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Effect of Application Delays on the Dentin Bond Strength and Interfacial Morphology of Different Adhesive Systems: An SEM Analysis

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Abstract

Objective: This study evaluated the effect of application delays on the dentin bond strength of different adhesive systems used in composite restorations.

Methods: Forty-five extracted human molars were randomly assigned to Clearfil SE Bond, G-Premio Bond, and Single Bond Universal (n=15). Each adhesive was subdivided according to application time: immediate use, 3-minute delay, and 5-minute delay (n=5). Adhesives were applied to occlusal dentin surfaces according to manufacturers' instructions, followed by composite build-up procedures. Microtensile bond strength (μTBS) was evaluated using a universal testing machine, and failure modes were examined microscopically. Selected specimens were analyzed using scanning electron microscopy (SEM).

Results: Adhesive system ($F=48.32$, $p<0.001$) and application time ($F=22.72$, $p<0.001$) significantly influenced μTBS. The highest bond strength was observed in the immediate Clearfil SE Bond group (34.5±4.8 MPa), whereas the lowest value was recorded in the 5-minute delayed G-Premio Bond group (13.4±2.8 MPa). A 5-minute delay significantly reduced bond strength in all adhesive systems, whereas a 3-minute delay generally did not affect bonding performance. More than 85% adhesive failures were observed in the 5-minute delay groups. SEM analysis revealed porosity and crater-like surface defects in delayed universal adhesive groups.

Conclusions: A 5-minute application delay significantly compromised dentin bond strength and increased adhesive failures. Immediate adhesive application is recommended to preserve bonding effectiveness and optimize restoration longevity**.**

Keywords: Universal adhesive; Application delay; Microtensile bond strength; Dentin bonding; Adhesive performance; Resin adhesion.

1. Introduction

Dental adhesive systems play a crucial role in the long-term success of composite resin restorations by providing durable bonding between restorative materials and dental tissues [1]. The clinical performance of adhesive restorations is highly dependent on the formation of a stable hybrid layer and adequate resin infiltration into dentin, both of which may be influenced by the composition of the adhesive system and application protocol [2,3].

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Contemporary adhesive materials, particularly universal adhesives, contain volatile solvents such as acetone, ethanol, and water to facilitate monomer penetration into the demineralized dentin substrate [4]. However, changes in solvent concentration caused by evaporation may alter adhesive viscosity, monomer diffusion, polymerization behavior, and ultimately bond strength [5,6]. Previous studies have demonstrated that solvent evaporation and inadequate handling procedures may negatively affect the physicochemical properties and bonding effectiveness of adhesive systems [7,8].

In daily clinical practice, adhesive materials are frequently dispensed into a mixing well before application. During restorative procedures, interruptions associated with cavity preparation, matrix placement, composite handling, or other clinical factors may result in unintended delays between adhesive dispensing and application. Such delays may increase solvent evaporation and potentially compromise adhesive performance [9,10]. Despite the clinical relevance of this situation, limited information is available regarding the influence of delayed adhesive application on dentin bond strength.

Universal adhesives have gained widespread popularity because of their simplified clinical protocols and versatility in different bonding strategies [11]. Nevertheless, their relatively high solvent content may render them more susceptible to compositional changes during clinical handling. Understanding the influence of application delays on bonding effectiveness is therefore important for optimizing restorative outcomes and establishing evidence-based clinical recommendations [12].

Previous studies have investigated [4,9,10] the influence of delayed adhesive application, solvent evaporation, and operator-related variables on the bonding performance of adhesive systems. However, these studies primarily focused on delays occurring during clinical application procedures or variations in adhesive protocols. To the authors' knowledge, no previous study has specifically evaluated the effect of pre-application holding time after dispensing adhesive materials onto a mixing plate prior to placement on dentin. Because volatile adhesive components may evaporate during this period, potentially altering adhesive composition and bonding effectiveness, this clinically relevant variable warrants further investigation.

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Such delays are not uncommon in routine clinical practice. During restorative procedures, clinicians may need to manage moisture contamination, control bleeding, replace applicators, adjust matrices, evaluate cavity margins, or restore multiple teeth simultaneously. In these situations, adhesive materials may remain on the mixing plate for several minutes before application. Because contemporary adhesive systems contain volatile solvents, even short waiting periods may alter their composition and potentially affect bonding performance. Therefore, evaluating the clinical consequences of this commonly encountered handling condition is of practical importance.

Therefore, the null hypotheses of the study were as follows:

H_{01}: There is no difference in the bond strength to dentin tissue between the different dental adhesives used in our study.

H_{02}: The duration between transferring the dental adhesives from the bond bottle to a mixing plate and their application to the tooth does not influence their bond strength to dentin.

2. Material and Methods

This study supported by the Akdeniz University Scientific Research Project Coordinatorship under project number TDH-2021-540.

2.1. Preparation and grouping of samples

The sample size was determined according to the methodological recommendations of the Academy of Dental Materials for micro-tensile bond strength (μTBS) testing, which state that when the tooth is considered the statistical unit, a minimum of five teeth per experimental group is acceptable. [13]. Accordingly, five teeth were allocated to each subgroup (n = 5), resulting in a total of 45 teeth distributed in a 3 × 3 factorial design (three adhesive systems × three time periods).

Post-hoc power analysis was performed using G*Power software based on the effect sizes derived from the two-way ANOVA results (Cohen's f = 1.64, 1.12, and 0.74 for adhesive system, application time, and their interaction, respectively). With $\alpha = 0.05$ and a total sample size of 45 teeth, the achieved statistical power exceeded 0.99 for both main effects and was approximately 0.98 for the interaction effect, confirming adequate statistical power despite the modest sample size per subgroup.

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The tooth was defined as the statistical unit. Following sectioning, approximately 10 non-trimmed stick specimens (cross-sectional area ≈ 1 mm²) were obtained from each tooth, depending on dentin surface availability and sectioning geometry. This number falls within the commonly reported range for μTBS testing [13]. Bond strength values obtained from sticks originating from the same tooth were averaged to generate a single tooth-level mean μTBS value before statistical analysis. Therefore, each tooth contributed equally to the analysis, regardless of the number of sticks obtained. This approach prevented teeth yielding a higher number of sticks from exerting disproportionate influence on subgroup means. Although variation in stick number may have affected the precision of individual tooth-level estimates to a limited extent, its potential influence on the final subgroup means was minimized by using tooth-level averaging.

