Journal of
Composites Science

MDPI

Article

Adhesive Evaporation Implication in Dentin Bond Strength and MMP Activation

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Abstract

(1) Background: This study aimed to assess how two different adhesive solvent evaporation techniques affect the microtensile bond strength (μTBS) of a universal adhesive to dentin, as well as the enzymatic activity of dentinal matrix metalloproteinases (MMPs) at baseline and after 6-month aging under simulated pulpal pressure. (2) Methods: Middle-depth dentin surfaces of 32 sound extracted human molars were bonded under simulated pulpal pressure using a universal adhesive (Clearfil Universal Bond Quick) in either etch-and-rinse (ER) or self-etch (SE) mode. Two solvent evaporation approaches were compared: evaporation with a disposable air/water syringe (air) and with a disposable suction device (suction), yielding four experimental groups (n = 8): ER/air, ER/suction, SE/air, and SE/suction. After light-curing for 10 s, a 4 mm composite build-up was created. Specimens were stored in distilled water under simulated pulpal pressure at 37 °C for either 24 h (T0) or 6 months (T6) and then sectioned into sticks and subjected to μTBS testing. Fracture surfaces were examined by scanning electron microscopy (SEM). An additional subset of teeth from each group (n = 3) underwent in situ zymographic analysis, in which bonded sticks were ground and exposed to fluorescein-conjugated gelatin, with enzymatic activity quantified by confocal microscopy. Statistical analysis was performed at a significance threshold of $p < 0.05$. (3) Results: Air-drying yielded significantly higher μTBS values than suction drying, irrespective of adhesive application mode ($p < 0.05$). At T6, SE groups retained bond strength values comparable to baseline ($p > 0.05$), whereas ER groups showed a significant reduction in bond strength with aging ($p < 0.05$). The ER mode and suction were associated with significantly increased MMP activity at T0 ($p < 0.05$), while there were no differences between SE/ER at T6 ($p > 0.05$), and air increased MMP activity ($p < 0.05$). (4) Conclusions: When using an ethanol-based universal adhesive under pulpal pressure, evaporation with a disposable air syringe combined with the self-etch application mode appears to offer greater bonding reliability and stability.

Keywords: universal adhesive; adhesive evaporation; bond strength; MMPs; pulpal pressure

Academic Editor: Mahdi Bodaghi

Received: 25 June 2026
Revised: 2 August 2026
Accepted: 4 August 2026
Published: 8 August 2026

1. Introduction

The evolution of adhesive formulations has markedly simplified clinical procedures and reduced technique sensitivity [1–3]. Universal adhesives represent the most recent generation of adhesive systems, and their "universality" is reflected in two principal features [1,2]. First, they support multiple bonding strategies, including etch-and-rinse (ER), self-etch (SE), and selective enamel etching (SEE) methods, depending on the clinical indication [1,3,4]. Second, they can be applied to a broad range of dental substrates [5,6]. These adhesives are typically formulated as single-bottle systems containing an ethanol- or acetone-based solvent, functional monomers, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP), photoinitiators, and a blend of hydrophilic and hydrophobic resin components [2–4].

The dentin bonding performance of universal adhesives is influenced by several formulation-related factors, including pH, solvent type, and monomer composition [2,4,7]. The type of solvent is particularly important in clinical practice because it directly influences adhesive infiltration into dentin [7,8]. However, incomplete solvent evaporation may compromise the mechanical properties of the adhesive, impair its polymerization [7,9], and increase water sorption and hydrolytic degradation within the adhesive layer [2,9]. These effects may be further exacerbated by the intrinsic hydrophilicity of dentin [2,7,8]. Therefore, an appropriate air-drying step after adhesive application is essential to facilitate solvent evaporation and promote resin monomer infiltration into the dentinal tubules [7,10]. Clinically, this is most commonly achieved using an air stream from a dental syringe [7,11], although negative-pressure suction has recently been proposed as an alternative or adjunctive approach [11]. Despite the well-recognized importance of solvent evaporation, the optimal method for enhancing this process in universal adhesives remains to be determined.

Dentin bonding relies on acid etching to demineralize the inter- and intra-fibrillar apatite crystallites. Ideally, resin monomers fully infiltrate the demineralized matrix and form a homogeneous, stable hybrid layer (HL), thereby providing immediate bond strength and contributing to long-term durability [2,8]. However, under clinical conditions, monomer infiltration often does not extend to the full depth of demineralization, and water within the collagen fibrils cannot be completely displaced, resulting in a water-rich zone of exposed collagen at the base of the HL [8,12,13]. Within this water-rich microenvironment, matrix metalloproteinases (MMPs) and cysteine cathepsins retained in the mineralized matrix may be activated, leading to progressive degradation of collagen fibrils that are inadequately infiltrated by resin [1,8,14]. At the same time, residual water can impair resin polymerization and accelerate hydrolytic degradation of the adhesive layer [1,8,15]. These synergistic processes increase nanoleakage, promote progressive loss of bond strength, and ultimately compromise the long-term success of the restoration [1,15,16].

