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Impact of Tooth Age and Adhesive Application Period on Dentin Microtensile Bond Strengths of Two Different Adhesive Systems: An In Vitro Comparative Study

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ABSTRACT

Objectives: The current research assessed the impact of dentin age and adhesive application period on the microtensile bond strength (µTBS) of two different adhesive systems.

Materials and Methods: A total of 96 human teeth were initially split into main groups (n=48) according to the age categories: young dentin (18–30 years) and aged dentin (≥55 years). Then, each main group was randomly split into two equal subgroups (n=24) based on the used adhesive system: a total-etch, 2-step system (Adper Single Bond 2) or a self-etch, 1-step system (Clearfil S³ Bond). Each subgroup was split into two divisions (n=12) based on the application time: the manufacturer's recommended time or an extended time (double the recommended duration). Following adhesive application, a nanohybrid resin composite (Filtek Z350 XT, 3M ESPE) was placed incrementally in 2-mm layers over the bonded dentin surface via a specially-made Teflon split disc (4×4 mm). Following 24 hours of immersion in distilled water, the samples were cut into sticks and subjected to µTBS testing by the universal testing machine.

Results: The results revealed that neither dentin age nor adhesive application time produced significant main effects. There was no statistically significant relationship between dentin age and application time. However, the total-etch adhesive demonstrated significantly greater µTBS values than the self-etch adhesive across all conditions.

Conclusions: The performance of adhesive systems was primarily determined by the bonding strategy rather than dentin age or extended application time. The total-etch, 2-step adhesive system consistently outperformed the self-etch adhesive irrespective of substrate aging or application protocol.

Keywords: *Dentin age; Application time; Microtensile bond strength; Total-etch; Self-etch.*

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1. INTRODUCTION

The longevity of tooth-colored restorations continues to be a central concern in restorative dentistry (1). The etch-and-rinse (total-etch) approach involves separate acid etching and rinsing steps and creates a deepest hybrid layer in dentin than the self-etch approach (2). However, due to its multiple steps and the greater impact of the acid etching process on the dentin tissue, the etch-and-rinse procedure necessitates an extended treatment duration, increases post-filling hypersensitivity, and is greater prone to failure than the self-etch approach (3). By minimizing clinical steps, cutting down on clinical utilization time, and lowering method sensitivity, modern adhesive technology tends to streamline bonding processes and improve standardization (4). Recent advancements in non-rinsing adhesives, referred to as self-etch systems, have made the conventional idea of bonding simpler. In comparison with multi-step etch-and-rinse adhesives, these materials are simple to utilize and need less time to apply. Another significant therapeutic advantage of self-etch adhesives is that patients have less or no post-operative sensitivity (5).

Although advances in adhesive systems have refined the predictability of resin-dentin bonding, achieving durable adhesion remains challenging, particularly when the underlying substrate is physiologically aged dentin (6). Dentin is a biologically dynamic and heterogeneous tissue, and as it ages, it undergoes structural changes that can significantly affect the efficacy of adhesive procedures (7).

With aging, dentin undergoes changes, notably a reduction in dentinal tubule patency due to mineral deposition, a process termed sclerosis. This is accompanied by intertubular dentin thickening and alterations in collagen structure, including tighter packing and decreased water content (8). These age-related transformations can compromise both the micromechanical and chemical interactions necessary for effective bonding. Yet, despite these physiological changes, it was reported that there was no substantial variation in microtensile bond strength (μTBS) between young and aged dentin when using conventional adhesive systems (6).

Another strategy proposed to enhance adhesion in aged or less permeable dentin is the extension of adhesive application time. The rationale is that prolonged application allows more solvents to evaporate and facilitates deeper resin infiltration into demineralized collagen matrices (9). It was demonstrated that increasing application time significantly improved bond strength, particularly for acetone-based adhesives, which are more volatile and technique-sensitive. Extending the application to 150 or even 300 seconds provided measurable improvements in μTBS, likely due to more effective solvent evaporation and monomer diffusion (10).

