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ORIGINAL ARTICLE

Role of adjunctive methacryloyloxydecyl dihydrogen phosphate primers on dentin bond strength of universal adhesives under saliva and artificial aging

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Abstract

This study evaluated whether adjunctive 10-methacryloyloxydecyl dihydrogen phosphate (MDP) primers enhance the microtensile bond strength (μTBS) of two universal adhesives (UAs) to dentin under artificial aging and repeated salivary contamination applied at each bonding step. One hundred fifty-two human molars were assigned to 24 groups based on UA type, MDP primer application, contamination, and aging period (1, 30, and 180 d). Resin–dentin beams were sectioned and loaded to failure to determine μTBS. Failure modes were analyzed, and scanning electron microscopy (SEM) was used to assess hybrid layer quality and interfacial morphology. FTIR–ATR evaluated the effect of MDP primers on the degree of conversion (DC). Data were analyzed using one and three-way ANOVA, Scheffé's test, and Weibull statistics. Under uncontaminated conditions, MDP primers significantly increased μTBS for both UAs after 180 d. Repeated contamination significantly reduced μTBS; however, adjunctive MDP priming restored bond strength to levels comparable to uncontaminated controls. FTIR–ATR demonstrated higher DC in MDP primer groups, suggesting enhanced interfacial polymerization. Weibull analysis confirmed improved bonding reliability with primer use. SEM revealed continuous hybrid layers in MDP groups regardless of contamination, whereas failure modes shifted toward more cohesive patterns. Adjunctive MDP priming mitigates the adverse effects of salivary contamination and aging on UA-dentin bonding.

KEYWORDS

adhesion, conditioning, dental tissue, saliva contaminations, universal adhesive

INTRODUCTION

The primary goal of adhesive dentistry is to establish durable bond to dental tissues in order to minimize microleakage, enhance retention, ensure the color stability of marginal interfaces, and improve the longevity of restorations. A major focus of adhesive research has been the simplification of clinical protocols while maintaining reliable adhesion to various substrates, including enamel, sound dentin, caries-affected dentin, and sclerotic dentin [1]. Early adhesive systems were based on the etch-and-rinse (ER) strategy, in which dental substrates are conditioned with phosphoric acid before adhesive application [2].

Although ER adhesives provide strong and consistent bonding, especially to enamel [1], their clinical application is technique-sensitive due to the need for precise control of dentin moisture after phosphoric acid etching. Self-etch (SE) adhesives were subsequently developed, incorporating acidic functional monomers that simultaneously demineralize and infiltrate dentin [3]. By eliminating the separate etching, rinsing, and drying steps, SE adhesives reduce the risk of collagen collapse associated with over-drying. Contemporary SE adhesives are generally recognized for their reliable bonding performance to dentin; however, their performance may vary depending on their formulation. Although simplified one-step SE systems may exhibit reduced long-term durability, particularly due to their increased hydrophilicity and permeability, two-step SE adhesives have demonstrated bond strength and durability comparable to ER systems [4]. More recently, a new class of "universal," "multimode," or "multipurpose" adhesives has been introduced to increase clinical flexibility. These materials can be applied using ER, SE, or selective enamel etching strategies, thereby offering greater versatility in different clinical situations [5, 6].

Universal adhesives (UAs) generally perform well under favorable laboratory and clinical conditions [5, 7, 8]. However, like their predecessors, they remain highly susceptible to contamination, especially by saliva, which is frequently encountered in clinical practice despite rubber dam isolation [9]. This problem is particularly relevant for cervical restorations, where access and isolation are challenging.

The deleterious effects of saliva contamination are well established. Saliva contains glycoproteins, enzymes, and mucins that form a proteinaceous film on dentin, impairing adhesive infiltration and polymerization. Enzymes such as collagenase, amylase, and esterase may further degrade exposed collagen fibrils, whereas the increased hydrophilicity of contaminated dentin reduces resin monomer penetration [10, 11]. These effects compromise the integrity of the adhesive interface and can accelerate restoration failure. Clinical studies confirm that inadequate isolation significantly reduces cervical restoration survival at 6 months (OR 2.29, 95% CI 1.05–4.99) [12]. Although most studies have investigated single contamination episode, clinical situations with compromised isolation often lead to repeated dentin contamination at multiple stages of the bonding procedure [9]. The effect of repeated saliva exposure has been insufficiently addressed, particularly in the context of UA [13, 14]. Various strategies have been proposed to manage saliva-contaminated dentin, including air-drying, rinsing, phosphoric acid, EDTA, chlorhexidine, ethanol, and sodium hypochlorite [15–18], as well as surfactant-based approaches, such as the use of amphiphilic cleaning agents or universal cleaning solutions designed to remove organic contaminants [19]. Although 10-methacryloyloxydecyl dihydrogen phosphate (MDP) has been a key functional monomer in adhesive dentistry for more than three decades, its application in dedicated primer formulations as adjuncts to UAs has only recently attracted research interest. Historically, MDP was functional monomer used in SE adhesives formulations [20]. Owing to their surfactant-like properties, MDP primers can help remove contaminants while promoting durable chemical bonding to hydroxyapatite through their phosphate groups. Many UAs are based on the "all-in-one" concept of previous one-step SE systems and incorporate functional monomers such as MDP in a single bottle containing primer and bonding [8]. However, although MDP primers are commercially available as part of self-adhesive resin cement (SARC) systems, their potential as adjuncts to UA remains largely unexplored. The effectiveness of MDP is known to depend on its concentration, purity, and formulation [5, 21]. Thus, dedicated MDP primers typically less hydrophilic and with higher monomer content than UA could enhance adhesion under compromised conditions such as repeated contamination. However, this hypothesis has not yet been systematically tested.

The laboratory study aims to evaluate whether the application of MDP primers—MDP primer 1 (G-Cem One Adhesion Enhancing Primer, GC) with the UA1 (G-Premio Bond, GC) and MDP primer 2 (Panavia V5 Tooth Primer, Kuraray) with the UA2 (Clearfil Universal Bond Quick, Kuraray)—can maintain or improve the microtensile bond strength (μTBS) of UAs to dentin when subjected to multiple saliva contaminations and long-term water aging individually and in combination.

Crucially, this study differs fundamentally from recent work on SARC materials [14, 22, 23] by focusing solely on the adhesion interface of UAs and the dentin, a distinct clinical challenge.

The four null hypotheses tested were that MDP primer application has not significantly improved μTBS of UAs to dentin; multiple saliva contaminations have not significantly reduced μTBS; water aging has not significantly reduced μTBS; and MDP priming has not significantly affected μTBS under conditions of multiple saliva contaminations and/or water aging.

TABLE 1 The commercial materials used.