Tested teeth had been extracted due to periodontal, prosthetic, or similar reasons, and were free of caries, fractures, cracks, and material loss. This study examined the impact of two universal adhesives (G-Premio Bond and Singlebond Universal) and one two-step self-etch adhesive (Clearfil Se Bond) on dentin bond strength when applied immediately, after a 3-minute delay, and after a 5-minute delay. The 3- and 5-minute delay periods were selected to reflect clinically plausible chairside interruptions; while 3 minutes represents a short delay, 5 minutes was included to simulate a slightly prolonged and less ideal, yet still possible, clinical scenario.

The materials used in the study are described in Table 1. The scheme of the subgroups formed is also shown in Figure 1.

Group 1: Clearfil SE Bond (n = 15)

Group 1A (Immediate application n = 5): For the immediate application subgroups, the adhesive was dispensed directly from the original bottle onto a disposable applicator and immediately applied to the dentin surface, without any holding time.Then, the adhesive was light-cured according to the manufacturer's instructions.

Group 1B (3-minute delay n = 5): The adhesive was dispensed into a mixing well and left undisturbed with the mixing plate cap closed for 3 minutes under ambient daylight conditions simulating routine clinical practice ambient laboratory conditions at room temperature (23 ± 1°C). During the delay period, the dispensed adhesive was covered with a black application cap to

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minimize ambient light exposure and prevent premature polymerization. The elapsed time was strictly monitored using a digital stopwatch. At the end of the 3-minute holding period, the adhesive was applied to the dentin surface using a disposable applicator. Then, the adhesive was light-cured according to the manufacturer's instructions.

Group 1C: 5-minute delay (n = 5): The adhesive was dispensed into a mixing well and maintained with the mixing plate cap closed for 5 minutes under ambient daylight conditions simulating routine clinical practice ambient laboratory conditions at room temperature (23 ± 1°C). During the delay period, the dispensed adhesive was covered with a black application cap to minimize ambient light exposure and prevent premature polymerization. The holding time was controlled with a digital stopwatch. After completion of the 5-minute interval, the adhesive was applied to the dentin surface using a disposable applicator. Then, the adhesive was light-cured according to the manufacturer's instructions.

The same experimental procedures and subgroup protocols were applied to **Group 2 (G-Premio Bond, n = 15)** and **Group 3 (Single Bond Universal, n = 15).**

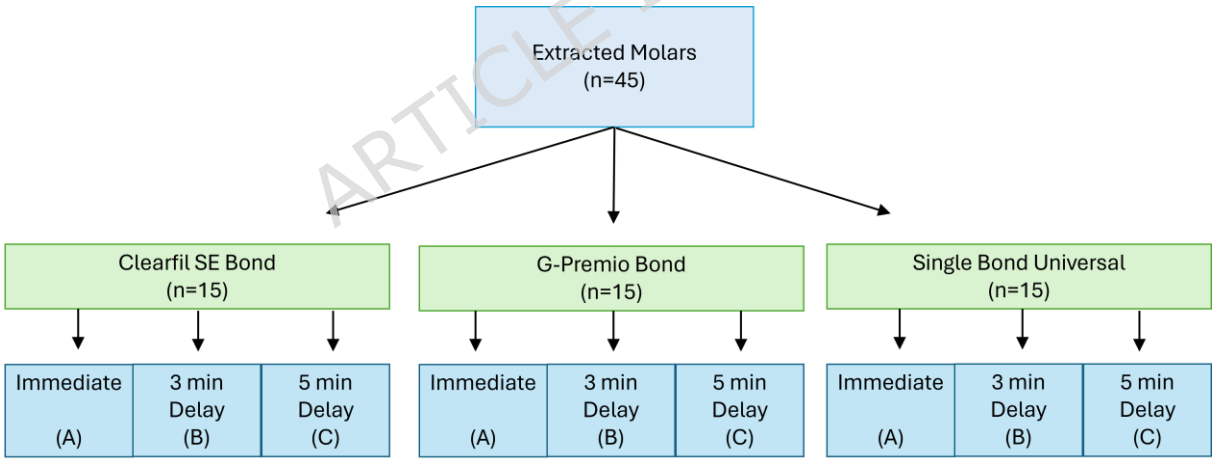


Figure 1. Experimental design flowchart illustrating the grouping of extracted molars according to bonding agent and holding time before light curing.

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2.3. Adhesive application procedure

Clearfil SE Bond (Two-step self-etch adhesive)

Clearfil SE Bond was applied according to the self-etch protocol. The primer was actively applied to the dentin surface using a microbrush with gentle agitation for 20 seconds, followed by gentle air-drying for approximately 5 seconds to evaporate the solvent. Subsequently, the bonding resin was applied in a uniform layer and gently air-thinned to ensure even distribution. The adhesive layer was then light-cured for 10 seconds using an LED curing unit operating at an intensity of approximately 1000 mW/cm².

G-Premio Bond (Universal adhesive)

G-Premio Bond was applied in self-etch mode. The adhesive was actively rubbed onto the dentin surface for 10–20 seconds using a microbrush to enhance infiltration. Gentle but continuous air-drying was performed for approximately 5 seconds to facilitate solvent evaporation, particularly due to its acetone-based composition. The adhesive was then light-cured for 10 seconds with the same LED curing unit.

Single Bond Universal (Universal adhesive)

Single Bond Universal was applied in self-etch mode. The adhesive was actively applied to the dentin surface with agitation for 20 seconds. A gentle air stream was applied for approximately 5 seconds to evaporate solvents and thin the adhesive layer. Light polymerization was subsequently performed for 10 seconds using the LED curing unit at approximately 1000 mW/cm².

After completion of the bonding procedures and composite build-ups, all specimens were stored in distilled water at 37 ± 1 °C for 24 hours to allow post-polymerization and to standardize conditions for immediate bond strength evaluation prior to microtensile testing.