While current dental adhesive protocols are often standardized, the relationship between dentin aging, adhesive choice, and application time is not fully understood. Given the increasing number of elderly patients needing restorative work, research into how adjusting adhesive application techniques, like extending application time, might improve results across different dentin ages is crucial, according to a recent study (11).

Therefore, this study aimed to assess the impact of dentin age and adhesive application period on the μTBS of two different adhesive systems (2-step total-etch and 1-step adhesive systems). The null hypothesis of the current research stated that dentin age and adhesive application time would not influence the μTBS of the two adhesive systems.

The current research assessed the impact of dentin age and adhesive application period on the microtensile bond strength (μTBS) of two different adhesive systems

2. MATERIALS AND METHODS

Materials

In this investigation, two adhesives were employed: 2-step total-etch: Adper Single Bond 2 (3M ESPE) – and 1-step self-etch: Clearfil S³ Bond (Kuraray Noritake Dental Inc.). Also, a nanohybrid resin composite (Filtek Z350 XT, 3M ESPE) was used in this study. The materials utilized in this investigation are enumerated in (**Table** 1).

Table 1: Composition and manufacturer information of materials utilized

Material	Type	Manufacturer	Composition
Adper Single Bond 2	Total-etch, 2-step adhesive	3M ESPE, St. Paul, MN, USA.	BisGMA, HEMA, dimethacrylates, ethanol, water, a specialized photoinitiator system, and a polyalkenoic acid copolymer (a methacrylate functional copolymer of polyacrylic and polyitaconic acids). It also contains a 10% weight of 5nm diameter silica nanofiller.
Clearfil S³ Bond	Self-etch, 1-step adhesive	Kuraray Medical Inc., Tokyo, Japan.	MDP, bis-GMA, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, silanated colloidal silica, ethyl alcohol, and water
Scotchbond Universal Etchant	Phosphoric acid etchant	3M ESPE, St. Paul, MN, USA	This etching gel contains 35% by weight phosphoric acid. Its viscosity is modified with fumed silica and a water-soluble polymer, and it has a distinct blue color to aid in rinsing.
Filtek Z350 XT	Nanohybrid resin composite	3M ESPE, St. Paul, MN, USA.	The resin contain: UDMA, Bis-GMA, Bis-EMA, TEGDMA. The fillers consist of: Silica (20 nm nonagglomerated/aggregated), zirconia (4-11 nm nonagglomerated/aggregated and agglomerated), clusters, zirconia/silica aggregated particles (20 nm silica particles combined with 4-11 nm zirconia 3).

BisGMA: Bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, UDMA: Urethane dimethacrylate, Bis-EMA: Ethoxylated bisphenol A dimethacrylate, TEGDMA: Triethylene glycol dimethacrylate, PEGDMA: Polyethylene glycol dimethacrylate.

Sample size calculation

Employing G*Power 3.1 (Heinrich Heine University, Düsseldorf, Germany), the necessary sample size was calculated according to data from a previous research evaluating the impact of dentin age on the µTBS using similar adhesives [(6)]. Considering an effect size of 0.40, a power of 0.80, and a significance level of $\alpha = 0.05$ for a two-way ANOVA (factors: dentin age and application time), the minimum number of specimens required per subgroup was calculated to be 10 teeth. To account for potential pre-test failures, each subgroup included 12 teeth, resulting in a total of 96 teeth for the study.

Tooth Selection and Storage

A total of 96 sound, human third molars extracted for orthodontic or periodontal reasons were collected following institutional ethical approval. All teeth were free from caries, cracks, restorations, and developmental defects. Immediately after extraction, the teeth were cleaned of soft tissue debris and kept in 0.5% chloramine-T solution at 4°C until use, for a maximum period of three months, as recommended in similar experimental protocols [(12)].