Abbreviated name	Commercial name (lot number)	Manufacturer	Composition
MDP primer 1	G-Cem One Adhesive Enhancing Primer (2308244)	GC	Ethyl alcohol (25%–50%), 2-hydroxy-1,3-dimethacryloxypropane (10%–20%), MDP (5–10), butylated hydroxytoluene (1%–2.5%), Monophosphate dodecyl trimethacrylate, vanadyl acetylacetonate, 4-methacryloxyethyl trimellitate anhydride, water. pH: 1.5
UA1	G-Premio Bond (2309204)	GC	Acetone (25%–50%), 2-hydroxy-1,3-dimethacrylaxypropane (10%–20%), MDP (5%–10%), 2,2-ethylenedioxydiethyl dimethacrylate (1–5%), diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (1%–5%), 2,6-di-*tert*-butyl-*p*-cresol (<0.5%), water, 4-methacryloxyethyl trimellitate anhydride, methacrylate monomer, and silica
Chemical activator	Clearfil DC Activator (1B0015)	Kuraray	Ethanol, aryl sulfinate salt, and accelerators
MDP primer 2	Panavia V5 Tooth Primer (1R0147)	Kuraray	Hydroxyethyl methacrylate, MDP, hydrophilic aliphatic dimethacrylate, vanadium-compound, and water
UA2	Clearfil Universal Bond Quick (5P0409)	Kuraray	Bisphenol A-glycidyl methacrylate (10%–25%), ethanol (10%–25%), 2-hydroxyethylmethacrylate (2.5%–10%), MDP, hydrophilic amid monomers, sodium fluoride, DL-camphorquinone, and colloidal silica
Resin composite	G-aenial Universal injectable A1 (230515A)	GC	Octahydro-4,7-methano-1*H*-indenediyl)bis(methylene) bismethacrylate (5%–10%), 2,2-dimethyl-1,3-propanediyl bismethacrylate (2.5%–5%), 1,3,5-triazine-2,4,6-triamine, polymer with formaldehyde (1%–2.5%), urethane dimethacrylate (0.5%–1%), titanium dioxide (0.2%–0.5%), 2,2′-ethylenedioxydiethyl dimethacrylate (0.2%–0.5%), 2-(2*H*-benzotriazol-2-yl)-*p*-cresol (0.2%–0.5%), diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide cresol (0.2%–0.5%), 6-*tert*-butyl-2,4-xylenol (0.1%–0.2%), filler load: 82% wt: glass-filler (300 nm barium glass) 16 nm (fumed silica), and organic filler
Orthophosphoric acid	G-Etch (071624)	Elsodent	38% orthophosphoric acid

Abbreviations: MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UA1, universal adhesive 1; UA2, universal adhesive 2.

By addressing these questions, the study seeked to provide clinically relevant insights into the performance of UAs under conditions commonly encountered in practice and to evaluated whether MDP primers could enhance bonding reliability and longevity.

MATERIAL AND METHODS

Materials

The materials used in this study are listed in Table 1 [13, 14, 19, 24, 25]. Two commercially available UAs, UA1 (G-Premio Bond, GC) and UA2 (Clearfil Universal Bond Quick, Kuraray), and two dedicated MDP primers, MDP primer 1 (G-Cem One Adhesive Enhancing Primer, GC) and MDP primer 2 (Panavia V5 Tooth Primer, Kuraray), were selected for this study. The composition and manufacturer details of these materials are summarized in Table 1.

Experimental design

This in vitro study followed a factorial design with four independent variables: (1) bonding system (UA1 or UA2), (2) adjunctive MDP primer application (with or without), (3) salivary contamination (with or without repeated contamination applied at all stages of the adhesive procedure), and (4) water aging period (24 h, 30 d, or 180 d). This yielded eight bonding protocols, evaluated at three aging periods, resulting in 24 experimental groups in total. A total of 152 human molars were allocated: 144 for μTBS testing (six molars per group) and eight for interfacial scanning electron microscopy (SEM) analysis. The bonding protocols and the specific number of teeth and microtensile beams obtained for each group and each aging period are illustrated in Figure 1 and summarized in Table 2. The control bonding protocols were designated as UA1 only and UA2 only.

Tooth collection and storage

These 152 sound caries-free human molars, extracted from patients aged 18–60 yr, were collected from the Dental Service of Pitié-Salpêtrière Hospital (Paris, France). All procedures were conducted in accordance with the Declaration of Helsinki. Teeth were obtained following oral informed consent, in compliance with French ethical regulations and under the authorization of the Faculty of Dentistry, Uni-

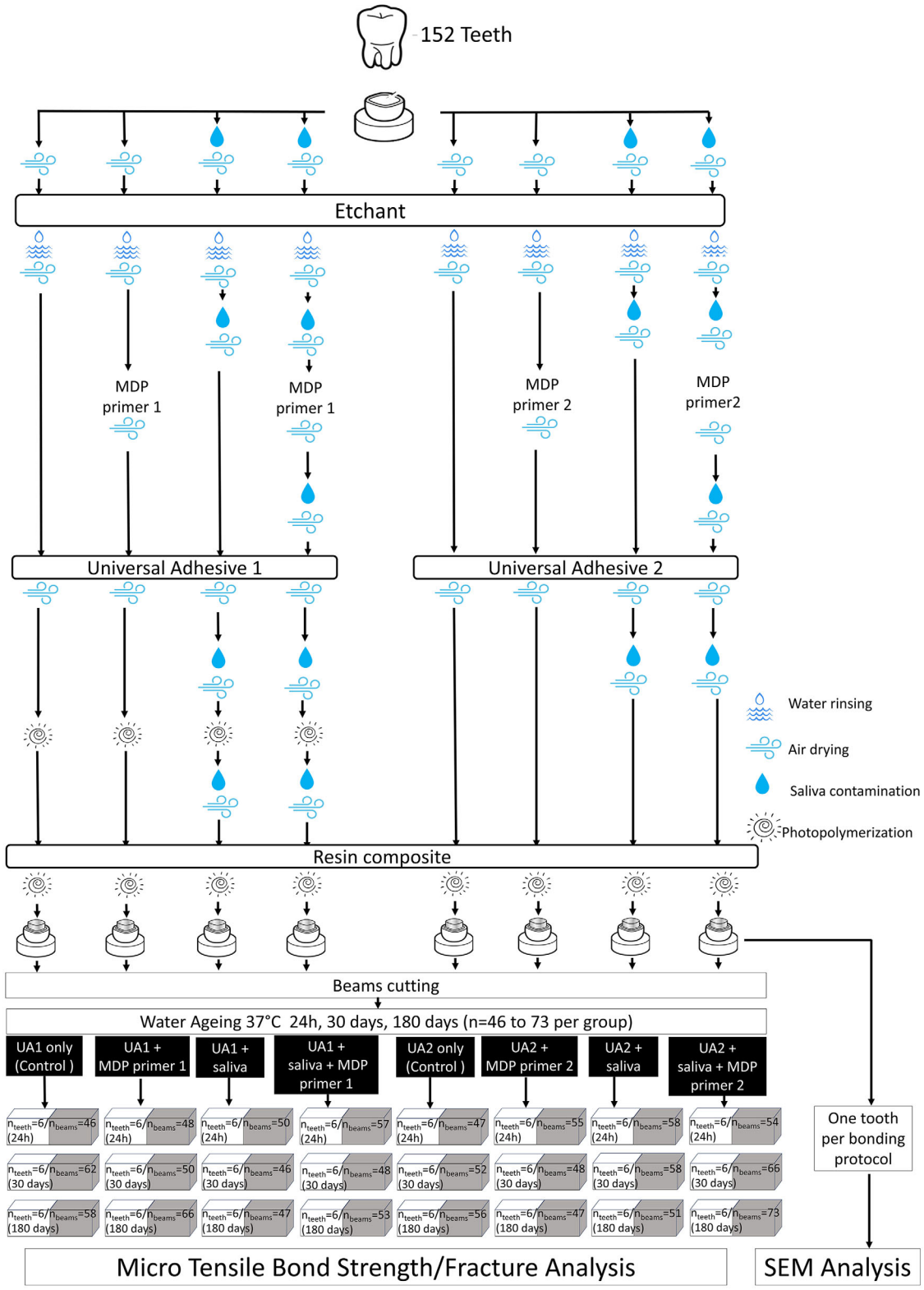

FIGURE 1 Experimental design and bonding protocols. Saliva contamination at various stages of the bonding process. Protocols UA1 only and UA2 only serve as controls. MDP, 10-methacryloyloxydecyl dihydrogen phosphate; SEM, scanning electron microscopy; UA1, universal adhesive 1; UA2, universal adhesive 2.

TABLE 2 Description of the bonding protocols according to adhesive type, 10-methacryloyloxydecyl dihydrogen phosphate (MDP) primer application, saliva exposures.