2.2. Micro-tensile Bond Strength Test

The 3- and 5-minute pre-application delay periods were selected to simulate clinically relevant waiting times after dispensing the adhesive into a mixing well before application to dentin. In

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routine restorative procedures, the adhesive may remain in the mixing well for a short period due to chairside interruptions or procedural steps. The 5-minute interval was included because at least one of the tested adhesives is reported by the manufacturer to have an extended working time of up to 5 minutes after dispensing. Therefore, the selected intervals allowed evaluation of whether short pre-application delays within a clinically plausible working period could affect dentin bond strength.

Forty-five teeth selected for microtensile testing were embedded in acrylic resin blocks. The occlusal surfaces of the teeth were removed in order to expose the dentin, a process known as underwater cooling. The bonding agent for each group was applied to the dentin surfaces in accordance with the manufacturer's instructions.

The restoration's height was standardised through the implementation of a layering technique (comprising two layers of 2 mm each) with 4 mm composite resin (Grandio, VOCO GmbH, Cuxhaven, Germany) on the bonded surfaces. Polymerization was performed using Valo Cordless (Ultradent, South Jordan, Utah, USA). The specimens were then placed within the IsoMet™ Low Speed Precision Cutter (Waukegan Road, Lake Bluff, IL, USA) device, and 10 sticks measuring 1x1 mm were produced from each specimen. The resultant sticks were then placed into the Micro Tensile Tester (Bisco Inc., Schaumburg, IL, USA), a micro tensile testing device. Each stick specimen was fixed to the microtensile testing jig using a cyanoacrylate adhesive (Pattex 2K Activator Rapid Adhesive, Henkel, Turkey) prior to μTBS testing.The specimens were subjected to microtensile bond strength (μTBS) testing using a universal testing machine at a crosshead speed of 1.0 mm/min with a 50-kgf load cell until failure occurred. Tension was applied until fracture occurred between the dentin and composite.

Following μTBS testing, 20 fractured stick specimens from each subgroup were selected for failure mode evaluation under stereomicroscopy (x25) (Zeiss, Jena, Germany) to determine the nature of the fracture [14]. The failure types were classified in accordance with the following categories. Adhesive failure was observed in the adhesive layer. Observations of cohesive failure have been made in dentin or composite resin, with a mixture of these two materials present. Mixed failure was observed in both adhesive resin and substrates.

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2.3. Scanning Electron Microscope (SEM) Examination

The surface of the samples that broke during micro-tension bond strength tests was analysed using a scanning electron microscope (Leo 1430, Zeiss, Jena, Germany). For SEM examination, one fractured stick specimen was randomly selected from each subgroup. The selected specimen was coated with gold-palladium and examined under SEM. No quantitative image analysis or morphometric measurements were performed. Representative specimens from each group were coated with 300 angstroms of gold-palladium using a Polaron SC7620 Sputter Coater (VG Microtech Inc., West Sussex, UK) coating device for SEM analysis. The specimens were then placed into the microscope for imaging, and images were obtained.

2.4. Statistical Analysis

Normality of tooth-level mean μTBS values was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene's test. The assumptions of normality and homoscedasticity were satisfied. For each tooth, bond strength values obtained from stick specimens were averaged to generate a single mean μTBS value prior to statistical analysis.

A two-way analysis of variance (ANOVA) was performed to evaluate the effects of adhesive system (three levels), application time (three levels), and their interaction on microtensile bond strength. Statistical analyses were performed using jamovi software and R software version 4.6.0 (R Foundation for Statistical Computing, Vienna, Austria). Post hoc pairwise comparisons were performed using Tukey-adjusted estimated marginal means. Mean differences (MD), 95% confidence intervals (CI), adjusted p values, and Cohen's d effect sizes were reported for pairwise comparisons. Cohen's d was calculated using the residual standard deviation from the ANOVA model. Failure mode distributions were compared among groups using the Fisher–Freeman–Halton exact test. The level of statistical significance was set at $\alpha = 0.05$.

3.Results

The present in vitro study examined the effects of delays during the application of dental adhesives on bond strength. The bond strength results of adhesives applied immediately, after a 3-minute wait, and after a 5-minute wait were measured, the fracture types were determined, and representative SEM images were analysed for each group.

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3.1. Micro-tensile Bond Strength Test Results of Dental Adhesives

Two-way ANOVA identified significant main effects of adhesive system ($F(2,36)=48.32$, $p<0.001$, partial $\eta^2=0.729$) and application time ($F(2,36)=22.72$, $p<0.001$, partial $\eta^2=0.558$) on μTBS. The adhesive system × application time interaction was also significant ($F(4,36)=4.93$, $p=0.003$, partial $\eta^2=0.354$), indicating that the influence of application time differed by adhesive.

Mean μTBS values (MPa) and standard deviations for all groups are shown in Figure 2. The Clearfil SE Bond control group showed the highest μTBS (34.5±4.8), whereas G-Premio Bond applied after 5min exhibited the lowest mean μTBS (13.4±2.8).

For Clearfil SE Bond, the 5-minute delay resulted in significantly lower μTBS compared with both immediate application (mean difference [MD] = 11.6, $p < .001$) and 3-minute delay (MD = 8.4, $p = .015$). No significant difference was observed between immediate application and 3-minute delay (MD = 3.2, $p = .872$).

For G-Premio Bond, immediate and 3-minute applications did not differ significantly (MD = −2.19, $p = .986$). However, the 5-minute delay significantly reduced bond strength compared with the 3-minute delay (MD = 9.00, $p = .007$).

For Single Bond Universal, immediate application yielded significantly higher μTBS than both 3-minute (MD = 8.9, $p = .008$) and 5-minute delays (MD = 7.3, $p = .049$). No significant difference was detected between 3-minute and 5-minute delays (MD = −1.60, $p = .998$). Compared with Clearfil SE Bond, Single Bond Universal exhibited significantly lower bond strength at all time intervals except the 3-minute comparison with Clearfil SE Bond ($p = .053$).

Overall, significantly lower μTBS values were observed in the 5-minute delay groups, particularly for Clearfil SE Bond and G-Premio Bond. The most pronounced decrease was observed between Clearfil SE Bond applied immediately and G-Premio Bond after a 5-minute delay (MD = 21.1, $p < .001$).