Teeth grouping

The 96 teeth included in this study were initially split equally into main groups (n=48) based on the age categories: young dentin (18–30 years) and aged dentin (≥55 years). After that, each main group was randomly split into two equal subgroups (n=24) based on the used adhesive approach: a 2-step total etch system (Adper Single Bond 2) or a self-etch, 1-step system (Clearfil S³ Bond). Then each subgroup was split into two equal divisions (n=12) based on the application time: the manufacturer's recommended time (control) or an extended time (double the recommended duration).

Mold Fabrication for Stabilization of Teeth:

A custom-made cylindrical plastic mold measuring 15 mm in internal diameter and 20 mm in height was created. The mold's inside surface was coated with a separating substance. To provide a smooth and level surface, the mold's base sat on a glass slab, and the mold itself had been filled using self-curing acrylic resin (Auto Clear, Dent Bras; Pirassununga, São Paulo, Brazil). Whereas the acrylic resin remained in the dough phase, every molar was vertically inserted in the mold up to the cemento-enamel junction, while the coronal surface protruded over the mold's surface.

Specimen Preparation

The occlusal enamel of every molar was cut perpendicular to the long axis via a slow-speed diamond saw (Ecomet 3 Grinder/Polisher machine, Buehler, Lake Bluff, IL.) under continuous water irrigation to expose flat mid-coronal dentin. The occlusal enamel was removed, and dentin was flattened 2 mm apically from the original occlusal surface. The exposed dentin surfaces were standardized by wet-polishing with 600-grit silicon carbide abrasive paper for 60 seconds to produce a uniform smear layer [(13)].

Adhesive Application Protocols

For each adhesive, two application protocols were employed: manufacturer's recommended application time (control) and extended application time (double the manufacturer's recommended duration)

2-step total etch adhesive system (Adper Single Bond 2):

The 35% phosphoric acid gel (Scotchbond Universal Etchant, 3M ESPE) was applied for 15 seconds, washed for 10 seconds, gently blotted the excess moisture, and maintained a visibly moist surface. The adhesive (Adper Single Bond 2) was applied with active agitation for 15 seconds (the manufacturer's recommended time) or double the recommended duration for 30 seconds (extended). Finally, the adhesive was light-cured for ten seconds utilizing an LED device (Elipar S10, 3M ESPE; USA) after being lightly air-thinned for five seconds with both periods (15 and 30 seconds).

Self-etch, 1-step (Clearfil S³ Bond):

The 1-step self-etch adhesive (Clearfil S³ Bond) was applied directly to the prepared dentin surface with active brushing for 20 seconds (the manufacturer's recommended time) or double the recommended duration for 40 seconds (extended). Then, the adhesive was light-cured for ten seconds utilizing an LED device after being lightly air-thinned for five seconds with both periods (20 and 40 seconds).

Composite Build-Up

Following adhesive application, a nanohybrid resin composite (Filtek Z350 XT, 3M ESPE) was placed incrementally in 2-mm layers over the bonded dentin surface via a specially made Teflon split disc (4×4 mm). Every increment was light-cured for 20 seconds, and the final build-up reached a height of about 4 mm.

Microtensile Bond Strength (µTBS) Testing

Following 24 hours of storage in distilled water at 37°C, every sample was sliced into multiple sticks with a cross-sectional area of about 1 mm² utilizing the Isomet saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA). Individual sticks were attached to a microtensile testing device (universal testing machine) with cyanoacrylate adhesive and loaded to failure in tension at a crosshead speed of 0.5 mm/min (EZ-Test, Shimadzu, Kyoto, Japan). Bond strength values (MPa) were determined by dividing the load at failure (N) by the cross-sectional area (mm²) [14].

Statistical Analysis

The Shapiro-Wilk test was employed to determine the normality of the data. Bond strength data were analyzed using a three-way analysis of variance (ANOVA) with factors 'adhesive system', 'dentin age', and 'application time'. Between-group comparisons of adhesive systems were performed using independent t-tests. Post-hoc multiple comparisons were made utilizing Tukey's HSD test ($\alpha = 0.05$). All statistical procedures were made utilizing SPSS version 25 (IBM Corp., Armonk, NY, USA).

