at each bonded stick resin-dentin interface. The relative percentages of NL along the adhesive and hybrid layers were evaluated using the public-domain ImageJ software.

Statistical Analysis

The experimental unit in the present study was the tooth for dentin and enamel, as all the specimens

from the same tooth were tested. The mean values of μTBS for dentin (MPa) and μSBS (MPa) for enamel, as well as the mean values of DC (%) and NL (%) for dentin and enamel from the same tooth, were averaged for statistical purposes. After observing the normality of the data distribution (Kolmogorov-Smirnov test) and the equality of the variances (Bartlett's test), all data were subjected to a two-way ANOVA statistical analysis (adhesive vs adhesive strategy) and Tukey's *post hoc* test for pairwise comparisons (α=0.05).

RESULTS

Resin-dentin Interface Evaluation

Most of the specimens were classified as adhesive/mixed failures (97.3%). A low percentage of cohesive failures (2.76%) was observed, for all adhesives and adhesive strategies (Table 2).

Regarding the resin-dentin microtensile bond strength, the results obtained in MPa were as follows: SBU (ER: 46.2±3.7; SE: 48.6±3.3), GBU (ER: 38.6±2.9; SE: 32.4±2.2), SUB (ER: 36.1±3.2; SE: 28.8±3.4), and ZIP (ER: 46.4±3.9; SE: 37.4±3.1). The cross-product interaction was statistically significant (p=0.001; Table 3). In terms of adhesives, SBU and ZIP exhibited higher μTBS values than those of GBU and SUB for both strategies (p=0.001; Table 3). However, SBU showed higher μTBS values in the SE strategy than ZIP (p=0.001; Table 3). Regarding the adhesive strategy, the μTBS values of the ER strategy were higher than those of the SE strategy (p=0.001; Table 3), except SBU, which exhibited the highest μTBS values in the SE strategy among all adhesives (p=0.001; Table 3).

Regarding the DC for the resin-dentin interface, the results obtained were as follows: SBU (ER: 59.5±3.7; SE: 48.2±3.3), GBU (ER: 36.1±1.5; SE: 35.4±3.6), SUB (ER: 31.4±3.4; SE: 31.7±2.3), and ZIP (ER: 53.7±3.5; SE: 42.7±1.0). The cross-product interaction was statistically significant (p=0.0001; Table 3). SBU and ZIP adhesives exhibited higher DC values for both strategies when compared to GBU and SUB (p=0.0001; Table 3). It is worth mentioning that SBU showed higher DC values than ZIP in both strategies (p=0.0001; Table 3). Regarding the adhesive strategy, only higher DC in the ER strategy than in the SE strategy was observed for SBU and ZIP (p=0.0001; Table 3). However, there was no significant difference in the DC for GBU and SUB in ER and SE strategies (p>0.05).

In terms of NL for the resin-dentin interface, the results obtained were as follows: SBU (ER: 4.6±1.7; SE: 3.3±0.7), GBU (ER: 9.1±1.1; SE: 7.7±1.1), SUB (ER: 8.4±2.9; SE: 10.5±1.8), and ZIP (ER: 6.0±0.9; SE: 2.9±0.7). The cross-product interaction, as well as the adhesive strategy, was not statistically significant (p=0.62 and p=0.78, respectively). Only the factor adhesive was statistically significant (p=0.001; Table 4). When comparing adhesives, SBU and ZIP exhibit lower NL values than those of GBU and SUB for both strategies (p=0.001; Table 3). Regarding the adhesive strategy, there was no significant difference in NL values when comparing ER and SE, for all adhesives (p>0.05; Table 3). In general, silver nitrate deposits were observed within the hybrid layer for all adhesives and strategies evaluated (Figure 1).