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Detailed Tukey-adjusted pairwise comparisons for the adhesive system × application time interaction are presented in Supplementary Table S1. The table includes mean differences, 95% confidence intervals, adjusted p values, and Cohen's d effect sizes for all pairwise comparisons.

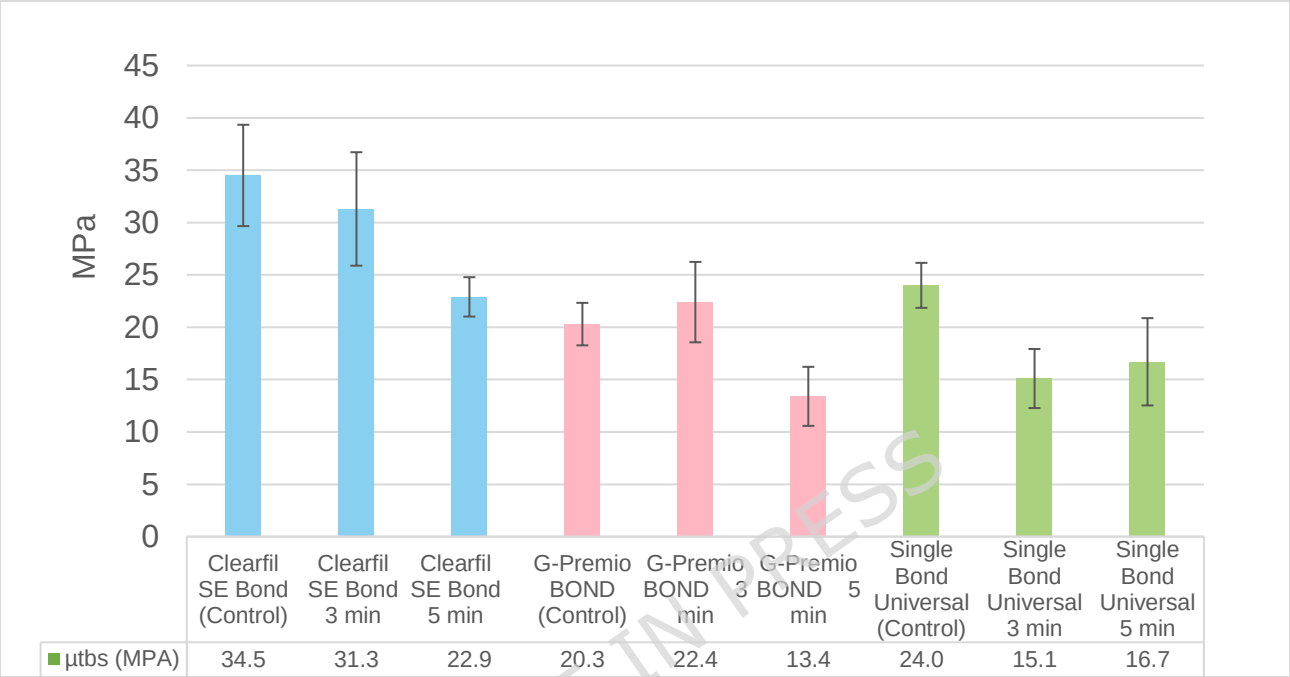


Figure 2. Mean bond strength values for each group. The error bars represent the standard deviation of the data.

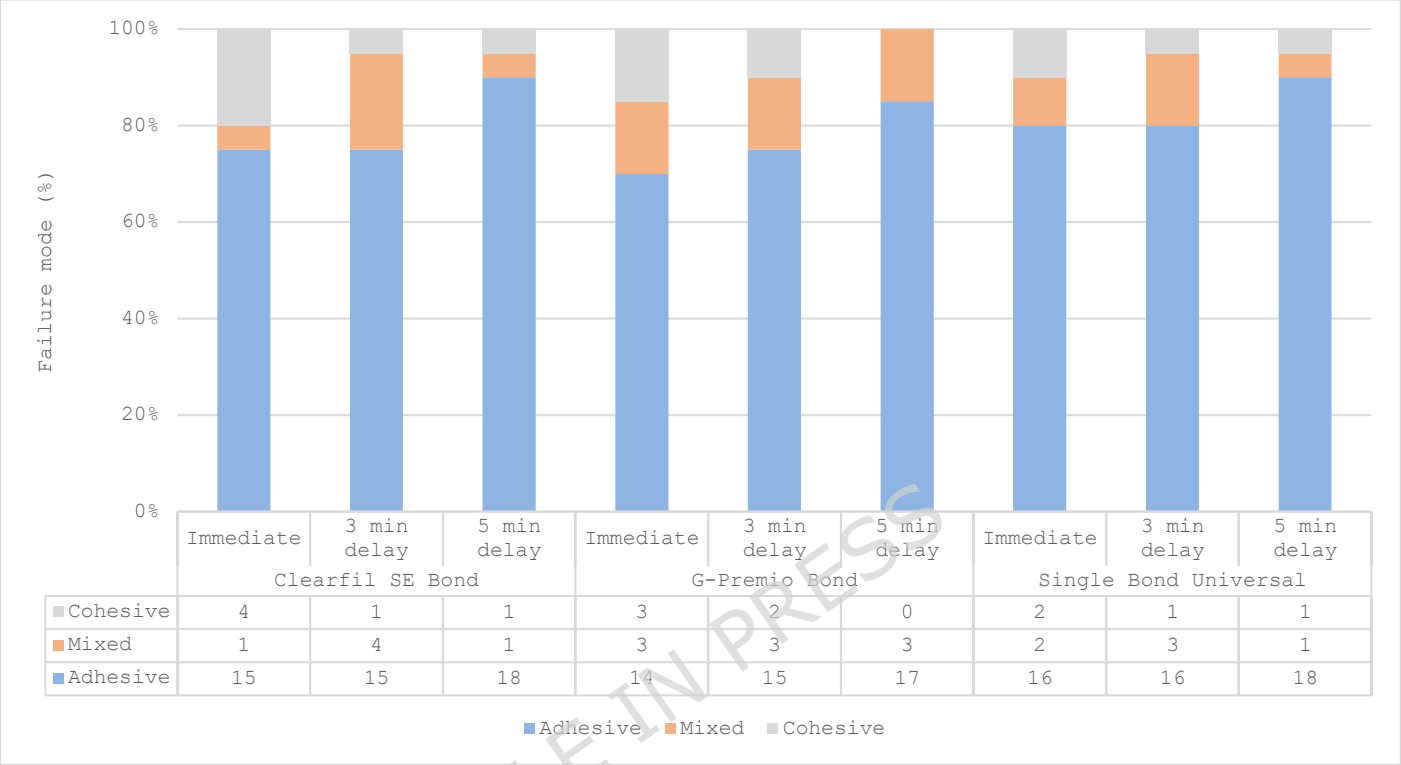
3.2. Analysis Results of Fracture Types

Following the implementation of the micro-tensile test, the surfaces of the fractured specimens were subjected to microscopic examination using a microscope to ascertain the nature of the fracturing in the samples.