3. RESULTS

Statistical Overview

Three-way ANOVA revealed statistically significant variations in microtensile bond strength (µTBS) across both adhesive systems ($p < 0.001$). However, neither dentin age ($p = 0.112$) nor adhesive application time ($p = 0.087$) produced significant main effects. There was no statistically significant relationship between dentin age and application time ($p = 0.241$) (**Figure 1 and Table 2)**.

Table 2. Three-way ANOVA findings for microtensile bond strength

Source	Sum of Squares	Mean Square	F-value	p-value	η^2
Adhesive System	4,821.3	4,821.3	156.7	<0.001*	0.463
Dentin Age	78.2	78.2	2.54	0.112 ns	0.014
Application Time	91.7	91.7	2.98	0.087 ns	0.016
Age × Time	42.4	42.4	1.38	0.241 ns	0.008

*; significant at P < 0.05. ns; non- significant P > 0.05.

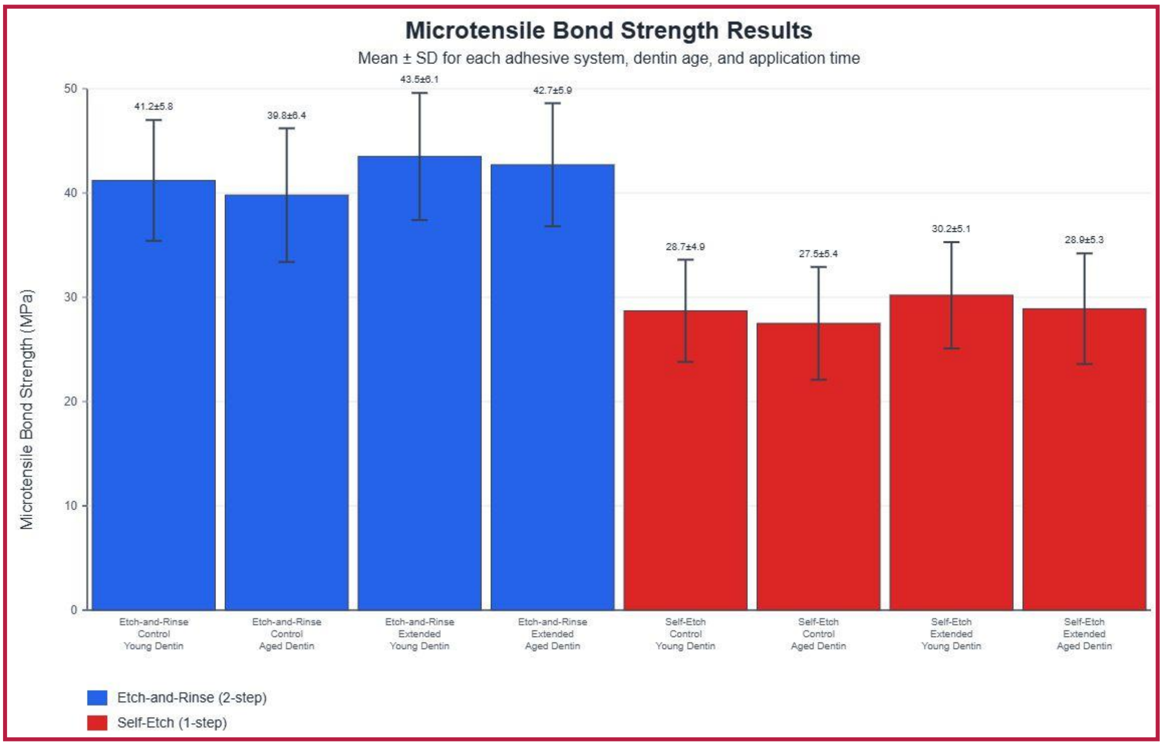

Figure 1. Microtensile bond strength (µTBS) of both adhesive systems under varying experimental conditions.