Resin-enamel Interface Evaluation

Most of the specimens were classified as adhesive/mixed failures (96.5%). A low percentage of cohesive

Table 2: *Number of Specimens (%) According to Fracture Mode and the Premature Failure of All Experimental Groups for Dentin and Enamel*

Adhesive System	Application Mode	Fracture Pattern					
		Dentin			Enamel		
		C	A/M	PF	C	A/M	PF
Scotchbond Universal	ER	2 (6.5)	30 (93.5)	0 (0)	2 (7.1)	26 (92.9)	0 (0)
	SE	1 (3.1)	31 (96.9)	0 (0)	3 (10.7)	25 (89.3)	0 (0)
Gluma Bond Universal	ER	0 (0)	32 (100)	0 (0)	1 (3.6)	27 (96.4)	0 (0)
	SE	0 (0)	26 (81.3)	4 (18.7)	0 (0)	24 (85.7)	4 (14.3)
Solare Universal Bond	ER	0 (0)	31 (96.9)	1 (3.1)	0 (0)	28 (100)	0 (0)
	SE	0 (0)	32 (100)	0 (0)	0 (0)	27 (96.4)	1 (3.6)
Zipbond Universal	ER	3 (9.3)	29 (90.7)	0 (0)	2 (7.1)	26 (92.9)	0 (0)
	SE	1 (3.1)	31 (96.9)	0 (0)	0 (0)	27 (100)	0 (0)

Abbreviations: C, cohesive fracture mode; A/M, adhesive or mixed fracture mode; PF, premature failure.

Table 3: *Means and Standard Deviations of Microtensile Bond Strength (MPa),* in situ *Degree of Conversion (%), and Nanoleakage (%) to Dentin for All Experimental Groups*[a]

Adhesives	Strategy	Microtensile Bond Strength (MPa)	*In Situ* Degree of Conversion (%)	Nanoleakage (%)
Scotchbond Universal	Etch-and-rinse	46.2 ± 3.7 A	59.5 ± 3.7 a	4.6 ± 1.7 AB
	Self-etch	48.6 ± 3.3 A	48.2 ± 3.3 b	3.3 ± 0.7 AB
Gluma Bond Universal	Etch-and-rinse	38.3 ± 2.9 B	36.1 ± 1.5 d	9.1 ± 1.1 C
	Self-etch	32.4 ± 2.2 C	35.4 ± 3.6 d	7.7 ± 1.1 C
Solare Universal Bond	Etch-and-rinse	36.1 ± 3.2 B	31.4 ± 3.4 d	8.4 ± 2.9 C
	Self-etch	28.8 ± 3.4 C	31.7 ± 2.3 d	10.5 ± 1.8 C
Zipbond Universal	Etch-and-rinse	46.4 ± 3.9 A	53.7 ± 3.5 b	6.0 ± 0.9 B
	Self-etch	37.4 ± 3.1 B	42.7 ± 1.0 c	2.9 ± 0.7 AB

[a]*For each test, different uppercase or lowercase letters mean differences statistically significant between groups for each test (two-way ANOVA; Tukey test,* p*<0.05; For each test, eight teeth were used for experimental condition).*

failures (3.5%) were observed in the enamel and resin composite, for all adhesives and adhesive strategies (Table 2).

Regarding resin-enamel microshear bond strength, the results obtained in MPa were as follows: SBU (ER: 12.5±1.1; SE: 13.9±1.5), GBU (ER: 13.6±1.8; SE: 8.5±1.6), SUB (ER: 7.8±1.6; SE: 6.1±0.9), and ZIP (ER: 11.4±1.1; SE: 5.9±1.7). The cross-product interaction was statistically significant (p=0.01; Table 4). Among adhesives, SBU exhibited the highest μSBS values in the SE strategy when compared to all adhesives in the SE mode (p=0.01; Table 4). In the ER strategy, SUB has the lowest μSBS values among all adhesives (p=0.01; Table 4). Regarding the adhesive strategy, GBU and ZIP showed higher μSBS values in the ER than in the SE mode (p=0.01; Table 4).

Regarding the DC for the resin-enamel interface, the results obtained were as follows: SBU (ER: 49.4±1.1; SE: 49.7±3.1), GBU (ER: 36.3±4.2; SE: 33.2±2.5), SUB (ER: 33.6±2.9; SE: 29.7±5.9), and ZIP (ER: 42.9±3.5; SE: 42.7±3.0). The cross-product interaction, as well as the adhesive strategy, were not statistically significant (p=0.32 and p=0.64, respectively). Only the factor adhesive was statistically significant (p=0.00001; Table 4). SBU and ZIP adhesives exhibited higher DC values when compared to GBU and SUB for both strategies (p=0.00001; Table 4). However, SBU showed higher DC values to enamel than ZIP in both adhesive strategies (p=0.00001; Table 4). Regarding the adhesive strategy, there was no significant difference in DC values when comparing ER and SE, for all adhesives evaluated (p>0.05; Table 4).