Importantly, these mechanisms of HL degradation all require a water-rich microenvironment, whereas dentin bonding is performed on an intrinsically moist substrate under continuous pulpal pressure [17,18]. Under in vivo conditions, pulpal pressure drives a continuous outward flow of dentinal fluid toward the dentin surface, thereby providing an additional source of moisture during adhesive application and solvent evaporation [16,18,19]. This additional moisture may compound the effect of residual interfacial water caused by incomplete solvent evaporation, further hindering resin monomer infiltration and polymerization while maintaining the hydrated conditions required for MMP activation and activity [14,18]. Therefore, simulating pulpal pressure under in vitro conditions is important for a more clinically relevant evaluation of adhesive performance [16,17]. However, direct evidence is still lacking as to whether persistent pulpal pressure has an effect on endogenous enzymatic activity in dentin, and this issue requires further clarification.

Accordingly, under in vitro conditions simulating physiological pulpal pressure, this study evaluated the effects of different solvent evaporation methods after the application of a universal adhesive in the ER or SE modes on dentin bond strength and endogenous MMP activity, at baseline and after 6 months of artificial aging in distilled water at 37 °C. The null hypotheses were that: (1) the solvent evaporation method (air-drying vs. negative-pressure suction) would not affect the dentin bond strength of the universal adhesive; (2) the solvent evaporation method would not affect endogenous MMP activity in dentin; and (3) aging would not influence bond strength or dentinal MMP activity. All hypotheses were tested at baseline and after 6 months of artificial aging in distilled water at 37 °C.

2. Materials and Methods

2.1. Specimen Preparation

A total of 44 sound, non-carious human molars from anonymous donors, freshly extracted for therapeutic needs after written informed consent was obtained, were collected in accordance with a protocol approved by the Ethics Committee of the University of Bologna, Italy (protocol N°: 71/2019/OSS/AUSLBO). The teeth were stored in distilled water at 4 °C and were used within 1 month after extraction. The roots were sectioned off at the cement–enamel junction (CEJ) using a low-speed, water-cooled diamond saw (Microremet, Remet, Casalecchio di Reno, Italy), while preserving the intact crown and pulp chamber. The occlusal enamel was then removed with a high-speed handpiece to expose mid-coronal dentin. The dentin surface was wet-ground with #180-grit silicon carbide paper to create a standardized smear layer, rinsed with distilled water, and gently air-dried for 5 s, leaving the surface uniformly moist and avoiding desiccation. The pulp tissue was removed using fine forceps, paying attention not to touch the chamber walls while preserving the integrity of the chamber.

2.2. Group Allocation

All specimens were randomly allocated according to a 2 × 2 factorial design, with the adhesive application mode (ER/SE) and solvent evaporation method (air-drying/negative-pressure suction) as the two main factors. All bonding procedures were performed under simulated physiological pulpal pressure (20 cm H_2O) [16]. Four experimental groups were established (n = 8): G1 (ER—air-drying), G2 (ER—negative-pressure suction), G3 (SE—air-drying), and G4 (SE—negative-pressure suction).

2.3. Bonding Procedure

The same universal adhesive, CLEARFIL Universal Bond Quick (Kuraray Noritake Dental Inc., Tokyo, Japan), was used in all groups. The adhesive was applied by one trained operator according to the assigned application mode, strictly in accordance with the manufacturer's instructions. In the ER groups (G1 and G2), dentin was etched with 37% phosphoric acid gel for 15 s, thoroughly rinsed with distilled water for 10 s, and gently air-dried until the surface appeared uniformly moist. The adhesive was then applied with a microbrush and gently rubbed for 20 s. In the SE groups (G3 and G4), the adhesive was directly applied to the unetched, moist dentin surface and gently rubbed for 20 s.

After adhesive application, solvent evaporation was performed using one of two methods. In the air-drying groups (G1 and G3), an oil-free air stream was delivered through a disposable air syringe tip positioned approximately 1.5 cm from the dentin surface at a 45° angle for 5 s at a pressure of 1 bar. In the negative-pressure suction groups (G2 and G4), a disposable high-volume suction tip was positioned approximately 1 cm from the adhesive-coated dentin surface, and negative pressure was applied for 5 s to remove excess

solvent and moisture [16]. All bonding procedures were performed by a single trained operator to minimize operator-related variability.