Bonding protocols	Adhesive	MDP primer	Saliva	Protocol steps (in order)
UA1 only	UA1	—	No	Air-dry 5 s → H_3PO_4 38% 15 s → rinse + air-dry 5 s → UA1—air-dry 5 s → light cure 10 s → resin composite—light cure 20 s
UA1 + MDP primer 1	UA1	MDP primer 1	No	Air-dry 5 s → H_3PO_4 15 s → rinse + air-dry 5 s → MDP primer 1 10 s, air-dry 5 s → UA1—air-dry 5 s → light cure 10 s → resin composite—light cure 20 s
UA1 + saliva	UA1	—	Yes	**Saliva 10 s—air-dry 5 s** → H_3PO_4 15 s → rinse + air-dry 5 s → **saliva 10 s—air-dry 5 s** → UA1—air-dry 5 s → **saliva 10 s—air-dry 5 s** → light cure 10 s → **saliva 10 s—air-dry 5 s** → resin composite—light cure 20 s
UA1 + saliva + MDP primer 1	UA1	MDP primer 1	Yes	**Saliva 10 s—air-dry 5 s** → H_3PO_4 15 s → rinse + air-dry 5 s → **saliva 10 s—air-dry 5 s** → MDP primer 1 10 s, air-dry 5 s → **saliva 10 s—air-dry 5 s** → UA1—air-dry 5 s → **saliva 10 s—air-dry 5 s** → light cure 10 s → **saliva 10 s—air-dry 5 s** → resin composite—light cure 20 s
UA2 only	UA2	—	No	Air-dry 5 s → H_3PO_4 15 s → rinse + air-dry 5 s → UA2 active rubbing 20 s, air-dry 5 s → resin composite—light cure 20 s
UA2 + MDP primer 2	UA2	MDP primer 2	No	Air-dry 5 s → H_3PO_4 15 s → rinse + air-dry 5 s → MDP primer 2 10 s, air-dry 5 s → UA2 active rubbing 20 s, air-dry 5 s → resin composite—light cure 20 s
UA2 + saliva	UA2	—	Yes	**Saliva 10 s—air-dry 5 s** → H_3PO_4 15 s → rinse + air-dry 5 s → **saliva 10 s—air-dry 5 s** → UA2 active rubbing 20 s, air-dry 5 s → **saliva 10 s—air-dry 5 s** → resin composite—light cure 20 s
UA2 + saliva + MDP primer 2	UA2	MDP primer 2	Yes	**saliva 10 s—air-dry 5 s** → H_3PO_4 15 s → rinse + air-dry 5 s → **saliva—air-dry 5 s** → MDP primer 2 10 s, air-dry 5 s → **saliva 10 s—air-dry 5 s** → UA2 active rubbing 20 s, air-dry 5 s → **saliva 10 s—air-dry 5 s** → resin composite—light cure 20 s

Note: H_3PO_4 is orthophosphoric acid (38%); UA is universal adhesive. Steps in bold indicate salivary contamination applications. Light curing: all steps performed with the same curing unit.

Abbreviations: UA1, universal adhesive 1; UA2, universal adhesive 2.

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After extraction, residual soft tissues and bone fragments were carefully removed using a curette. Teeth were then stored in 0.5% chloramine-T solution at 4°C in a temperature-controlled environment and used within 3 months of extraction.

Dentin surface preparation

The occlusal enamel and coronal portion of each tooth were removed under water cooling with a polishing device (ESC300 GT, Escil) using sequential silicon carbide papers (grit #220 and #600) to obtain flat, standardized mid-coronal dentin surfaces.

Salivary contamination protocol

The saliva contamination protocol followed previously established methods [14, 26]. The saliva was collected from one of the authors (J.-F.N.), on the same time in the morning. It was fresh, whole, unstimulated human saliva, and it was used within 30 min. The saliva was applied to the dentin surface with a microbrush for 10 s, followed by thorough air-drying for 5 s. This contamination procedure was repeated after each bonding step to simulate multiple exposures (Figure 1 and Table 2).

Bonding procedures

All bonding protocols followed an ER mode and are detailed in Table 2 and illustrated in Figure 1. For UA2, the adhesive was mixed with a chemical activator (Clearfil DC Activator, Kuraray) at a 1:1 ratio for 10 s prior to application. Resin composite (G-ænial, GC) was applied in 2-mm horizontal increments using a transparent matrix strip (Hawe Striproll, Kerr) to obtain a final build-up height of 6 mm. Each increment was light-cured for 20 s using an LED curing unit (Valo Cordless, Ultradent) with an irradiance of 890 mW/cm^2.

Specimen sectioning and water aging

Following adhesive and resin composite application, all specimens were stored overnight in 100% humidity, then sectioned

under water cooling using a diamond saw (Isomet, Buehler) into 47–73 rectangular beams (1 mm × 1 mm × 10 mm) per group. Beams dimensions were verified using a digital caliper (Mitutoyo) prior to testing. Beams were stored in distilled water at 37°C for 24 h, 30 d, or 180 d to simulate intraoral artificial aging through water storage.

Microtensile bond strength

Bond strength was assessed using the μTBS method in accordance with ISO/TS 11405:2015. Each specimen was bonded to a testing fixture using a cyanoacrylate adhesive (Cyanboard) and subjected to tensile loading in a computer-controlled universal testing machine (Instron Model 5943, BLUEHILL software) at a crosshead speed of 0.5 mm/min until failure occurred. The failure load (in Newton) was recorded, and μTBS values (in MPa) were calculated based on the bonded cross-sectional area.

Failure mode at the adhesive interface was analyzed under a stereomicroscope (Leica S9D, Wetzlar) at 50× magnification. Fractures were categorized into five types: cohesive failure within dentin, cohesive failure within the resin composite, interfacial failure (at the adhesive interface), mixed failure involving both the adhesive interface and dentin, and mixed failure involving both the adhesive interface and the resin composite [27].

Scanning electron microscopy (SEM) analysis

One tooth per group was selected for SEM analysis. After application of the UA and the resin composite following protocols described above, specimens were re-embedded, and a new cut was made through the center to obtain a cross-sectional view of the adhesive interface. Each specimen was trimmed into approximately 1 cm × 1 cm squares and polished using silicon carbide paper up to 4000 grit. The samples were then dehydrated in absolute ethanol for 1 h, followed by gold sputter-coating. SEM observation was performed at 2000× magnification using an SEM (JSM-6400, JEOL).

Determination of the degree of conversion by FTIR spectrometer

The degree of conversion (DC) at 30 min was quantified using attenuated total reflection Fourier-transform infrared spectroscopy (ATR–FTIR) with a Nicolet IS10 spectrometer (Thermo Scientific, Madison, WI, USA). All spectra were acquired with 32 scans at a resolution of 8 cm^{-1}. The method used for DC determination followed the procedure described by Yoshihara *et al.* [28]. Measurements were performed for four groups: UA1, UA1 combined with MDP primer 1, UA2, and UA2 combined with MDP primer 2 ($n = 8$ per group). The DC was calculated based on the change in the aliphatic C = C absorption peak at 1638 cm^{-1} relative to the aromatic C = O reference peak at 1715 cm^{-1}, comparing spectra obtained before and after polymerization using the following equation:

$$\mathrm{DC}\ (\%) = \left[1 - \frac{\left(\frac{A_{C=C}}{A_{C=O}}\right)\text{polymer}}{\left(\frac{A_{C=C}}{A_{C=O}}\right)\text{monomer}}\right] \times 100 \qquad (1)$$

where $A_{C=C}$ is the ratio after light-curing (i.e., polymer) and $A_{C=O}$ is the ratio before light-curing (i.e., monomer).