Table 4: *Means and Standard Deviations of Microshear Bond Strength (MPa),* in situ *Degree of Conversion (%), and Nanoleakage (%) to Enamel for All Experimental Groups*[a]

Adhesives	Strategy	Microshear Bond Strength (MPa)	*In Situ* Degree of Conversion (%)	Nanoleakage (%)
Scotchbond Universal	Etch-and-rinse	12.5 ± 1.1 A	49.4 ± 3.1 a	0.0 ± 0.0 A
	Self-Etch	13.9 ± 1.5 A	49.7 ± 3.1 a	0.0 ± 0.0 A
Gluma Bond Universal	Etch-and-rinse	13.6 ± 1.8 A	36.3 ± 4.2 c	0.02 ± 0.04 A
	Self-Etch	8.5 ± 1.6 B	33.2 ± 2.5 cd	0.03 ± 0.02 A
Solare Universal Bond	Etch-and-rinse	7.8 ± 1.6 B	33.6 ± 2.9 cd	0.02 ± 0.01 A
	Self-Etch	6.1 ± 0.9 B	29.7 ± 5.9 d	0.03 ± 0.03 A
Zipbond Universal	Etch-and-rinse	11.4 ± 1.0 A	42.9 ± 3.5 b	0.04 ± 0.04 A
	Self-Etch	5.9 ± 1.7 B	42.7 ± 3.0 b	0.08 ± 0.04 A

[a] *For each test, different uppercase or lowercase letters mean differences statistically significant between groups (two-way ANOVA; Tukey test,* p*<0.05; For each test, six teeth were used for experimental condition).*

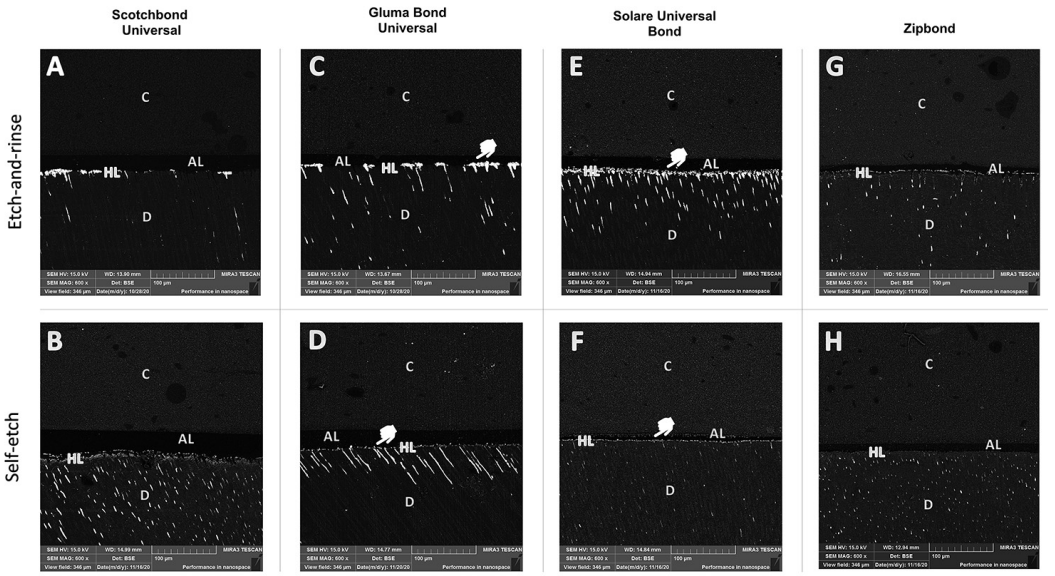

Figure 1. *Representative backscattered scanning electron microscope (SEM) images of the resin-dentin adhesive interfaces of each experimental group. Observe that the amount of nanoleakage was lower and practically occurred within the hybrid layer for Scotchbond Universal (A-B) and Zipbond Universal (G-H). For Gluma Bond Universal (C-D) and Solare Universal Bond (E-F), the amount of NL was higher than that of the other materials, with most silver nitrate uptake occurring throughout the entire thickness of the HL (white hands indicator). Abbreviaitons: C, composite; AL, adheslive layer, HL, hybrid layer, D, dentin.*