After solvent evaporation, the adhesive was light-cured for 10 s using an LED curing unit (1470 mW/cm^2; Elipar DeepCure-L, 3M ESPE, St. Paul, MN, USA). Composite resin (CLEARFIL MAJESTY ES-2, Kuraray Noritake Dental Inc.) was then placed incrementally in 2 layers approximately 2 mm thick, with each layer light-cured for 20 s. The resin–dentin-bonded specimens were then stored at 37 °C in distilled water under simulated pulpal pressure for 24 h (T0) or 6 months (T6).

2.4. In Vitro Pulpal Pressure Simulation System

An in vitro pulpal pressure simulation system was established according to the method described by Feitosa et al. [16]. Briefly, each bonded specimen was fixed with wax to the inner surface of the lid of a cylindrical container, with the pulp chamber opening facing the interior of the container, which was kept patent and unobstructed. Distilled water was then introduced into the container to create a 20 cm water column. After the lid was secured, the container was inverted so that the water column was positioned above the specimen. Under this configuration, the fluid within the pulp chamber was subjected to a hydrostatic pressure of 20 cm H_2O ($P = \rho gh$, where P is hydrostatic pressure, ρ is fluid density, g is gravitational acceleration, and h is the height of the water column), thereby continuously driving fluid outward through the dentinal tubules toward the exposed dentin surface and reproducing the physiological pulpal pressure conditions of vital teeth. The specimens were stored in distilled water under pulpal pressure at 37 °C for 24 h (T0) or 6 months (T6).

2.5. Microtensile Bond Strength (μTBS) Testing

After aging (at T0 or T6), the specimens were sectioned with a low-speed, water-cooled diamond saw (Microremet, Remet) along the X and Y directions, perpendicular to the bonding interface, to obtain resin–dentin sticks with a cross-sectional area of approximately 0.9 mm^2. Specimen preparation was performed according to the non-trimming method for microtensile bond strength (μTBS) testing [20]. Each stick was then attached at both ends to the testing jig of a μTBS testing machine (Bisco, Schaumburg, IL, USA) using the cyanoacrylate adhesive and loaded to failure at a crosshead speed of 1 mm/min. The load at failure was recorded in Newtons (N) and converted into bond strength values (MPa) according to the following formula: bond strength (MPa) = load (N)/bonded area (mm^2). After testing, all fractured sticks were examined under a stereomicroscope at 50× magnification by a trained examiner blinded to group allocation in order to determine the failure mode. Failure modes were classified as adhesive (A, at the dentin/adhesive interface), cohesive in dentin (CD, within dentin), cohesive in composite resin (CC, within the composite resin), or mixed (M, involving both adhesive and cohesive components).

In addition, two representative fractured specimens from each group, with bond strength values close to the group mean, were selected for observation using a digital microscope (Keyence VHX-7000, Osaka, Japan). The specimens were mounted on a custom-made silicon support, and sputter-coated with gold/palladium. The fractured interfaces were then examined at different magnifications in 2D and 3D using a digital microscope for descriptive morphological analysis.

2.6. In Situ Zymography

Additional extracted molars (n = 3 per group) were prepared for in situ zymography. After completion of the bonding and restorative procedures as described in Section 2.3, the teeth were stored under simulated pulpal pressure in distilled water in a laboratory oven at 37 °C for 24 h (T0) or 6 months (T6). After aging, each specimen was sectioned

longitudinally using a low-speed, water-cooled diamond saw to obtain approximately 1 mm thick slabs (n = 4 per tooth), exposing the resin–dentin interface.

Each slab was fixed onto a glass slide with cyanoacrylate adhesive and progressively ground under water cooling with increasingly finer silicon carbide papers to a final thickness of approximately 50 μm, thereby fully exposing the hybrid layer [15]. Self-quenched fluorescein-conjugated gelatin (DQ-gelatin; E-12055, Molecular Probes, Eugene, OR, USA) was used as the fluorescent substrate. The stock solution was diluted 1:8 in a dilution buffer, applied to the polished specimen surface, and covered with a coverslip. The specimens were then incubated overnight at 37 °C in a humidified dark chamber. After incubation, the resin–dentin interface/hybrid layer region was examined using confocal laser scanning microscopy (excitation wavelength: 488 nm; emission wavelength: 530 nm). Multiple z-stack images (n = 3 per slab) were acquired from each specimen under standardized acquisition settings (scanning depth approximately 15 μm; step size, 1 μm). The integrated density of the fluorescent signal in each image was quantified using ImageJ software v. 2.9.0 (National Institutes of Health, Bethesda, MD, USA), and green fluorescence intensity was used as a relative indicator of endogenous gelatinolytic activity.

2.7. Statistical Analysis

Normality and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. The μTBS and in situ zymography fluorescence intensity data (with sticks used as a statistical unit) were analyzed by three-way analysis of variance (3-way ANOVA), with application mode (ER/SE), solvent evaporation method (air-drying/negative-pressure suction), and testing time point (T0/T6) as fixed factors. Main effects and interaction effects were examined, and pairwise comparisons were performed using Tukey's honestly significant difference (HSD) post hoc test. All statistical analyses were performed using SigmaPlot 16.0 software (Systat Software Inc., San Jose, CA, USA). The level of significance was set at $\alpha = 0.05$.