Statistical analysis

μTBS data for each adhesive system were analyzed using one-way analysis of variance (ANOVA) followed by Scheffé's post hoc test for multiple comparisons ($\alpha = 0.05$) to identify statistically homogeneous subgroups across all bonding protocol × aging period combinations. As the two adhesive systems (UA1 and UA2) were analyzed independently, each with its own dedicated MDP primer, statistical comparisons were performed separately for each UA. Within each adhesive system, three independent factors were analyzed: MDP primer application (P), salivary contamination (S), and aging time (A), using three-way ANOVA ($\alpha = 0.05$). This approach allowed examination of all main effects and their two- and three-way interactions ($P \times S$, $P \times A$, $S \times A$, $P \times S \times A$). All analyses were performed using SPSS version 21 (IBM).

Specimens that failed during pretesting were assigned a bond strength value of 0 MPa for the subsequent statistical analysis. Furthermore, bond strength values associated with cohesive failures were retained and included in the statistical analyses, following the recommendation established by Franz *et al.* [29].

As the Weibull distribution cannot handle zero values, pretested failures were replaced with a random value between zero and the lowest measured value in the respective group, and the resulting Weibull parameters were obtained [30, 31]. Weibull statistical parameters were calculated for the bond strength data. The description of the Weibull distribution is given by the following equation:

$$P_f = 1 - e^{-\left(\frac{t}{\eta}\right)^{\beta}} \qquad (2)$$

where P_f is the fracture probability, defined by the relation estimated using Bernard's estimation according to the

following equation:

$$P_f = \frac{K - 0.3}{N + 0.4} \quad (3)$$

where K is the rank of strength from least to greatest, N is the total number of specimens in the sample, β is the shape parameter (Weibull modulus), t is bond strength, and η is the scale parameter or characteristic strength (63.2% probability of failure) [30]. Weibull modulus, characteristic strength, and strength at 10% probability of failure were obtained using the Weibull statistics option in Excel (MICROSOFT). The DC values for each UA were analyzed using t-tests with a significance level of 0.05.

RESULTS

The μTBS and DC results are summarized in Tables 3–5, together with the statistical analyses.

For UA1, μTBS was significantly affected by saliva contamination, MDP primer 1 application, and water aging ($p < 0.05$). The highest μTBS was recorded for UA1 combined with MDP primer 1 after 1 d of water aging (74.85 ± 16.40 MPa; 95% CI: 70.09; 79.61), whereas the lowest value was observed for saliva-contaminated UA1 after 180 d of water aging (9.51 ± 8.32 MPa; 95% CI: 7.08; 11.96). Across all conditions, MDP primer 1 application resulted in significantly higher μTBS values, whereas groups without MDP primer 1 exhibited pronounced bond degradation after aging ($p < 0.05$).

For UA2, similar trends were observed. The highest μTBS was recorded for UA2 combined with MDP primer 2 after 30 d of water aging (86.79 ± 22.28 MPa; 95% CI: 80.32; 93.26), whereas the lowest value was observed for saliva-contaminated UA2 after 180 d of water aging (9.24 ± 9.26 MPa; 95% CI: 6.64; 11.84). Saliva contamination significantly reduced μTBS under all conditions ($p < 0.05$), particularly in the absence of MDP primer 2. Although water aging led to progressive bond strength reduction for all groups, this effect was less pronounced when MDP primer was applied ($p < 0.05$).

Three-way ANOVA showed that, for UA1, MDP priming (yes/no), saliva contamination (yes/no), and water aging time (24 h/30 d/180 d) had significant effects on μTBS ($p < 0.05$), and all two-way and three-way interactions (MDP × saliva, MDP × aging, saliva × aging, and MDP × saliva × aging) were also significant ($p < 0.05$). For UA2, all main factors were significant ($p < 0.05$), whereas the interactions MDP × aging and saliva × aging were not significant ($p > 0.05$).

Failure mode analysis in Figure 2 revealed a higher incidence of mixed and interfacial failures in saliva-contaminated and non-primed groups for both adhesives, whereas cohesive failures predominated in MDP primer-treated groups. Pretest failures occurred exclusively in saliva-contaminated groups without MDP primer and were observed during beam preparation.

Weibull analysis (Figure 3 and Table 4) showed trends consistent with μTBS values, with higher Weibull moduli in non-contaminated, MDP primer-treated groups for both UAs.

SEM observations revealed interfacial gaps in saliva-contaminated groups without MDP primer (red arrows, Figures 4C and 5C), whereas a continuous adhesive interface was observed when MDP primer was applied. Resin tags were present in all bonding protocols (blue arrows, Figures 4 and 5) and voids within the adhesive layer were also detected (green arrows, Figures 4A,B,D and 5A,B,D).

FTIR–ATR analysis revealed a significant increase in DC following MDP primer application for both UAs ($p < 0.05$) (Table 5).

DISCUSSION

This study evaluated the effects of MDP primer application and water aging on the μTBS of two UAs, UA1 and UA2, under both favorable (no saliva exposure) and unfavorable (multiple saliva contaminations) conditions on dentin. The results supported the rejection of first null hypothesis confirming that the application of an MDP primer significantly increased μTBS for both UAs. Second null hypothesis was likewise rejected, indicating that saliva contaminations led to a significant decrease in μTBS regardless of the adhesive used. The third null hypothesis, stating that water storage would not affect bond strength, was partially rejected. Although the three-way ANOVA identified aging as a significant factor overall, post hoc analysis (Scheffé test) revealed that this effect was not statistically significant for certain bonding protocols, including UA1 only, UA1 + saliva + MDP1, UA2 only, and UA2 + saliva. These findings indicate that the impact of aging on μTBS was not uniform across all experimental conditions. Furthermore, fourth null hypothesis, the interaction hypothesis, was also rejected. This finding indicates that the influence of saliva contaminations on μTBS was significantly dependent on the presence of MDP primer 2 priming for the second UA2, whereas both the effect of saliva contaminations and water aging were modified by MDP primer 1 priming for the first UA1. Collectively, these results highlight that MDP priming, repeated saliva contamination, and water aging all have a substantial impact on the dentin bonding performance of both UAs.

MDP, developed in the 1980s (Kuraray), is widely incorporated into adhesives due to its ability to chemically interact with both hydroxyapatite and collagen [32–34]. The adhesives

TABLE 3 Microtensile bond strength (μTBS) (mean ± SD, MPa).

	μTBS in MPa (mean ± SD) [95% CI] (*n* teeth/*n* beams)		
Bonding protocols	**24 h water aging**	**30 d water aging**	**180 d water aging**
Universal adhesive 1			
UA1 only	32.95 ± 15.22^{c} [28.42; 37.46] *(6/46)*	31.57 ± 15.45^{c} [27.65; 35.49] *(6/62)*	34.20 ± 13.33^{c} [30.70; 37.70] *(6/58)*
UA1 + MDP primer 1	74.85 ± 16.40^{a} [70.09; 79.61] *(6/48)*	58.88 ± 18.47^{b} [53.62; 64.12] *(6/50)*	52.62 ± 11.87^{b} [49.70; 55.54] *(6/66)*
UA1 + saliva	12.23 ± 9.73^{ef} [9.46; 15.00] *(6/50)*	18.25 ± 10.56^{de} [15.11; 21.39] *(6/46)*	9.51 ± 8.32^{f} [7.08; 11.96] *(6/47)*
UA1 + saliva + MDP primer 1	28.20 ± 11.21^{c} [25.23; 31.17] *(6/57)*	29.81 ± 14.54^{c} [25.59; 34.03] *(6/48)*	25.98 ± 12.40^{cd} [22.56; 29.40] *(6/53)*
Universal adhesive 2			
UA2 only	59.10 ± 24.33^{cd} [51.96; 66.24] *(6/47)*	50.86 ± 16.37^{def} [46.31; 55.43] *(6/52)*	40.80 ± 20.88^{f} [35.22; 56.40] *(6/56)*
UA2 + MDP primer 2	80.11 ± 23.60^{ab} [62.83; 86.50] *(6/55)*	86.79 ± 22.28^{a} [80.32; 93.26] *(6/48)*	68.67 ± 24.44^{bc} [61.49; 75.85] *(6/47)*
UA2 + saliva	16.09 ± 11.88^{g} [12.97; 19.21] *(6/58)*	11.58 ± 10.28^{g} [8.88; 14.28] *(6/58)*	9.24 ± 9.26^{g} [6.64; 11.84] *(6/51)*
UA2 + saliva + MDP primer 2	66.67 ± 23.09^{c} [60.37; 72.97] *(6/54)*	57.29 ± 21.43^{cde} [52.02; 62.56] *(6/66)*	45.44 ± 18.34^{ef} [41.16; 49.72] *(6/73)*
3-way ANOVA—factors: MDP primer (*P*) × saliva (*S*) × aging time (*A*)			
Source of variation	**UA1**		**UA2**
MDP primer (*P*)	*		*
Saliva (*S*)	*		*
Aging (*A*)	*		*
P × *S*	*		*
P × *A*	*		ns
S × *A*	*		ns
P × *S* × *A*	*		*