The fracture mode analysis revealed that adhesive failure was the predominant pattern across all groups. In all adhesive systems, the proportion of adhesive failure increased with prolonged application delay, with the highest percentages observed in the 5-minute groups. Conversely, cohesive failure decreased as the delay time increased and was completely absent in the 5-minute G-Premio Bond group. Mixed failures were observed at lower rates and did not show a consistent trend among the groups.

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The distribution of failure modes is presented in Figure 3. Values are expressed as percentages of specimens in each failure category.



	Clearfil SE Bond			G-Premio Bond			Single Bond Universal		
	Immediate	3 min delay	5 min delay	Immediate	3 min delay	5 min delay	Immediate	3 min delay	5 min delay
Cohesive	4	1	1	3	2	0	2	1	1
Mixed	1	4	1	3	3	3	2	3	1
Adhesive	15	15	18	14	15	17	16	16	18

Figure 3: Distribution of adhesive, mixed, and cohesive failure modes according to adhesive system and application time. Stacked columns represent percentage distributions of failure modes, whereas the embedded data table presents the actual number of specimens in each category. Twenty fractured stick specimens from each subgroup were evaluated for failure mode analysis. No statistically significant differences were observed among groups (Fisher–Freeman–Halton exact test, $p = 0.768$).

3.3. SEM Analysis Results

Images were acquired at magnifications of 175x and 1000x from composite fragments fractured post-microtensile testing of samples prepared for SEM examination.

In Figure 4, images A1–A2 represent the Clearfil SE Bond group immediate, B1–B2 the group with a 3-minute delay, and C1–C2 the group with a 5-minute delay. At 175× magnification, fractures were observed on the composite surfaces in A1, B1, and C1. At 1000× magnification, microscopic examination revealed delamination on the composite surfaces.

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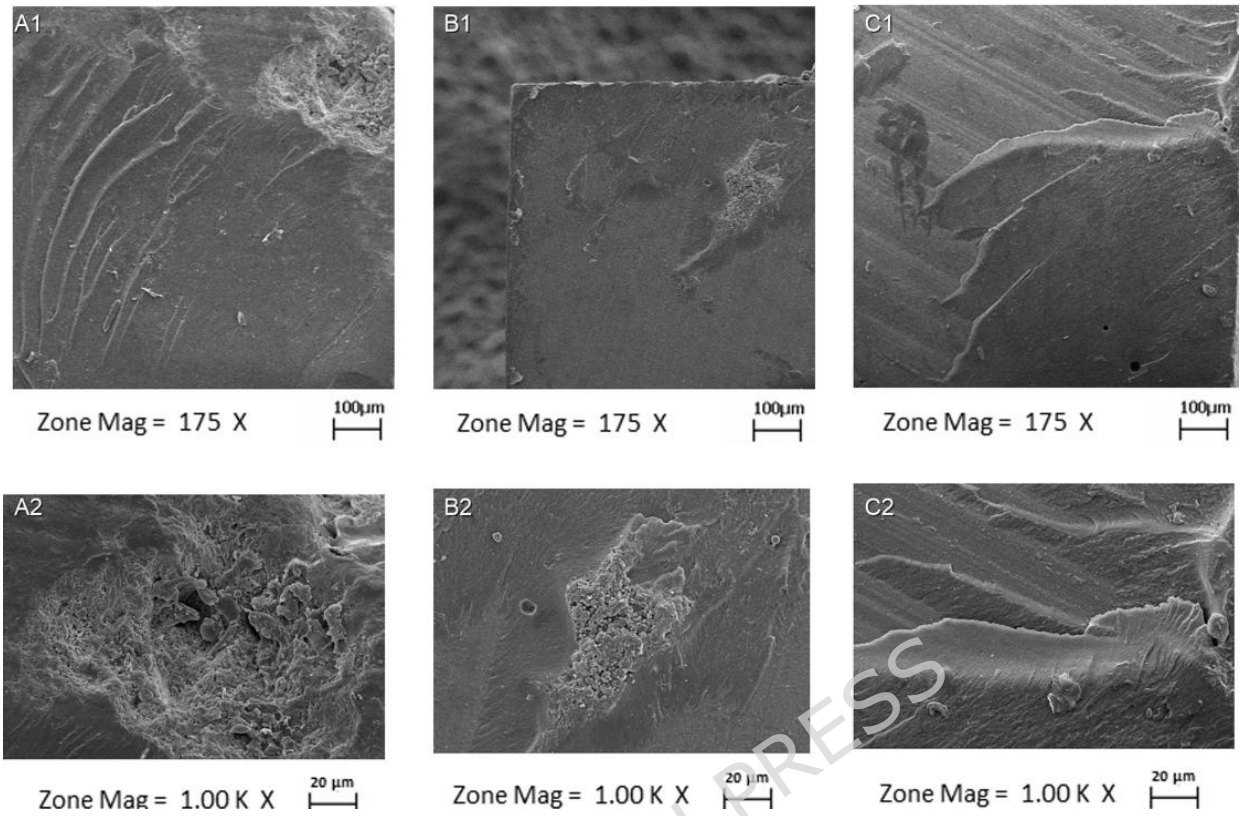


Figure 4 . Representative SEM micrographs of Clearfil SE Bond specimens. A1–A2: immediate application; B1–B2: 3-minute delay; C1–C2: 5-minute delay. Upper images were obtained at 175× magnification and lower images at 1000× magnification.