3. Results

3.1. Microtensile Bond Strength and Failure Mode

Descriptive results are summarized in Table 1.

Table 1. Microtensile bond strength (MPa) with failure modes of a universal adhesive applied in ER and SE modes using air-drying or suction under pulpal-pressure conditions at baseline and after 6 months of artificial aging. Different lowercase letters indicate significant differences within the rows, while different uppercase letters indicate significant differences within columns.

Adhesive Mode	Evaporation Method	Baseline (T0) Mean ± SD	6 Months (T6) Mean ± SD
ER	Air	33.44 ± 13.09 ^{aA} 56.4% M, 33.3 CC, 5.1% A, 5.1% CD	17.92 ± 9.77 ^{bB} 26% M, 42% A, 32% CC
ER	Suction	27.93 ± 10.79 ^{aB} 78% M, 15.2 CC, 5.1% A, 1.7 CD	16.39 ± 4.63 ^{bB} 52% M, 40% A, 8% CC
SE	Air	30.71 ± 10.39 ^{aA} 62.5% M, 37.5% CC, 0%A, 0% CD	25.95 ± 2.72 ^{aA} 66.6% M, 30.3% A, 3.1% CC
SE	Suction	22.36 ± 7.72 ^{aB} 93.3% M, 6.7% A, 0% CC, 0% CD	23.05 ± 6.02 ^{aA} 64% M, 32% A, 4% CC

ER, etch-and-rinse method; SE, self-etch method; SD, standard deviation. Failure modes: Adhesive (A, occurring at the dentin/adhesive interface), cohesive in dentin (CD, occurring within dentin), cohesive in composite resin (CC, occurring within the composite resin), or mixed (M, involving both adhesive and cohesive components).

Three-way ANOVA revealed significant main effects of the evaporation method ($p < 0.001$) and aging time ($p < 0.001$) on μTBS, whereas the main effect of the application mode was not significant ($p = 0.184$). Air-drying produced significantly higher μTBS than negative-pressure suction across all conditions ($p < 0.001$). No significant interaction was found for application mode × evaporation method ($p = 0.441$), evaporation method × aging ($p = 0.072$), or the three-way interaction ($p = 0.723$). A significant interaction was observed for application mode × aging ($p < 0.001$).

Within the ER mode, μTBS was significantly lower at T6 than at T0 ($p < 0.001$), whereas no significant difference was found between time points within the SE mode ($p = 0.270$). At T0, ER showed higher μTBS than SE ($p = 0.003$), while at T6, SE showed higher μTBS than ER ($p < 0.001$).

Mixed failure predominated across all groups and time points, except for the ER air group at T6, where adhesive failure predominated. The remaining failures were predominantly cohesive in composite resin at T0, while at T6 there was a significant increase in the adhesive failure percentage. Cohesive failure in dentin was confined to the ER groups at T0 (≤5.1). Figure 1 demonstrates failure modes of representative specimens found in each group at each aging time. Cohesive failures or mixed failures are more evident at T0, where the morphology of composite resins with fillers is notable; conversely, after aging, the failures shift more towards adhesive failures with remnants of adhesive resin on the surface.