Note: Superscript letters indicate statistically homogeneous subgroups within a material category (1-way analysis of variance followed by Scheffé test, $\alpha = 0.05$). 3-way ANOVA: *P* is MDP primer; *S* is salivary contamination; *A* is water aging time. * is $p < 0.05$; ns is not significant ($p \geq 0.05$). Analyses were performed separately for UA1 and UA2.

Abbreviations: ANOVA, analysis of variance; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UA1, universal adhesive 1; UA2, universal adhesive 2.

selected in this study were paired with their corresponding manufacturer supplied MDP-containing primers, originally designed for use with SARCs. This allowed a direct comparison between the additional MDP provided by the primer and the MDP already incorporated within the adhesive formulation [14, 25].

Under favorable clinical conditions (i.e., without saliva contamination), the application of an MDP primer significantly improved μTBS and the Weibull modulus in both UAs, indicating enhanced bonding reliability. The MDP monomer reacts chemically with hydroxyapatite in dentin, forming stable calcium–MDP salts at the mineral surface through a self-assembled nanolayering phenomenon. This process involves superficial dissolution of hydroxyapatite followed by the precipitation of low-solubility Ca–MDP salts. The resulting stable and durable chemical bond creates a resilient biochemical interface, providing superior mechanical and chemical retention compared with other functional monomers [32, 33, 35]. In addition, MDP undergoes chemical adsorption with dentinal collagen mainly through hydrogen bonding and hydrophobic interactions involving its long carbon chain [36, 37]. These interactions allow MDP to associate with the helical structure of collagen fibrils, promoting deeper infiltration and stabilization of the organic matrix [33].

Accordingly, previous reports have consistently shown that adhesives containing MDP achieve higher bond strengths and more predictable results in terms of bonding effectiveness and durability [34, 38]. Fehrenbach *et al.* further demonstrated the overall superiority of MDP-based adhesives when compared with materials formulated with other acidic resin monomers [20].

TABLE 4 Weibull parameters for microtensile bond strength (μTBS) data (β, PF10, and characteristic strength η with 95% confidence intervals) of all bonding protocol groups at three water aging time points.

	24 h			30 d			180 d		
Bonding protocol	**β [95% CI]**	**PF^{10} MPa [95% CI]**	**$PF^{63.2\%}$ (η) MPa [95% CI]**	**β [95% CI]**	**PF^{10} MPa [95% CI]**	**$PF^{63.2\%}$ (η) MPa [95% CI]**	**β [95% CI]**	**PF^{10} MPa [95% CI]**	**$PF^{63.2\%}$ (η) MPa [95% CI]**
Universal adhesive 1									
UA1 only	2.21 [2.11; 2.31]	13.49 [13.01; 13.93]	37.37 [36.53; 38.36]	2.35 [2.22; 2.48]	13.61 [12.96; 14.24]	35.52 [34.50; 36.60]	2.73 [2.61; 2.85]	16.88 [16.21; 17.56]	38.50 [36.54; 40.86]
UA1 + MDP primer 1	5.15 [4.93; 5.38]	52.57 [50.68; 54.54]	81.34 [77.82; 85.38]	3.13 [2.90; 3.35]	32.24 [30.43; 33.64]	66.24 [64.74; 69.58]	5.18 [5.00; 5.36]	37.01 [36.55; 38.36]	57.15 [54.10; 57.56]
UA1 + saliva	1.42 [1.33; 1.50]	2.85 [2.58; 3.13]	13.86 [12.94; 15.00]	1.63 [1.53; 1.72]	5.41 [5.02; 5.80]	21.59 [20.61; 22.62]	0.99 [0.93; 1.04]	1.08 [0.94; 1.20]	10.62 [9.98; 11.70]
UA1 + saliva + MDP primer 1	2.30 [2.19; 2.43]	12.17 [11.61; 12.72]	32.30 [30.97; 33.82]	2.17 [2.06; 2.28]	11.94 [11.32; 12.56]	33.72 [31.70; 36.22]	2.04 [1.93; 2.13]	9.80 [9.31; 10.28]	29.58 [28.65; 30.60]
Universal adhesive 2									
UA2 only	2.51 [2.45; 2.57]	27.28 [26.63; 27.91]	66.82 [65.11; 68.65]	3.20 [3.08; 3.32]	28.22 [27.83; 28.56]	57.99 [56.09; 58.03]	2.02 [1.94; 2.09]	15.13 [14.45; 15.83]	46.20 [42.82; 50.70]
UA2 + MDP primer 2	3.67 [3.54; 3.79]	48.09 [47.37; 48.81]	88.85 [88.67; 89.12]	4.41 [4.24; 4.58]	57.11 [56.20; 57.97]	95.11 [94.54; 95.77]	3.23 [3.08; 3.38]	38.13 [37.45; 38.78]	76.55 [75.41; 77.79]
UA2 + saliva	0.95 [0.87; 1.03]	1.75 [1.43; 2.08]	18.54 [16.61; 21.11]	0.83 [0.77; 0.88]	0.81 [0.67; 0.96]	12.43 [11.21; 13.50]	1.07 [1.01; 1.14]	1.28 [1.13; 1.42]	10.43 [9.70; 11.25]
UA2 + saliva + MDP primer 2	3.07 [2.99; 3.15]	35.87 [35.16; 36.60]	74.44 [73.70; 75.19]	3.03 [2.93; 3.13]	30.48 [30.27; 30.57]	64.07 [60.34; 68.72]	2.54 [2.49; 2.59]	21.11 [20.91; 21.33]	51.42 [49.40; 53.52]

Note: β is Weibull modulus (shape parameter): reflects scatter of bond failures. Higher β means more homogeneous distribution. PF^{10} is characteristic bond strength at 10% probability of failure (MPa). $PF^{63.2\%}$ is scale parameter (η), characteristic bond strength at 63.2% probability of failure (MPa). Values in brackets are 95% confidence intervals.

Abbreviations: MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UA1, universal adhesive 1; UA2, universal adhesive 2.