Images D1–D2 represent the Single Bond Universal group applied immediately, E1–E2 the group with a 3-minute delay, and F1–F2 the group with a 5-minute delay. Images obtained from the immediate and 3-minute delay groups showed composite surface fractures and delamination features. In contrast, specimens in the 5-minute delay group exhibited porous areas and crater-like surface formations (Figure 5).

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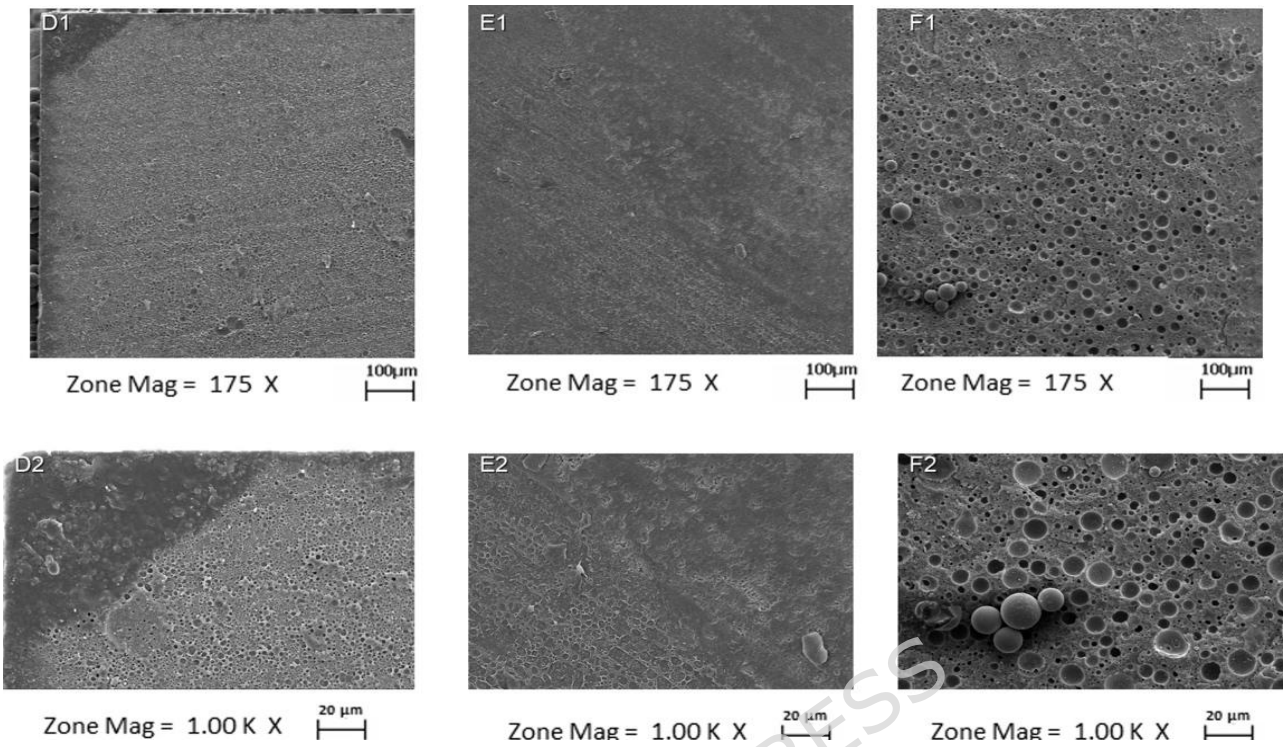


Figure 5. Representative SEM micrographs of Single Bond Universal specimens. D1–D2: immediate application; E1–E2: 3-minute delay; F1–F2: 5-minute delay. Upper images were obtained at 175× magnification and lower images at 1000× magnification.

Images G1–G2 represent the G-Premio Bond group applied immediately, H1–H2 the group with a 3-minute delay, and J1–J2 the group with a 5-minute delay. Composite surface fractures and delamination features were observed in the immediate and 3-minute delay groups, whereas the 5-minute delay group exhibited porous areas and crater-like surface formations (Figure 6).

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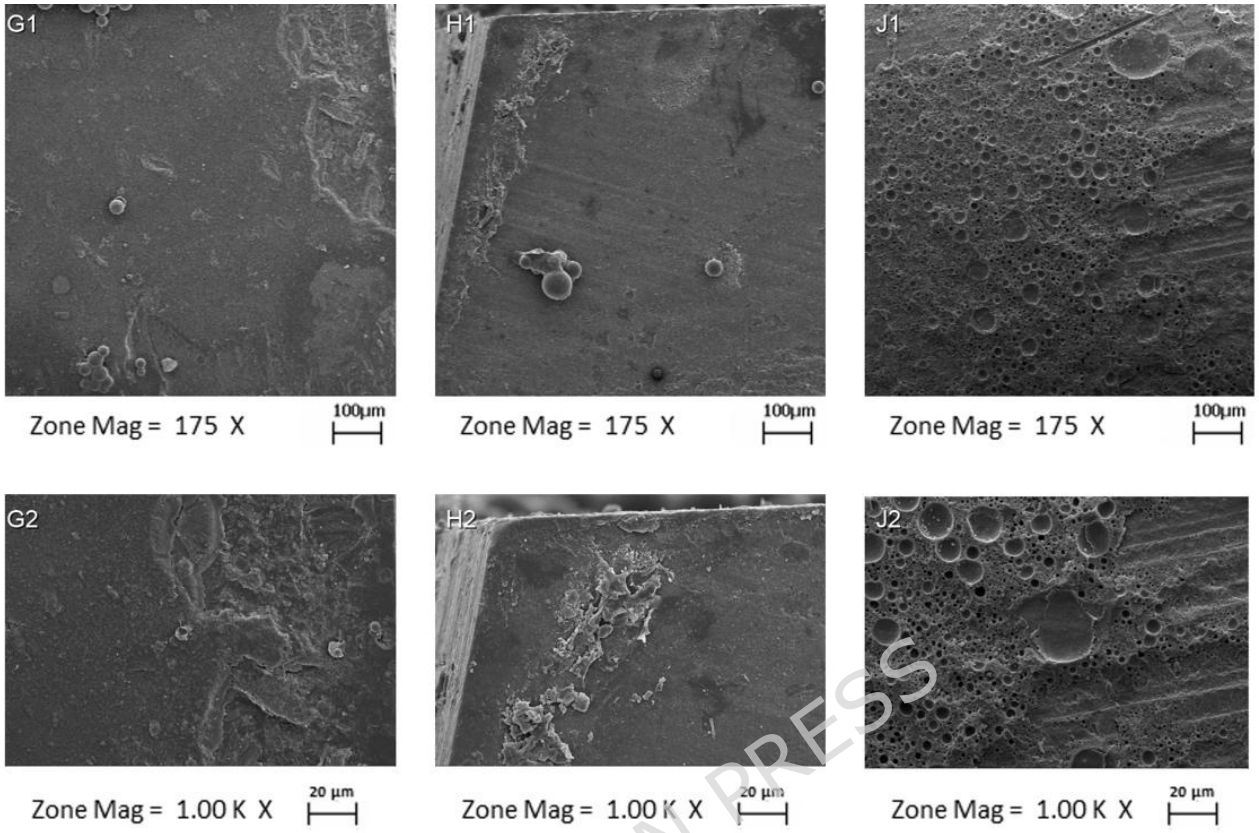