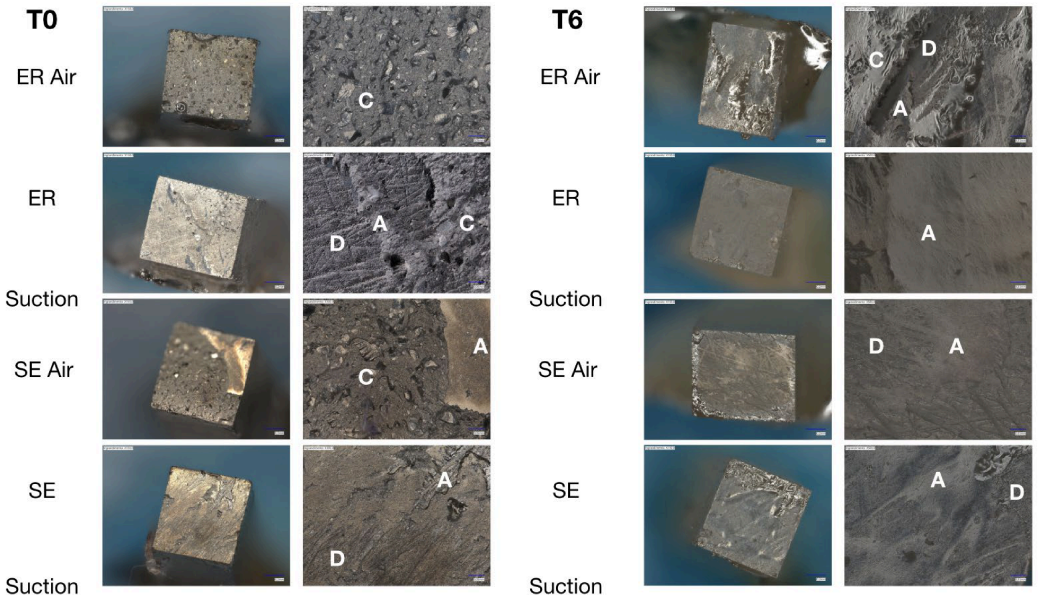

Figure 1. Representative images (150× and 500×) of the fractured resin–dentin specimens. A—adhesive resin; D—dentin; C—composite resin.

3.2. In Situ Zymography

Three-way ANOVA revealed significant main effects of application mode ($p < 0.001$) and aging time ($p < 0.001$) on fluorescence intensity, whereas the main effect of the evaporation method was not significant ($p = 0.884$). No significant interaction was found for application mode × evaporation method ($p = 0.822$) or the three-way interaction ($p = 0.698$). Significant interactions were found for application mode × aging ($p = 0.046$) and evaporation method × aging ($p < 0.001$).

Descriptive results are presented in Figures 2 and 3.

Fluorescence intensity (Figure 2) was significantly lower at T6 than at T0 in both application modes (both $p < 0.001$). At T0, ER showed higher fluorescence intensity than SE ($p < 0.001$), whereas no significant difference was found at T6 ($p = 0.152$). A significant

reduction from T0 to T6 was also observed in both evaporation subgroups (with $p < 0.001$). At T0, the suction subgroup showed higher fluorescence intensity than the air-drying subgroup ($p = 0.015$), whereas the reverse was observed at T6 ($p = 0.017$).

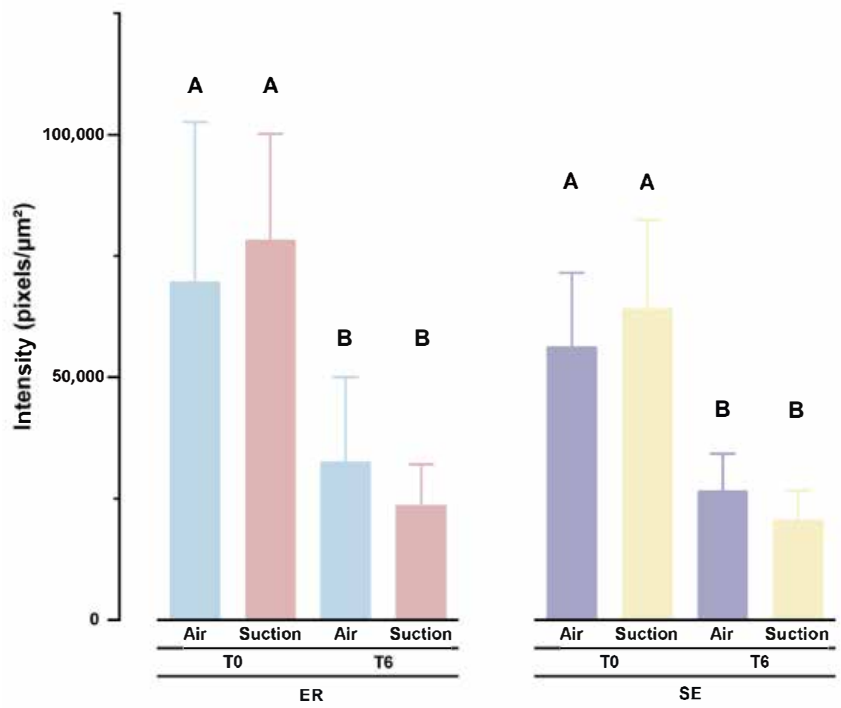

Figure 2. In situ zymography fluorescence intensity in the hybrid layer in ER and SE application modes with air-drying or suction under simulated pulpal pressure at T0 and T6. Bars represent m. Different uppercase letters indicate significant differences between the groups within the ER or SE mode ($p < 0.05$).