In terms of NL for the resin-enamel interface, the cross-product interaction, as well as the main factors were not statistically significant (p=0.23; p=0.39 and p=0.72, respectively; Table 4). As observed in Table 4, the NL values were so low on groups GBU, SUB, and ZIP they didn't differ from the zero NL observed on SBU. This was confirmed in Figure 2 where only a few spots of silver nitrate uptake were observed.

DISCUSSION

This study was conducted to investigate three different MDP-containing and HEMA-free UAs in comparison

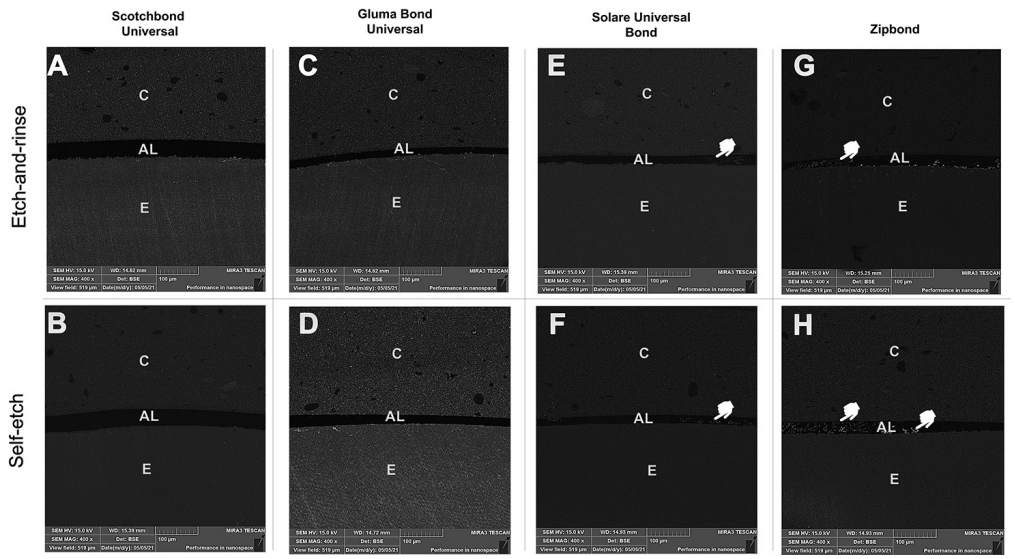

Figure 2. *Representative backscattered SEM images of the resin-enamel adhesive interfaces of each experimental group. Despite, no nanoleakage was observed in the Scotchbond Universal adhesive (A-B), usually, the amount of NL was so low in the other groups (Gluma Universal Bond; C-D, Solare Universal Bond, E-F and Zipbond Universal; G-H) that it was not possible to observe some significant difference among them. Only a few spots of silver nitrate uptake were observed (white hands indicators in E-H). Abbreviations: C, composite; AL, adheslive layer, E, enamel*

with one UA containing MDP and HEMA, considering their immediate bonding properties to enamel and dentin. GBU and SUB, two HEMA-free UAs, demonstrated lower adhesive performance (μTBS, DC, and NL to dentin and DC to enamel) when compared to ZIP, a HEMA-free UA, and SBU, a HEMA-containing adhesive and the first universal adhesive launched in the market. This resulted in the partial rejection of all null hypotheses.