Figure 6. Representative SEM micrographs of G-Premio Bond specimens. G1–G2: immediate application; H1–H2: 3-minute delay; J1–J2: 5-minute delay. Upper images were obtained at 175× magnification and lower images at 1000× magnification.

4.Discussion

In our study, a sixth-generation self-etch adhesive (Clearfil SE Bond) and two universal adhesives were tested. Among our test groups, the highest average bond strength values were obtained in the control group treated with Clearfil SE Bond. Compared to the control groups, this two-step self-etch system was found to have statistically higher average bond strength values than the universal bonds (G-Premio Bond and Singlebond Universal) ($p<0.05$). Therefore, the H01 hypothesis of our study was rejected.

The lowest average bond strength value was measured in the G-Premio Bond group in samples 5 minutes delay. In all adhesive groups, the 5-minute delay groups statistically significantly reduced bond performance. Therefore, the second null hypothesis of our study was rejected too.

During adhesive procedures, certain delays may occur due to operator errors or unforeseen clinical circumstances, potentially prolonging the time before adhesive application. Previous studies have

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extensively investigated parameters such as the duration of air-drying and their influence on solvent evaporation, adhesive infiltration, and bond strength [15-18]. Our findings are consistent with those of Awad et al. [17], who reported that adhesive air-drying protocols significantly influence dentin bond strength. Similarly, Luque-Martinez et al. [19] demonstrated that solvent evaporation time affects the immediate bonding properties of universal adhesives. Although those studies focused on air-drying and solvent evaporation procedures, the present study evaluated a different clinical scenario involving pre-application holding time on a mixing plate. However, to the best of our knowledge, no studies have specifically examined the effect of leaving adhesives in a mixing plate for different time intervals prior to application. This represents an unexplored research gap that may have significant clinical implications, as prolonged exposure to the ambient environment could alter the physical and chemical properties of adhesives, ultimately compromising their bonding performance.

The selection of the 3- and 5-minute delay intervals was designed to simulate a realistic clinical scenario in which adhesive materials are dispensed onto a mixing plate at the beginning of the restorative procedure and remain exposed on the dental unit tray while other operative steps are performed. In routine practice, clinicians may prepare the adhesive material prior to cavity preparation; however, before reaching the adhesive application stage, procedures such as caries removal, cavity refinement, and acid etching are completed.

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The volatile solvents present in adhesive formulations, such as acetone and ethanol, may gradually evaporate once the material is dispensed from the original bottle, leading to alterations in its composition over time [19,20]. Similar observations have been reported by Gutiérrez et al. [15], who demonstrated that prolonged solvent evaporation may negatively affect the clinical performance of universal adhesives. Likewise, Klein-Junior et al. [20] reported that solvent evaporation conditions influence adhesive layer characteristics and resin-dentin bond strength.Furthermore, prolonged exposure of adhesive systems in a mixing plate may result in the degradation of functional monomers such as dimethacrylate and 10-MDP, accompanied by the release of by-products such as free methacrylic acid and free phosphoric acid. These chemical changes are likely to compromise the physicochemical properties of the adhesive and its bonding effectiveness [21,22].

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In the present study, samples in which adhesives were left in a mixing plate for 5 minutes exhibited a markedly higher percentage of adhesive failures compared with both the control group and the 3-minute groups. This outcome may be associated with alterations in adhesive composition during the waiting period and the presence of unbound acidic by-products, which may have weakened bonding efficiency [23].

The adhesive systems evaluated in this study were deliberately chosen to represent contemporary bonding strategies with different chemical compositions and solvent systems. Clearfil SE Bond, a two-step self-etch adhesive containing the functional monomer 10-MDP, was included as a well-established reference material with documented clinical reliability, durable chemical interaction with hydroxyapatite, and stable bonding performance over time [24–26]. G-Premio Bond and Single Bond Universal, on the other hand, are widely used universal adhesives characterized by simplified clinical protocols and distinct solvent and monomer formulations [27,28]. G-Premio Bond contains a multifunctional monomer system and a solvent composition predominantly based on acetone, which is known for its high volatility and technique sensitivity [29]. In contrast, Single Bond Universal incorporates ethanol/water as solvent components along with 10-MDP and additional functional monomers, which may influence water sorption, solvent evaporation dynamics, and long-term bonding stability [9,30]. The inclusion of these adhesives enabled comparison of whether differences in solvent volatility and monomer chemistry influence the material's susceptibility to environmental exposure prior to application.

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The SEM analysis provided detailed insight into the fracture characteristics and interfacial integrity of the tested adhesive systems. In the Clearfil SE Bond groups, fractures observed at 175× magnification were predominantly located on the composite surfaces, while higher magnification (1000×) revealed delamination patterns. These findings suggest that although the adhesive interface was involved, failure propagation extended into the composite layer, indicating a relatively stable bonding interface in the immediate and 3-minute delay groups.