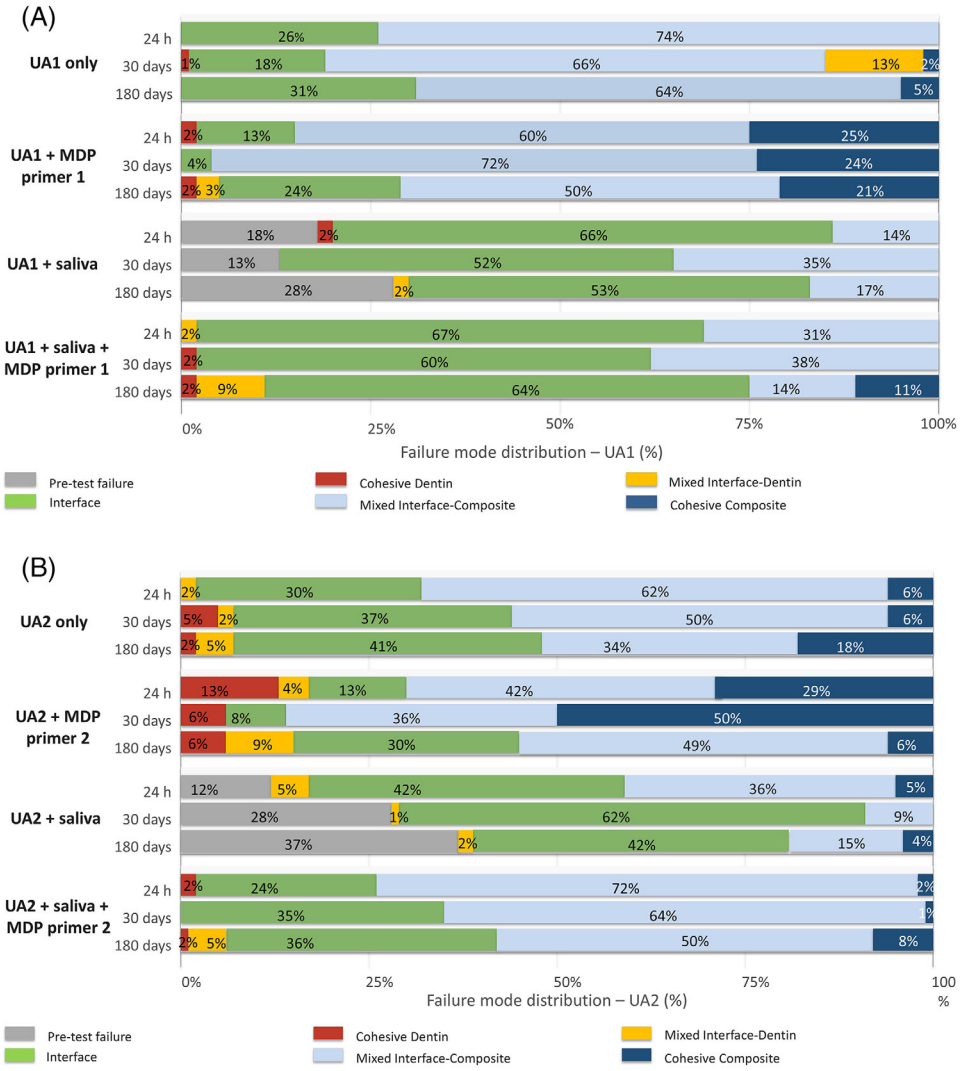

FIGURE 2 Failure mode analysis of specimens subjected to microtensile bond strength (μTBS) testing for UA1 (A) and UA2 (B). MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UA1, universal adhesive 1; UA2, universal adhesive 2.

TABLE 5 Degree of conversion of universal adhesive 1 (UA1) and universal adhesive 2 (UA2) (mean ± SD; $n = 8$) and results of statistical analysis.

Degree of conversion ± SD (in %) [95% CI]	
Universal adhesive 1	
Without MDP primer 1	With MDP primer 1
81.78 ± 3.06 [79.22; 84.34]	88.40 ± 4.80* [84.39; 92.41]
Universal adhesive 2	
Without MDP primer 2	With MDP primer 2
84.20 ± 3.14 [81.57; 86.83]	94.10 ± 2.82* [91.74; 96.46]

Abbreviations: MDP, 10-methacryloyloxydecyl dihydrogen phosphate.
*Identifies significantly different ($p < 0.05$) results based on pairwise *t*-test comparisons within an UA category.

Interestingly, μTBS was significantly enhanced by additional MDP primer application despite the presence of MDP within the tested adhesives. This may be explained by a higher local concentration of functional phosphate groups at the interface, more extensive and uniform nanolayer formation, and pre-polymerization interactions between MDP and dentin [21, 35].

First, the primer increased the local concentration of functional phosphate groups at the adhesive interface, thereby enhancing the formation of stable, hydrolytically resistant Ca–MDP salts with hydroxyapatite [35]. Second, the extent of nanolayering, which refers to the density and the formation of organized nanolayers of MDP–Ca salts at the adhesive interface, is reported to be directly related to the concentration of MDP [21]. When applied as a primer, these nanolayers could have been likely more extensive and uniform than when MDP is delivered only within a diluted adhesive formulation. Finally, applying the primer before adhesive placement allows MDP to chemically interact with dentin prior to polymerization, ensuring deeper and more effective chemical bonding. Collectively, these mechanisms could explain why the use of

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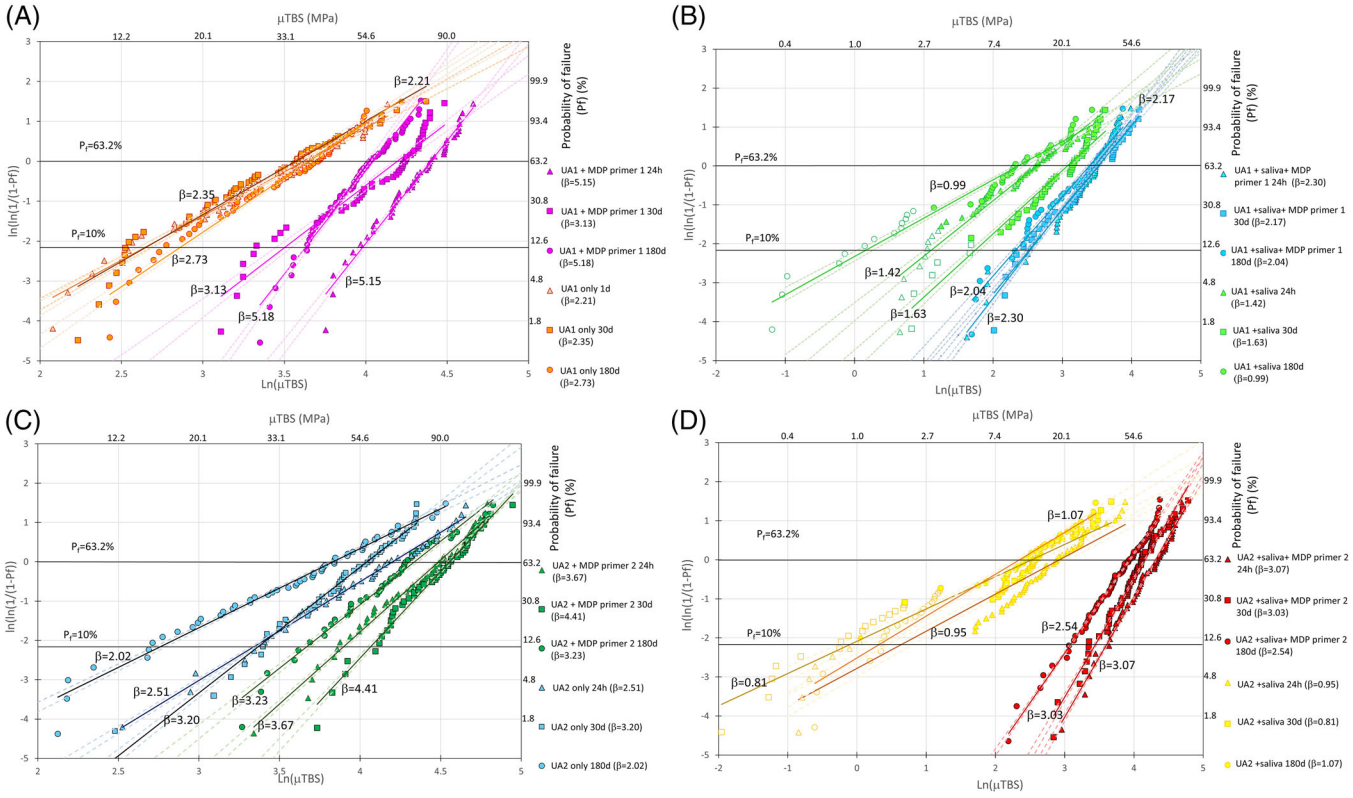

FIGURE 3 Weibull plot for UA1 without saliva contamination (A), with saliva contaminations (B) and UA2 without saliva contamination (C), with saliva contaminations (D). Dotted lines represent the 95% confidence limits for each group. True values are represented by filled symbols, whereas each value representing pretest failures is represented by open symbols. β is Weibull modulus. μTBS microtensile bond strength; UA1, universal adhesive 1; UA2, universal adhesive 2.

an MDP primer induced improvement in μTBS, even when MDP was already present in the adhesive formulations.