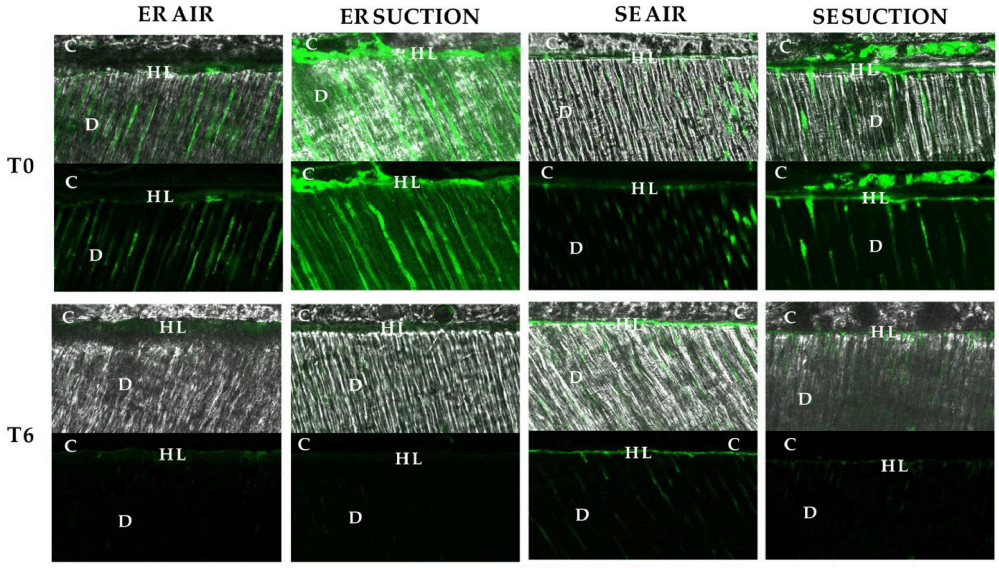

Figure 3. Representative images from in situ zymography for each group at T0 and T6. Resin–dentin interfaces exposed to quenched fluorescein-labeled gelatin at T0 and T6 months. In each group, the (**upper**) image was generated by overlaying the differential interference contrast (DIC) image, which depicts the optical density of the resin–dentin interface, with the corresponding green channel image. The (**lower**) image was captured in the green channel, revealing fluorescence within dentinal tubules and the hybrid layer. Abbreviations: D—dentin; HL—hybrid layer; C—resin composite; T0—24 h aging; T6—6-month aging.

4. Discussion

This study evaluated the effects of adhesive application mode, solvent evaporation method, and aging time on μTBS and interfacial endogenous MMP activity under simulated pulpal pressure. The results showed that solvent evaporation method significantly affected μTBS, with air-drying producing higher values than negative-pressure suction, indicating that more effective solvent removal improves interfacial mechanical performance. In contrast, solvent evaporation had no significant overall effect on interfacial endogenous MMP activity, although its influence varied with aging time, suggesting a time-dependent effect on interfacial biological behavior. The interaction of adhesive application mode with aging time was significant, indicating that the two application modes followed different aging trajectories: ER produced a higher bond strength than SE at baseline, whereas SE outperformed ER after 6 months. Similarly, application mode significantly affected interfacial endogenous MMP activity, with ER showing greater enzymatic activity at baseline but converging with SE after aging. Overall, aging time was a major factor influencing both the mechanical stability and biological activity of the dentin bonding interface. Therefore, all three null hypotheses were rejected. It is important to note that the three-way ANOVA revealed that the main effect of the evaporation method on MMP activity was not statistically significant ($p = 0.884$), while the evaporation method × aging interaction ($p < 0.001$) was. Hence, the second null hypothesis was rejected on the basis of the interaction rather than the main effect.

Dentin is inherently moist, porous, and rich in organic components, and its wet condition after acid etching makes bonding particularly challenging [8,21]. Modern dentin adhesives therefore typically dissolve hydrophilic and hydrophobic monomers in organic solvents such as acetone or ethanol [8,9]. These volatile solvents reduce resin viscosity, help displace water from the dentin surface and hydrated collagen network, and promote monomer infiltration into the microporosities of the demineralized collagen matrix exposed by acid etching [22,23], thereby improving bond strength [22,24]. Ideally, both solvent and water should be thoroughly removed before light curing, as their retention can compromise resin polymerization [22,25]. However, as water evaporates, the monomer-to-water ratio increases and vapor pressure decreases, which, in turn, limits the escape of both solvent and water [26,27]. Residual water and solvents may then become trapped within the resin during curing, leading to a porous interface, dilution of resin components, incomplete polymerization, and even phase separation, ultimately impairing bond quality and mechanical performance [7,9,22,28].

However, moisture management remains particularly challenging in dentin bonding, as adhesive protocols must balance the need to preserve essential water with the need to limit excess moisture. On the one hand, water is required for ionization of acidic monomers in self-etch systems; the use of wet-bonding techniques and hydrophilic monomers is essential for the adequate impregnation of collagen fibers with the adhesive; and the continuous presence of dentinal fluid from the pulp chamber cannot be prevented [1,8,16,29]. On the other hand, incomplete water removal leaves residual solvent and moisture within the adhesive layer, increasing its permeability and creating pathways for subsequent water transport and sorption, thereby accelerating degradation of the resin–dentin interface [11,15]. This intrinsic conflict remains a central challenge in the design of modern dentin adhesive systems.