As described in the introduction section, HEMA is the most-used hydrophilic monomer in simplified adhesives, mainly because HEMA allows higher miscibility among different components. Owing to its low molecular weight, HEMA allows higher diffusion inside the dentin.[16] However, due to greater water sorption,[25,26] adhesives containing HEMA are highly susceptible to hydrolytic degradation[18,19] resulting in pronounced degradation of the adhesive interface in the oral cavity; HEMA-free adhesives do not degrade to this extent.[27,28]

Furthermore, the interaction between HEMA and MDP causes a drastic decrease in MDP chemical bonding.[13] Yoshida and others[13] evaluated experimental adhesives with different concentrations of HEMA using HAp powder. Higher HEMA concentrations inhibit interfacial nanolayering, owing to the inability of HEMA to increase the demineralization performance of MDP.[20]

However, contrasting results were observed when HEMA-free UAs were compared. ZIP exhibited higher μTBS and DC, as well as lower NL to dentin for both strategies when compared to GBU and SUB. These dissimilar results for different HEMA-free UAs demonstrate that factors other than the presence of HEMA should be considered when choosing a UA.

A closer view of the composition of the HEMA-free UAs reveals significant differences. ZIP is ethanol-based, while GBU and SUB are acetone-based UAs. According to the material safety data sheet of each manufacturer, GBU and SUB contain approximately 25-50% acetone, whereas ZIP contains 30-35% ethanol.[29-31] Acetone is a solvent recognized for its higher vapor pressure compared to solvents such as ethanol and water.[17] However, acetone undergoes rapid evaporation, which increases the concentration of monomers. This may not allow sufficient time for the monomers to adequately infiltrate the dentin. As a result, pores are formed in the cured adhesive interface.[32,33] These pores displayed higher NL values in dentin and enamel for GBU and SUB when compared to ZIP, in agreement with previous studies.[32]

The pH of self-etching primers is a parameter used to classify the adhesives; additionally, it is a factor that accounts for the higher NL values in GBU and SUB when compared to that in ZIP.[5] While GBU and SUB exhibited pH values of 1.5 and 1.3, respectively, the pH of ZIP was 2.5.[29-31] According to Van Meerbeek and others,[5] GBU and SUB are considered intermediary strong adhesives, whereas ZIP is considered an ultra-mild UA. Intermediary strong adhesives result in a more profound demineralization of enamel and dentin than mild adhesives.[5] However, owing to the dissimilar depth of penetration and demineralization, a partially demineralized, uninfiltrated zone of dentin was observed beneath the hybrid layer when intermediary strong adhesives were applied.[34,35] The same phenomenon was previously observed for universal adhesives.[32] These regions of incomplete monomer penetration are infiltrated by silver nitrate, leading to a higher amount of NL for GBU and SUB when compared to ZIP.

In addition, these NL regions potentially represent areas of suboptimal conversion within the polymer matrix, due to incomplete solvent removal.[36] GBU and SUB showed lower *in situ* DC values in the enamel and dentin when compared to ZIP. The higher NL values associated with lower *in situ* DC for GBU and SUB significantly impact the bond strength at the dentin interface, when compared to ZIP, as previously observed in the literature.[32,37,38]

Despite differences in the composition of HEMA-free UAs evaluated in the present study, no significant difference was observed in the bond strength at the enamel interface when HEMA-free UAs (GBU and ZIP) were compared to HEMA-containing UA (SBU), in the ER strategy. However, SUB showed the lowest results in ER strategy among all adhesives tested. We hypothesized that it occurs due to the low application time recommended by the manufacturer, and evidence indicates that an active and prolonged application time may increase monomer diffusion.[39,40]

All HEMA-free UAs showed a slight amount of NL at the enamel interface. Silver nitrate uptake was observed in the partially demineralized uninfiltrated zones (classic NL) on dentin. Whereas, on enamel, NL was observed inside the adhesive layer, in the phenomenon known as "water trees".[36] These factors are related to the presence of water droplets on the adhesive layer, indicating the phenomenon of phase separation, in which adhesive monomers separate from water upon evaporation of ethanol or acetone.[17] In fact, HEMA-free adhesives are highly prone to phase separation at the interface,[18] which may be the limiting factor for the improved performance of these materials. However, as shown by statistical analysis, no significant difference was observed when HEMA-free UAs were compared with the evaluated HEMA-containing UAs. The use

of strong air-drying during the evaporation step for all adhesives tested may explain the lower amount of silver nitrate uptake observed in the enamel substrate, in the present study.[17]