Effect of Dentin Age

Analysis of dentin age effects revealed no statistically significant influence on µTBS across both adhesive systems. For the etch-and-rinse adhesive, young dentin demonstrated non statically significant higher mean µTBS values compared to aged dentin under both application conditions ($p = 0.342$). Similarly, the self-etch adhesive showed minimal age-related differences ($p = 0.189$). The overall effect of dentin age across all experimental conditions was not significant ($F = 2.54$, $p = 0.112$, $\eta^2 = 0.014$), indicating that the structural and compositional changes associated with aging did not substantially compromise the bonding potential under the tested conditions **(Table 3)**.

Table 3. The impact of dentin age on the microtensile bond strength

Adhesive System	Young Dentin (MPa ± SD)	Aged Dentin (MPa ± SD)	Mean Difference	95% CI	P-value	Cohen's d
Etch-and-rinse	42.4 ± 6.0	41.3 ± 6.2	1.1	-1.2 to 3.4	0.342 ^{ns}	0.18
Self-etch	29.5 ± 5.0	28.2 ± 5.4	1.3	-0.7 to 3.3	0.189 ^{ns}	0.25
Overall	**35.9 ± 8.5**	**34.8 ± 8.9**	**1.1**	**-0.4 to 2.6**	**0.112** ^{ns}	**0.13**

ns; non- significant $P > 0.05$.

Impact of Application Time

Extended application time produced non-statistically significant higher bond strength values across both adhesive systems and dentin age groups. For the etch-and-rinse system, extended application resulted in mean increases in young dentin ($p = 0.168$) and in aged dentin ($p = 0.089$). The self-etch adhesive showed smaller improvements in young dentin ($p = 0.284$) and in aged dentin ($p = 0.316$). Overall analysis revealed no significant main effect of application time ($p = 0.087$), suggesting that doubling the manufacturer's recommended application time does not provide clinically meaningful improvements in bond strength under these experimental conditions **(Table 4)**.

Table 4. Effect of application time on microtensile bond strength

Adhesive System	Control Time (MPa ± SD)	Extended Time (MPa ± SD)	Mean Difference	95% CI	P-value	Cohen's d
Etch-and-rinse	40.5 ± 6.1	43.1 ± 6.0	2.6	0.3 to 4.9	0.28 ^{ns}	0.43
Self-etch	28.1 ± 5.2	29.6 ± 5.2	1.5	-0.5 to 3.5	0.142 ^{ns}	0.29
Overall	**34.3 ± 8.2**	**36.4 ± 8.8**	**2.1**	**0.7 to 3.5**	**0.087 ^{ns}**	**0.25**

ns; non- significant P > 0.05.

Comparative Adhesive Performance

In both tested adhesives, Post-hoc analysis displayed no statistically significant variations between application times within the same dentin age group, though extended application consistently produced numerically higher values. Similarly, dentin age had no significant impact on the µTBS within the same application time.

Across all experimental subgroups, the etch-and-rinse adhesive system consistently produced significantly greater µTBS values in comparison to the self-etch adhesive (overall comparison: $p < 0.001$). This performance advantage was maintained regardless of dentin age (young dentin: $p < 0.001$; aged dentin: $p < 0.001$) or adhesive application time (control time: $p < 0.001$; extended time: $p < 0.001$), with the etch-and-rinse system demonstrating approximately 40-50% higher bond strengths. The magnitude of this difference suggests fundamental mechanistic advantages of the 2-step total etch approach over the self-etch technique under the tested conditions **(Table 5)**.