Moreover, the higher μTBS values observed in MDP primer groups may be explained by a "touch-cure" effect at the dentin–adhesive interface. In this context, "touch-cure" refers to an interfacial chemical interaction that can enhance polymerization through potential redox reactions between components of the primer and the adhesive system, leading to increased radical generation and, consequently, a higher DC. This increase in DC at the adhesive interface has been associated with improved bond strength, reduced water sorption, and enhanced resistance to hydrolytic degradation in SARCs [28]. In the present study, FTIR analysis demonstrated an increased DC in groups receiving adjunctive MDP primer application (MDP primer 1 with UA1; MDP primer 2 with UA2), supporting the hypothesis that MDP primer promotes interfacial polymerization and thereby contributes to the superior bond strength values recorded in these groups.

This likely arises from residual ethanol (from MDP primer 1 or from the UA2/chemical activator mixture), which, even after air-drying, may act as a hydrogen donor in radical photopolymerization. Ethanol could have reacted with reactive species generated from diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (in UA1) or camphorquinone (in UA2/chemical activator), forming secondary radicals that propagate polymerization [39, 40]. Additionally, the vanadyl compound in MDP primer 1 and MDP primer 2 may have further promoted radical generation through redox reactions in alcoholic environments and LED light curing [41]. These combined mechanisms plausibly could explain the enhanced radical density, higher DC, and resulting μTBS improvements observed with MDP primers. Further mechanistic and spectroscopic studies would be needed to confirm these pathways.

Under unfavorable conditions (i.e., in the presence of saliva exposures), repeated saliva contamination without primer markedly reduced μTBS, consistent with previous reports [10, 11, 13, 26, 42]. SEM images (Figures 4C and 5C) revealed adhesive-composite decohesion, supporting the interpretation that salivary proteins interfere with adhesive infiltration by reducing surface energy and forming biofilm [43–46], ultimately impairing bonding performance [19, 26]. Moreover, the presence of voids within the adhesive layer, observed across groups regardless of the bonding protocol, suggests that these features are more likely related to the application procedure such as microbrush-induced air entrapment or solvent evaporation dynamics rather than the experimental variables [47].

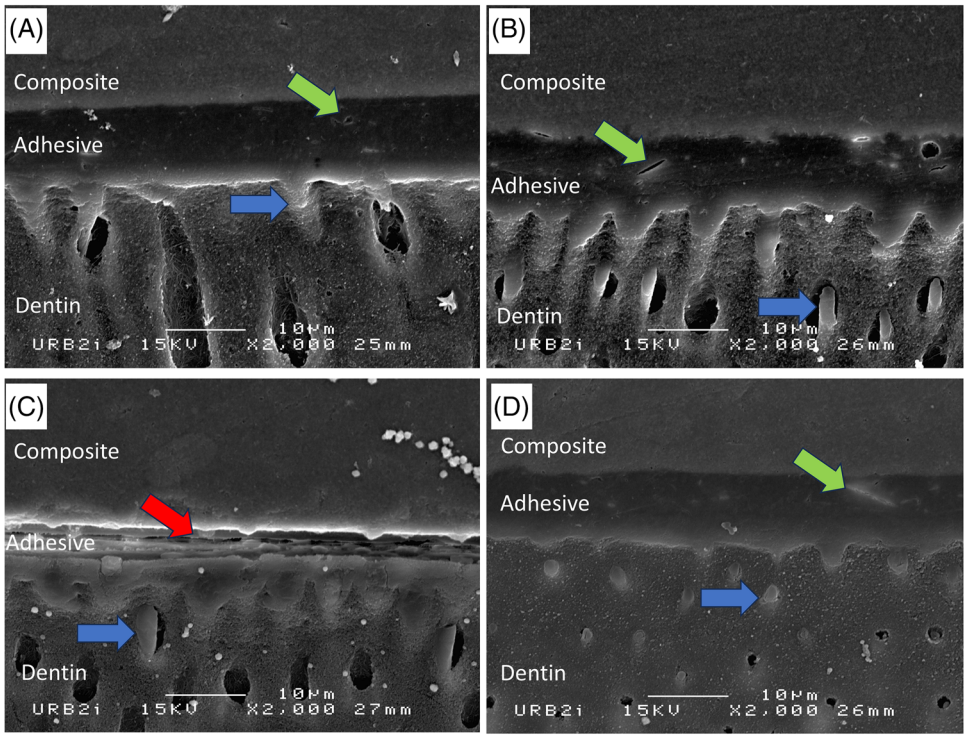

FIGURE 4 Scanning electron microscope images (×2000 magnification) of dentin substrates. (A) UA1 only, (B) UA1 + MDP primer 1, (C) UA1 + saliva, and (D) UA1 + saliva + MDP primer 1. A generally continuous adhesive interface was observed in all groups except the saliva-contaminated condition without primer (C), where interfacial gaps between the adhesive layer and resin composite were detected (red arrow). Resin tag penetration into dentinal tubules was observed in all groups (blue arrow). Voids within the adhesive layer were occasionally detected (green arrows), suggesting possible air entrapment or solvent-related effects. MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UA1, universal adhesive 1; UA2, universal adhesive 2.

These findings highlight the critical importance of implementing effective surface decontamination strategies such as the use of MDP-containing cleaners or MDP salts to restore the chemical reactivity of contaminated substrates prior to bonding [48]. Previous studies have demonstrated the effectiveness of both MDP amine salts and MDP monomers in decontaminating dentin surfaces before bonding [14, 19, 23]. The distinction is noteworthy: MDP monomers are primarily responsible for establishing chemical bonding, whereas MDP amine salts contribute mainly to surface cleaning and recovery of bonding capacity after contamination.

In the present study, μTBS values were significantly higher in groups exposed to multiple saliva contaminations with MDP primer application than in groups exposed to multiple saliva contaminations without MDP priming for both UAs at the same water aging time. Furthermore, no significant differences in μTBS were observed between the saliva-contaminated, MDP-primed groups (UA1 + saliva + MDP primer 1 and UA2 + saliva + MDP primer 2) and their respective control groups (UA1 only and UA2 only) at the same aging time, indicating that MDP priming effectively mitigated the deleterious effects of salivary contamination. The persistence of these μTBS values despite repeated contamination cycles suggests a residual protective or restorative effect of the MDP primer.

In addition to bond strength, bonding reliability followed the same pattern for both UAs. Without application of an MDP primer, multiple saliva exposures led to a reduction in the Weibull modulus compared with the control bonding protocol. Conversely, when an MDP primer was applied, the Weibull modulus remained comparable to that of the control protocol.

This is particularly relevant given that salivary exposure occurred at each bonding step, amounting to five exposures for the first UA1 and four for the second UA2. Such findings may have clinical relevance by demonstrating the potential of MDP primers to maintain reliable bonding effectiveness even under challenging operative conditions.