In this study, the air-drying group showed significantly higher immediate μTBS than the suction group. The main difference between the two methods lies in the direction of airflow: the air syringe delivers positive pressure toward the dentin surface, whereas suction drying applies negative pressure by drawing air away. Although suction drying is gentler and might reduce collagen collapse, its negative pressure may be insufficient to

prevent the accumulation of residual solvent and water [11], thereby increasing surface moisture and interfacial fluid movement. This may hinder adhesive infiltration into demineralized dentin and impair polymerization, ultimately weakening interfacial sealing and bond strength [11,15]. This finding differs from that of Magne et al. [11], who reported under conventional in vitro conditions using a three-step ER adhesive system that suction drying produced slightly lower bond strength than air-drying, although the difference was not statistically significant. One possible explanation for this is that aging in the present study was conducted under simulated pulpal pressure, where continuous outward dentinal fluid flow may have further increased interfacial moisture.

For the universal adhesive used in this study, a certain amount of water is required in the formulation to maintain ionization of the acidic monomers [2,19]. In the present study, the μTBS of Clearfil Universal Bond Quick ranged from 16.1 to 33.4 MPa, with an overall immediate mean of 28.6 MPa, which decreased to 20.8 MPa after 6 months of aging. These values were clearly lower than those reported under conventional in vitro conditions without simulated pulpal pressure (approximately 30–40 MPa). This difference suggests that continuous outward dentinal fluid flow under simulated pulpal pressure substantially interferes with interfacial bond longevity, consistent with previous reports showing the reduced μTBS of universal adhesives under pressure conditions [30,31]. Therefore, the present experimental model more closely resembles bonding conditions in vital teeth and may provide a more clinically relevant estimate of bond strength.

Endogenous MMPs in the dentin matrix are a major host-derived factor in degradation of the resin–dentin interface over time [8,14]. These enzymes are activated under acidic conditions through the cysteine switch mechanism, in which low pH induces conformational changes in the pro-peptide domain of latent pro-MMPs and exposes the catalytic site [14,32,33]. In the ER mode, phosphoric acid etching creates a strongly acidic environment and more effectively triggers this mechanism. In contrast, although the SE mode also relies on acidic monomers for demineralization, its milder acidity results in weaker MMP activation [14,34]. In addition to differences in activation, the two bonding modes also differ markedly in the extent of collagen exposure. The ER mode completely removes the smear layer and creates a demineralized zone approximately 5–10 μm deep. If resin monomers fail to infiltrate this full depth, a substantial amount of collagen remains exposed and unprotected at the base of the hybrid layer [1,8,35]. In contrast, in the SE mode, demineralization and resin infiltration occur simultaneously, resulting in a smaller mismatch between demineralization depth and resin penetration depth and, therefore, significantly less unprotected collagen [1,8,36]. As a result, the ER mode involves not only greater MMP activation but also more collagen substrate available for degradation, which may explain the significantly higher endogenous MMP activity observed in the ER mode than in the SE mode [1,14]. This interpretation is consistent with the findings of Mazzoni et al. [14], who used in situ zymography to directly assess MMP-2 and MMP-9 activity after application of ER and SE adhesive systems and found increased enzymatic activity in both modes, with generally higher activity in the ER mode, consistent with the present findings. This ongoing collagen degradation is an important biological mechanism underlying the decline in bond strength over time [37].

However, the lack of a significant difference in μTBS in support of MMP activity between the ER and SE modes may be attributable to several factors. At the immediate stage, each bonding strategy may offer distinct advantages. The ER mode exposes a larger collagen network through phosphoric acid etching, which may enhance initial micromechanical interlocking [37]. By contrast, in the SE mode, demineralization and infiltration occur simultaneously [1], reducing the amount of unprotected collagen at the base of the hybrid layer. In addition, 10-MDP can form stable chemical bonds with

hydroxyapatite [1,19]. These factors may result in comparable immediate bond strength between the two modes. Furthermore, solvent evaporation and interfacial moisture may have a more direct effect on immediate μTBS than the bonding mode itself [7,9,22]. Together, these factors may explain the absence of a statistically significant difference in immediate bond strength between the ER and SE groups. These findings are consistent with previous studies using universal adhesives under simulated pulpal pressure. Vichianrat et al. [38] reported no significant difference in immediate μTBS between the two modes for Single Bond Universal, and Gonçalves et al. [19] likewise found that bonding mode did not significantly affect the μTBS of the Scotchbond Universal Adhesive(Solventum; St Paul, MN, USA) .