Table 5. Overall adhesive system comparison

Parameter	Etch-and-Rinse	Self-Etch	Difference	Effect Size (Cohen's d)	P-value
Overall Mean ± SD (MPa)	41.8 ± 6.1	28.8 ± 5.2	13.0	2.31	<0.001*
Young Dentin Mean ± SD	42.4 ± 6.0	29.5 ± 5.0	12.9	2.33	<0.001*
Aged Dentin Mean ± SD	41.3 ± 6.2	28.2 ± 5.4	13.1	2.28	<0.001*
Control Time Mean ± SD	40.5 ± 6.1	28.1 ± 5.2	12.4	2.20	<0.001*
Extended Time Mean ± SD	43.1 ± 6.0	29.6 ± 5.2	13.5	2.41	<0.001*
Range (MPa)	26.8-54.9	16.9-41.3	-	-	-
Coefficient of Variation (%)	14.6	18.1	-	-	-

Parameter	Etch-and-Rinse	Self-Etch	Difference	Effect Size (Cohen's d)	P-value
95% Confidence Interval	40.5-43.1	27.8-29.8	-	-	-

*; significant at P < 0.05.

4. DISCUSSION

The strength of the bond between resin and dentin is indeed influenced by the microstructure of the dentin at the bonding location, and this is further affected by the aging process, as dentin is a dynamic tissue [15]. Dentin age is closely connected to the quantity of secondary dentin; as teeth age, dentin continues to be produced, leading to the contraction of dentinal tubules. This elevated the calcification, whether in response to external factors like attrition or cavities, is a component of physiological aging, also known as physiological sclerosis [16]. The rate at which monomers diffuse into demineralized dentin following smear layer removal can influence the binding strength of adhesive resins to dentinal substrates. The adhesive solution's diffusibility and the dentinal substrate's penetrability dictate this diffusion rate, with active application techniques and appropriate solvent systems enhancing deeper infiltration [17]. Consequently, some studies have reported that prolonged application time of bonding agents can improve dentin bond strengths compared to shorter application times [18].

The present results displayed that there was no statistically significant variation in µTBS between young and aged dentin. According to our results, the first part of the null hypothesis was accepted. This finding aligns with previous reports [6,7] indicating that physiological aging does not necessarily compromise immediate bonding effectiveness when contemporary adhesives are used. Although aged dentin exhibits increased mineral deposition within tubules, reduced permeability, and more densely packed collagen fibrils [19], the etch-and-rinse adhesive, phosphoric acid etching, eliminates mineral deposits that accumulate with age, including intratubular and peritubular sclerosis, thereby exposing the underlying collagen network for resin infiltration [20]. While phosphoric acid aims to demineralize and expose collagen, the effectiveness in highly sclerotic or aged dentin can be challenged due to the increased mineral content and acid-resistant layers, which may require adjustments to etching time or may not always lead to complete infiltration of the demineralized zone [21].

In contrast, self-etch adhesives (like Clearfil S³ Bond) contain the functional monomer 10-MDP, which chemically bonds to the residual hydroxyapatite in partially demineralized dentin, forming stable calcium–phosphate–MDP salts [22]. This chemical interaction, attributed to the phosphate group of 10-MDP and its stable calcium-MDP salt formation, contributes to a durable bond to the tooth structure [22]. These differing bonding mechanisms may explain why, in the current research, the µTBS of the two tested adhesive systems in aged dentin did not significantly differ from those in young dentin. This finding is consistent with prior reports showing that both adequate micromechanical retention and chemical adhesion can mitigate the effects of dentin aging [15,16].

However, it should be noted that other studies have reported lower bond strengths in aged dentin, particularly with certain three-step etch-and-rinse adhesives as found by Perdigão et al.[6] or when bonding to naturally sclerotic root dentin according to Tay et al. [25]. These discrepancies may be attributed to differences in adhesive chemistry, substrate location, and preparation technique.

Additionally, our results indicated that extending the adhesive application time did not lead to significant improvements in (µTBS) for both adhesive systems. According to our results, the second part of the null hypothesis was accepted. This finding aligns with several studies suggesting that simply prolonging application time beyond manufacturer recommendations may not always enhance bonding efficacy and, in some cases, can even be detrimental [20,25]. For Adper Single Bond 2, a 2-step total etch system adhesive, the bonding mechanism primarily relies on the micromechanical interlocking of resin within the demineralized dentin collagen network [28]. Phosphoric acid etching creates a porous collagen fibril network, and the following application of the primer/adhesive allows monomers to infiltrate these porosities [28]. While adequate time is crucial for proper infiltration, extending this beyond the manufacturer's recommended duration (typically 15 seconds for application, followed by air thinning for 5 seconds) may not lead to further significant resin penetration if the collagen network is already saturated or if sufficient solvent evaporation has occurred [29].