According to the present study, only one HEMA-free UA (ZIP) showed results approaching those of the control group SBU, mainly in terms of NL to the enamel and dentin interfaces in the ER strategy. As ZIP is an ethanol-based and ultra-mild UA, similar results were expected when compared to SBU, as observed by Fu and others.[41]

However, some differences were discovered. For instance, ZIP showed a lower DC on dentin and enamel substrates than SBU, for both adhesive strategies. A higher amount of solvent in ZIP (30-35%) compared to that in SBU (10-15%) may have contributed to the deficient DC. Complete evaporation of this higher amount of solvent is often not feasible, even when extended for larger durations of time; this is described in the application instructions of ZIP in comparison with that of SBU. Although the presence of the solvent did not interfere with the NL values, it had a high impact on the DC, as observed in the present study. Also, it is important to mention that SBU showed higher DC on dentin and enamel substrates than all HEMA-free adhesives and in both adhesive strategies.

In addition, differences in bond strength to dentin and enamel were observed when ZIP was compared to SBU, mainly in the SE strategy, with SBU showing better results than ZIP. The presence of a second monomer with the potential for chemical bonding may help to explain these differences. According to the manufacturers, in addition to MDP, SBU contains a methacrylate-modified polyalkenoic acid copolymer that improves the chemical bonding to calcium in HAp.[42] No significant differences were observed between ZIP and SBU when both adhesives were used in the ER strategy. The demineralization created by phosphoric acid seemingly diminished the role of the copolymer. Actually, SBU showed higher bond strength to dentin than all HEMA-free adhesives in the etch-and-rinse and self-etch strategies. Furthermore, SBU showed higher bond strength to enamel than all HEMA-free adhesives in the self-etch strategy and higher bond strength to enamel than SUB in the etch-and-rinse strategy.

The results showed that all adhesive properties are material-dependent, mainly because the HEMA-free universal adhesives are a heterogeneous group of UAs. Therefore, not only the absence of HEMA in the universal adhesives should be considered. Factors such as pH and solvent applied to HEMA-free adhesives and the presence of a second monomer with potential for chemical bonding in the composition of HEMA-free universal adhesives, should be considered.

However, the most important factor for the removal of HEMA is the decrease in the degradation of the adhesive interface. Therefore, future studies entail long-term bonding evaluation of enamel and dentin to assess the behavior of HEMA-free universal adhesives in comparison with HEMA-containing adhesives. Despite all progress in terms of the production of HEMA-free and MDP-containing universal adhesives, it is worth mentioning that, overall, the best immediate adhesive performance to dentin and enamel was obtained when a HEMA-and MDP-containing universal adhesive (SBU) was used.

It is important to mention the reason why the authors chose to apply microtensile bond strength (μTBS) for dentin and microshear bond strength (μSBS) for enamel tests. One of the most used tests to evaluate the bond strength to dentin is microtensile test.[43] This occurs due to several advantages of the test when compared to macro tests, which the most important are the following: 1) producing many specimens from the same extracted tooth; 2) higher versatility to evaluate regional differences intra- and intertooth; and 3) better stress distribution over a very small surface during loading, generating more interface failures (ie, fewer cohesive failures) in dentin.[44] However, microtensile bond strength test is considering a laborious and technique-sensitive procedure, mainly because it is necessary to cut and/or trim the specimens before testing. According to Armstrong and others,[45] the cutting/trimming can induce additional stress as reflected in the number of specimens that fail prior to testing, especially in weaker bonds or specimens with relatively brittle behavior. Taking in account that enamel is a brittle substrate, the trimming of resin-enamel interface is particularly susceptible to the specimen preparation effects of μTBS testing.[9,23,24,46] This is the main reason to choose μSBS test, instead μTBS test to evaluate the bond strength to enamel.

CONCLUSIONS

The immediate adhesive performance of commercial universal adhesives, MDP-containing, and HEMA-free, on enamel and dentin, varied greatly, appearing to be material-dependent. However, the best immediate adhesive performance to dentin and enamel was obtained for the HEMA- and MDP-containing universal adhesive.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the human subjects oversight committee guidelines and policies of ethics committee of State University of Ponta Grossa, Paraná, Brazil. The approval code issued for this study is 4.650.921.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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