Solvent evaporation by suction induced higher endogenous enzymatic activity at baseline, while air-blowing groups showed higher activity after aging. Hence, the effects of evaporation methods on MMP activity after aging are not in accordance with their effect on μTBS. Because direct evidence on the relationship between solvent evaporation and MMP activity remains limited, this discrepancy suggests that the two outcomes may reflect different aspects of interfacial performance. More effective solvent evaporation by air blowing helps remove residual moisture and promotes monomer polymerization, thereby improving immediate bond strength [7,39,40]. MMP activation depends mainly on the low-pH environment created by acidic monomers during the early bonding stage and on the presence of exposed collagen within the hybrid layer [14], and air suction entails the residues of solvent and water within the hybrid layer [11], allowing for a more prolonged etching of the dentin surface and a higher activation of MMPs. On the other hand, more efficient air blowing evaporates the solvent more efficiently, blocking the acidic reaction of functional monomers. However, this trend was not maintained after aging, where the general activity of MMPs decreased, and remained higher in the air-blowing group. This suggests that the two outcomes were not directly coupled in this study. Given that the endogenous dentinal MMPs were dependent on Ca and Zn ions in the saliva, distilled water may not have provided all the elements necessary for their prolonged activation. The initially more active MMPs in the air-suction groups may have depleted all the available substrate faster than the air-blowing groups, resulting in a lower activity after aging. On the other hand, this did not translate into a higher stability of bond strength after aging, because the degradation by MMPs is only one piece of the puzzle in the complex process of hybrid layer degradation. When investigating solvent evaporation efficacy, it is plausible that the inefficient solvent evaporation resulted in lower polymerization efficacy, and the reduction in bond strength after aging could be attributed more to hydrolytic degradation of the adhesive resin than to the activity of the MMPs. This aspect should be investigated in future studies.

This study used a three-factor design to systematically evaluate the effects of adhesive application mode, solvent evaporation method, and aging time on μTBS and interfacial endogenous MMP activity. Overall, both outcomes changed significantly over time, indicating that aging is a key factor in the long-term behavior of the bonding interface. These effects were not uniform, however. The significant interaction between bonding mode and time suggests that the ER and SE modes followed different aging trajectories, while the effects of the solvent evaporation method and application mode on endogenous MMP activity were likewise modulated by aging. Previous studies have shown that, although the ER mode may provide higher initial bond strength, its long-term stability is often inferior to that of the SE mode [41]. This difference may be related to variations in HL structure. The ER mode is more likely to leave exposed collagen at the base of the HL, making it more susceptible to hydrolytic and enzymatic degradation [8], whereas in the SE mode, demineralization and resin infiltration occur simultaneously, providing better collagen

protection [1]. As a result, the SE mode tends to show a more gradual decline over time. Taken together, long-term changes in the bonding interface are determined not by aging alone, but by the interaction between aging and the bonding strategy.

Several limitations of this study should be acknowledged. Although physiological pulpal pressure was simulated, this was still an in vitro study and did not reproduce the complex mechanical, thermal, and biological conditions of the oral environment; therefore, the findings should be extrapolated to clinical situations with caution [41]. Second, only one brand of universal adhesive was tested, and formulation differences among products may limit the generalizability of the findings [7]. In addition, the perfusion medium used to simulate pulpal pressure was distilled water rather than a physiological medium, which may have somewhat overestimated the effect of moisture interference [19]. Furthermore, similarly to a recently published study [42], sticks were used as statistical units; hence, pseudoreplication cannot be excluded, and the findings should be interpreted with caution. Finally, a 6-month aging period may not have been sufficient to fully reflect long-term changes in bond strength. Future studies should include longer aging periods and multiple adhesive systems to provide a more comprehensive assessment of long-term interfacial stability.

5. Conclusions

When employing an ethanol-based universal adhesive under simulated pulpal pressure, solvent evaporation via a disposable air syringe conferred higher immediate and long-term bond strength. The level of MMP activity can depend on the choice of evaporation method but does not seem to be the only mechanism underlying hybrid-layer degradation over time.

Author Contributions: Conceptualization, C.M. and T.M.; methodology, C.M. and A.M.; validation, F.F.; formal analysis, T.M. and X.B.; investigation, G.C., V.C., L.G., U.J. and X.B.; data curation, U.J.; writing—original draft preparation, T.M., X.B., F.F., C.M. and V.C.; writing—review and editing, L.B., A.M., U.J. and L.G.; supervision, A.M. and L.B.; project administration, C.M.; funding acquisition, L.B. All authors have read and agreed to the published version of the manuscript.

Funding: The research leading to these results received funding from the European Union—NextGenerationEU through the Italian Ministry of University and Research under PNRR—M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to Lorenzo Breschi, CUP J33C22002920006.

Institutional Review Board Statement: University Ethical Committee Approval (protocol N°: 71/2019/OSS/AUSLBO) was obtained for the use of human teeth.

Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset is available on request from the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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