For Clearfil S³ Bond, the mechanism involves the simultaneous demineralization of dentin and infiltration of monomers due to its acidic components, notably the 10-MDP monomer, which forms a chemical bond with residual hydroxyapatite [30]. This self-etching process is generally rapid and self-limiting [31]. Once the acidic monomers have sufficiently reacted with the dentin and the adhesive has penetrated the partially demineralized layer to form a hybrid layer, additional application time may offer little to no further benefit [31]. Additionally, the chemical reactivity of the monomers and the adhesive's inherent acidity (pH ≈ 2.7 for Clearfil S³ Bond) might already be sufficient to reach its maximum bonding potential within the manufacturer's recommended time [32].

Similarly, a prior research conducted by Kimmes et al. [33] stated that extending the treatment time of total-etch and self-etch adhesives didn't consistently lead to a relevant increase in bond strength [33]. Furthermore, Hass et al. [31] found that while extended application periods for certain 1-step self-etch adhesive systems increased the degree of conversion and reduced nanoleakage, it did not consistently lead to higher immediate bond strength.

On the other hand, Saeed et al. [34] found that expanding the application period of the 1-step self-etch adhesive can lead to a significant increase in µTBS to dentin. The apparent contradiction can be resolved by examining the quality of the application technique, rather than the time-strength relationship. Application time is a variable that facilitates a more complete infiltration of the dentinal substrate, and studies on active application or scrub technique show more reliable bond performance improvements [35].

Furthermore, our results demonstrating that the etch-and-rinse adhesive consistently produced significantly greater µTBS values in comparison to the self-etch adhesive (Clearfil S³ Bond) align with a common trend observed in the research by Ghajari et al. [36] that compared these two adhesive philosophies, particularly with one-step self-etch systems. This difference can be largely attributed to their distinct bonding mechanisms and the resulting characteristics of the resin-dentin interface [37]. The high immediate bond strengths for etch-and-rinse adhesive is because of this extensive micromechanical retention [38]. While the one-step self-etch adhesive simultaneously demineralizes the dentin and infiltrates it without a separate rinsing step, this process results in a milder, less aggressive etching pattern than the etch-and-rinse adhesives, leaving residual hydroxyapatite integrated inside the hybrid layer [39]. Additionally, the hybrid layer developed by one-step self-etch adhesive systems is typically thinner, and resin tag formation might be shorter or less numerous than with etch-and-rinse systems [40].

The limitations of the present research include its primary focus on immediate bond strengths, which may not fully reflect the long-term clinical durability of the adhesive interface. The study was also limited to two specific adhesive systems, potentially limiting the comprehensive understanding of diverse adhesive behaviors. Finally, its in vitro nature, which means that crucial in vivo factors like pulpal pressure, salivary contamination, and intraoral moisture were not simulated. Therefore, future research should aim to evaluate long-term bond durability, include a broader spectrum of adhesive systems, and consider clinical experimental designs to enhance clinical relevance.

5. CONCLUSIONS

Within the limitations of the present research, it may be concluded that dentin age and application time beyond the manufacturer's recommended duration didn't significantly improve the µTBS when using both tested adhesive systems. In contrast, the choice of adhesive system played a decisive role, with the etch-and-rinse adhesive consistently achieving significantly greater µTBS than the self-etch adhesive, regardless of dentin age or application protocol.

Clinical significance: This study suggests that selecting the right adhesive strategy is crucial for optimizing dentin bonding performance, with clinicians recommending 2-step total-etch adhesives, especially for aged dentin, for predictable and durable bonding outcomes.

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