In contrast, the Single Bond Universal and G-Premio Bond groups demonstrated a more pronounced time-dependent deterioration in surface morphology. Specimens from the immediate and 3-minute delay groups exhibited characteristics consistent with cohesive failure, indicating that the adhesive interface remained relatively intact under these conditions. However, in the 5-

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minute delay groups, distinct morphological alterations were observed, including increased porosity and crater-like surface formations. These features may indicate compromised structural integrity and may be associated with solvent evaporation, phase separation, or incomplete polymerization of the adhesive layer during prolonged pre-application exposure [9,31]. Additionally, incomplete polymerization and phase separation phenomena have been associated with interfacial defects and reduced bonding performance [20,31]. The presence of porosity and crater-like defects in the 5-minute delay groups is particularly noteworthy, as such features can act as stress concentration sites and facilitate crack initiation and propagation.

This observation is consistent with the failure mode analysis, which demonstrated a marked increase in adhesive failure rates and a reduction in cohesive failures in delayed groups. The shift toward predominantly adhesive failure may suggest that the integrity of the adhesive interface was compromised. Similar relationships between interfacial degradation and failure mode distribution have been reported in previous studies evaluating dentin bonding performance [19,31].

Collectively, these findings suggest that extended application delay may negatively influence adhesive performance and contribute to deterioration of the adhesive interface, leading to a shift toward weaker, interface-dominated failure patterns. It should be noted that SEM evaluation was performed on fractured surfaces and was intended only as a qualitative assessment of fracture morphology. Therefore, no direct conclusions regarding adhesive infiltration, hybrid layer formation, or monomer effectiveness can be drawn from these observations.

Despite the valuable findings of the present study, certain limitations should be acknowledged. First, this investigation was conducted under in vitro conditions, which cannot fully replicate the complex intraoral environment, including variations in temperature, humidity, pulpal pressure, and saliva contamination. Second, the delay periods were standardized and performed under controlled laboratory conditions; however, in clinical practice, environmental factors and operator-related variables may further influence solvent evaporation and adhesive stability. Additionally, a limitation of this study is that only immediate bond strength was evaluated, without assessment of long-term durability or aging effects; therefore, the results should be interpreted with caution, as bond strength values may decrease over time under clinical conditions. The chemical alterations hypothesized to occur during the waiting period were inferred based on bonding performance and

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failure modes, but no direct physicochemical analyses (e.g., spectroscopy) were performed to confirm these changes. Therefore, further studies incorporating long-term aging protocols and analytical evaluation of chemical stability are required to better elucidate the mechanisms underlying the observed reduction in bond strength.

5.Conclusion

Under standard application conditions, Clearfil SE Bond demonstrated significantly higher dentin bond strength than G-Premio Bond and Single Bond Universal ($p < 0.001$). A 3-minute delay on the mixing plate did not significantly affect the bond strength of Clearfil SE Bond or G-Premio Bond compared with their respective control groups ($p > 0.05$), whereas a 5-minute delay resulted in a significant reduction in bond strength across all adhesive systems ($p < 0.05$). Failure mode analysis revealed that the 5-minute delay subgroups exhibited the highest percentage of adhesive failures. SEM observations demonstrated porous, bubble-like, and crater-like surface features, particularly in G-Premio Bond and Single Bond Universal specimens after a 5-minute delay. Within the limitations of this study, immediate adhesive application is recommended to maintain optimal dentin bonding performance.

Clinical Significance: This study highlights that even short delays in adhesive application can weaken dentin bonding and increase failure risk. Adhesives should be applied immediately after dispensing to preserve their effectiveness and ensure long-term restoration durability.

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Conflict of Interest Statement

The authors declare that they do not have any financial interest in the companies whose materials are included in the article.

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Ethics Committee Approval

This study was carried out in accordance with the ethical standards laid out in the 1964 declaration of Helsinki and its later amendments and approved by the Akdeniz University Ethics Committee in Antalya,Turkey ; approval number: 616 on August 26, 2020. All participants provided written informed consent prior to participation in the study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Table 1. Materials used in the study with their manufacturers, application procedures according to manufacturers' instructions, main chemical components, and corresponding lot numbers.

Material and Manufacturer	Manufacturer's Instructions	Chemical Content	Lot Number
Clearfil SE Bond Kuraray Noritake Dental Inc., Japan	Use a light-blocking tray to protect from operating light or natural light and use within 3 minutes of mixing. The irradiation tip of the dental curing light should be held as close to the resin surface as possible and perpendicular to it. If the adherent surface covers the uncut surface, apply phosphoric acid agent and wait 10 seconds before rinsing and drying.	10-MDP, Bis-GMA, HEMA, hydrophobic aliphatic dimethacrylate, di-camphorquinone and colloidal silica, N,N-diethanol-p-tolidine	9G0586
G-Premio BOND GC,Tokyo, Japan	Shake the bottle before use. Pour a few drops from the bottle into a clean well. Close the cap immediately. Use the bond applied to the well within 5 minutes. If the adherent surface covers the uncut surface, etch it with 35-40% phosphoric acid for 10-15 seconds. Wait 10 seconds after bond application. Dry the surface with oil-free air at maximum air pressure for 5 seconds. Cure with a halogen/LED light for 10 seconds and a high-energy LED light for 5 seconds.	10-MDP, 4-MET, MDTP (Methacryloyloxydecyl dihydrogen thiophosphate), distilled water, acetone, photoinitiators	2009011
Singlebond Universal Bond 3M ESPE, St. Paul, MN, USA	It can be applied to both moist and dry dentin. Avoid contact with light before use. Apply the adhesive to the tooth surface, applying force for 20 seconds. Air dry for 5 seconds, then light cure for 10 seconds.	10-MDP, HEMA, silane, dimethacrylate resin, Vitrebond copolymer, filler, ethanol, water, initiator	01216A
Grandio Nanohybrid Resin Composite VOCO GmbH, Cuxhaven, Germany	Apply in 2 mm layers. Light power should be at least 500 mW/cm for halogen curing devices and 300 mW/cm for LED devices. Curing time is 20 seconds for each layer and at least 40 seconds for opaque colors such as OA2 and OA3.5. The light bar should be held as close to the filler as possible.	BIS-GMA, TEGDMA, UDMA, Universal light-cured 87% filled nanohybrid composite	1939193