As widely reported, water aging led to a reduction in μTBS [49–51]. For UA2, μTBS decreased significantly by 31% in the UA2 only bonding protocol but by only 14% (not statistically significant) in the UA2 + MDP primer 2 bonding protocol. This indicates that the application of an MDP primer not only improved immediate bonding but also enhanced resistance to hydrolytic degradation. The protective effect of MDP is attributed to its ability to form poorly soluble MDP–Ca salts through interaction of its phosphate group

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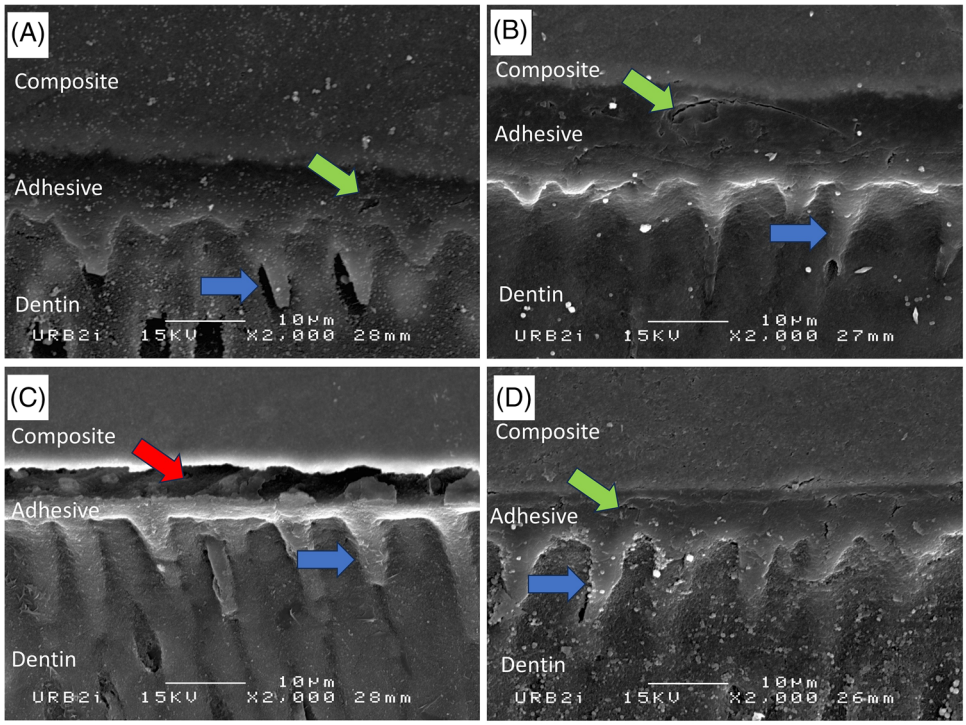

FIGURE 5 Scanning electron microscope images (×2000 magnification) of dentin substrates. (A) UA2 only, (B) UA2 + MDP primer 2, (C) UA2 + saliva, and (D) UA2 + saliva + MDP primer 2. A continuous adhesive interface was observed in all groups except the saliva-contaminated condition without primer (C), where interfacial gaps between the adhesive layer and resin composite were noted (red arrow). Resin tag formation was evident in all groups (blue arrow). Voids within the adhesive layer were also observed (green arrows), indicating possible application-related artifacts. MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UA1, universal adhesive 1; UA2, universal adhesive 2.

with calcium from hydroxyapatite, contributing to chemical bonding and improved hydrolytic stability at the interface. It should be noted, however, that these salts may be susceptible to dissolution under the acidic conditions of the oral environment, and their long-term stability in vivo remains to be fully established. Furthermore, in vitro investigations on synthetic hydroxyapatite have suggested that MDP monomers may self-assemble into organized molecular layers with a preferential orientation of their hydrophobic methacrylate chains, potentially limiting water sorption and interfacial degradation [35, 52]. Experimental evidence, including the work of Yoshida *et al.* [35], has demonstrated the formation of such nanolayered MDP–Ca structures on hydroxyapatite following water storage at 37°C, supporting the plausibility of this mechanism under controlled conditions. It should be acknowledged, however, that direct in situ confirmation of nanolayer formation on human dentin remains limited, and that oral temperature conditions (35–37°C) may influence both the kinetics of MDP–Ca salt formation and the stability of these organized structures compared to standard laboratory conditions. Together, these features likely account for the superior preservation of μTBS after water aging for UA2. Beyond the concentration of MDP monomers, its mode of delivery also appears to influence performance; applying MDP monomers as a separate primer may provide a chemical advantage over relying solely on the MDP incorporated into the adhesive formulation.

This study deliberately introduced saliva contamination at every bonding step to simulate the most challenging clinical conditions, in contrast to previous studies that considered only single contamination events [13, 19, 26]. This design enabled evaluation of the robustness of primer-assisted protocols under conditions that closely approximate clinical reality.

This work has certain limitations. The μTBS test is inherently variable, as shown by the relatively high coefficients of variation (CV) values observed. Under control conditions, CVs ranged from 32% to 51%, which aligns with previous reports and the known variability of μTBS testing (20%–50%) linked to dentin heterogeneity, specimen preparation, and technique sensitivity [27, 53, 54]. Higher CVs were recorded when saliva contamination was introduced, also reported by other authors [26, 42], reflecting the difficulty of achieving stable bonding under contaminated conditions rather than methodological imprecision. To address these limitations, evaluating interfacial fracture toughness may offer valuable advantages. This method is more sensitive to interfacial defects, better concentrates debonding stresses at the adhesive interface, provides insight into crack propagation resistance, and may therefore offer a more reliable assessment of the true mechanical performance of adhesive bonds

to dentin [31, 53, 54]. In addition, the 6-month aging period provides useful insight but does not reflect long-term clinical aging. Moreover, the findings apply only to the two UAs tested and cannot be generalized. Future studies should include longer aging protocols, thermocycling, and a wider range of adhesives to better validate the effect of MDP priming under repeated saliva contamination. Another limitation of this study is that saliva was obtained from a single donor. Because salivary composition varies among individuals, the use of pooled saliva from multiple donors could better represent clinical conditions. Thus, although the present study focused on μTBS as the primary outcome, it is important to acknowledge that bond strength and marginal sealing are not systematically correlated [55]. The adjunctive application of an MDP-based primer may contribute to improved interfacial integrity by increasing the local concentration of MDP monomers at the resin–dentin interface compared with the UA alone. This is hypothesized to promote a more stable and organized nanolayer of MDP–calcium salt complexes, which may be associated with enhanced resistance to hydrolytic degradation. Nevertheless, as marginal sealing and microleakage were not directly evaluated in this study, further research is required to assess the sealing efficacy of this adjunctive priming strategy.

Finally, from a clinical standpoint, adhesive systems have progressively evolved toward simplification, with UAs demonstrating generally favorable performance. However, the present findings suggest that the adjunctive use of MDP primers may further enhance bonding effectiveness and improve tolerance to salivary contamination. Although this additional step may partially offset the time-saving advantage typically associated with UAs, it may provide a clinically relevant benefit in terms of bonding reliability, particularly under less-than-ideal operative conditions. Compared with conventional SE adhesives, this approach may offer improved recovery of bond strength after contamination while preserving the versatility of UAs across different bonding strategies and substrates. Within the limitations of this study, the use of dedicated MDP primers with UAs appears to represent a simple and effective strategy to improve the predictability and durability of adhesive procedures particularly in clinically challenging situations.

AUTHOR CONTRIBUTIONS

Conceptualization: Amélie Mainjot, Jean-François Nguyen, and Marjorie Zanini. **Methodology**: Amélie Mainjot, Stéphane Le Goff, and Jean-François Nguyen. **Formal analysis**: Amélie Mainjot, Jean-François Nguyen, and Marjorie Zanini. **Investigation**: Stéphane Le Goff and Jean-François Nguyen. **Writing—original draft**: Stéphane Le Goff, Jean-François Nguyen, and Marjorie Zanini. **Writing and review and editing**: Amélie Mainjot and Marjorie Zanini.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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